Committee for Risk Assessment

RAC

Annex 1
Background document
to the Opinion proposing harmonised classification and labelling at EU level of

cymoxanil (ISO); 2-cyano-N-[(ethylamino) carbonyl]-2-(methoxyimino)acetamide

EC Number: 261-043-0
CAS Number: 57966-95-7

CLH-O-0000007044-81-01/F

The background document is a compilation of information considered relevant by the dossier submitter or by RAC for the proposed classification. It includes the proposal of the dossier submitter and the conclusion of RAC. It is based on the official CLH report submitted to consultation. RAC has not changed the text of this CLH report but inserted text which is specifically marked as 'RAC evaluation'. Only the RAC text reflects the view of RAC.

Adopted

16 September 2021

European Commission

Combined Renewal Assessment Report prepared according to Regulation (EC) N° 1107/2009
and
Proposal for Harmonised Classification and Labelling (CLH Report) according to Regulation (EC) N° 1272/2008

CYMOXANIL

Volume 1

Rapporteur Member State: Lithuania
Co-Rapporteur Member State: Finland

Version History

When	What
February 2020	First version of Draft Renewal Assessment Report (RAR) submitted to EFSA
March 2020	First version of Combined Renewal Assessment Report and Proposal for Harmonised Classification and Labelling (CLH report) submitted to ECHA
March 2020	Second version of Combined Renewal Assessment Report and Proposal for Harmonised Classification and Labelling (CLH report) submitted to EFSA
June 2020	Third version of Combined Renewal Assessment Report and Proposal for Harmonised Classification and Labelling (CLH report) submitted to EFSA
	Third version of Combined Renewal Assessment Report and Proposal for Harmonised Classification and Labelling (CLH report) submitted to ECHA

In addition, the following sections are considered necessary for the harmonised classification and labelling according to the CLP criteria:

- **RAR Volume 3 B.2 (AS) Physical and chemical properties**
- **RAR Volume 3 B.6 (AS) Toxicology and metabolism data**
- **RAR Volume 3 B.8 (AS) Environmental fate and behaviour**
- **RAR Volume 3 B.9 (AS) Ecotoxicology**

The RMS is the author of the Assessment Report. The Assessment Report is based on the validation by the RMS, and the verification during the EFSA peer-review process, of the information submitted by the Applicant in the dossier, including the Applicant's assessments provided in the summary dossier. As a consequence, data and information including assessments and conclusions, validated and verified by the RMS experts, may be taken from the applicant's (summary) dossier and included as such or adapted/modified by the RMS in the Assessment Report. For reasons of efficiency, the Assessment Report should include the information validated/verified by the RMS, without detailing which elements have been taken or modified from the Applicant's assessment. As the Applicant's summary dossier is published, the experts, interested parties, and the public may compare both documents for getting details on which elements of the Applicant's dossier have been validated/verified and which ones have been modified by the RMS. Nevertheless, the views and conclusions of the RMS should always be clearly and transparently reported; the conclusions from the applicant should be included as an Applicant's statement for every single study reported at study level; and the RMS should justify the final assessment for each endpoint in all cases, indicating in a clear way the Applicant's assessment and the RMS reasons for supporting or not the view of the Applicant.

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Level 1

CYMOXANIL

1 STATEMENT OF SUBJECT MATTER AND PURPOSE FOR WHICH THIS REPORT HAS BEEN PREPARED AND BACKGROUND INFORMATION ON THE APPLICATION

1.1 CONTEXT IN WHICH THIS DRAFT ASSESSMENT REPORT WAS PREPARED

1.1.1 Purpose for which the draft assessment report was prepared

This document is a summary of assessments of the dossiers submitted in accordance with the requirements of Regulation (EU) No 844/2012 to support the renewal of approval of cymoxanil as provided for by Regulation (EC) No. 1107/2009.
In addition, MRL application form to maintain the current MRL of 0.3 mg/kg for representative use on grapes was also submitted by CTF and included in the RAR.

1.1.2 Arrangements between rapporteur Member State and co-rapporteur Member State

Lithuania as RMS made assessment and prepared the RAR, Finland acting as Co-RMS, agreed to review the RAR before the submission to EFSA.

1.1.3 EU Regulatory history for use in Plant Protection Products

Cymoxanil was included in Annex I of Directive 91/414/EEC (2008/125/EC) on 1 September 2009 and is approved under Regulation (EC) No. 1107/2009 in accordance with the Commission Implementing Regulation (EU) No 540/2011. Austria were designated Rapporteur Member State for the first approval and the Draft Assessment Report (DAR) was issued in 2007. The European Food Safety Authority's conclusion on peer review was published on 17 September 2008.
2011 - Modification of the existing MRL for cymoxanil in spinach (EFSA Journal 2011;9(3):2093):
2015 - Review of the existing maximum residue levels for cymoxanil according to Article 12 of Regulation (EC) No 396/2005 (EFSA Journal 2015;13(12):4355);
2017 - Approval extended until 31/08/2021 (COMMISSION IMPLEMENTING REGULATION (EU) 2017/195 of 3 February 2017 amending Implementing Regulation (EU) No 540/2011 as regards the extension of the approval periods of several active substances listed in Part B of the Annex to Implementing Regulation (EU) No 686/2012 (AIR IV renewal programme));
2017 - Modification of the existing maximum residue level for cymoxanil in beans without pods (EFSA Journal 2017;15(12):5066);
2019 - Evaluation of confirmatory data following the Article 12 MRL review for cymoxanil (EFSA Journal 2019;17(10):5823); proposed MRLs not yet implemented. RMS LT does not agree with the proposed lowered MRL of 0.05 mg/kg for grapes.

1.1.4 Evaluations carried out under other regulatory contexts

No JMPR report available for cymoxanil.

1.2 APPLICANT INFORMATION

1.2.1 Name and address of applicant(s) for approval of the active substance

Name:	**Cymoxanil (AIR4) Task Force (CTF)**
Address	C/o Battelle UK Limited Avenue des Morgines, 12 1213 Petit-Lancy Geneva Switzerland
Contact	**<confidential>**
Tel:	<confidential>
Fax:	<confidential>
E-mail	<confidential>
Contact	**<confidential>**

Tel: <confidential>
E-mail <confidential>

Name **Agria SA**
Address: Asenovgradsko Shose
Plovdiv 4009
Bulgaria
Contact: <confidential>
Telephone: <confidential>
Email: <confidential>

Name: **Société Financière de Pontarlier (SFP)**
Address 11, Boulevard de la Grande Thumine
Parc d'Ariane – Bât B
F-13090 Aix en Provence
France
Contact <confidential>
Tel: <confidential>
Fax: <confidential>
E-mail -

1.2.2 Producer or producers of the active substance

CONFIDENTIAL information - data provided separately. Please refer to the relevant Volume 4.

1.2.3 Information relating to the collective provision of dossiers

Five applicants – Belchim, Du Pont, Indofil, Oxon, UPL submitted their joint Task Force dossier of cymoxanil. Two applicants - Agria and SFP - submitted their separate dossiers for the EU renewal of cymoxanil approval.

1.3 IDENTITY OF THE ACTIVE SUBSTANCE

The information under point 1.3.1 – 1.3.9 should be provided in a table compatible with Vol. 3 and LoEP.

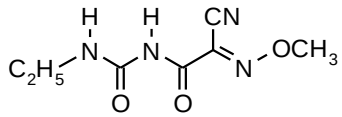
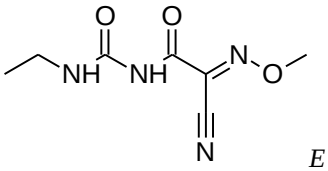
1.3.1 Common name proposed or ISO-accepted and synonyms	Cymoxanil (ISO)
1.3.2 Chemical name (IUPAC and CA nomenclature)	
IUPAC	1-[(*E/Z)*-2-cyano-2-methoxyiminoacetyl]-3-ethylurea
CA	2-cyano-N-[(ethylamino)carbonyl]-2-(methoxyimino)acetamide [1] (2*E*)-2-cyano-N-[(ethylamino)carbonyl]-2-(methoxyimino)acetamide [2][1]
1.3.3 Producer's development code number	*This item should be also included in the LoEP* **Task Force members** Producer: Belchim Crop Protection NV/SA — Develpoment code: BCP356F

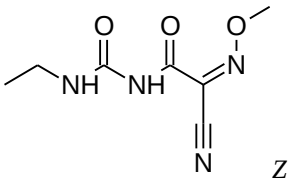
[1] Based on ECHA SID Team conlcusion. A multi-constituent substance cymoxanil is described by a name of its main constituent.

<table>
<tr><td></td><td>
<table>
<tr><td>DuPont International Operations S.a.r.l.</td><td>DPX-T3217

IN-T3217
T3217</td></tr>
<tr><td>Indofil Industries Limited</td><td>(none)</td></tr>
<tr><td>Oxon Italia S.p.A.</td><td>(none)</td></tr>
<tr><td>United Phosphorus Limited</td><td>FDJ06</td></tr>
</table>

Agria SA: none

SFP: none</td></tr>
<tr><td colspan="2">1.3.4 CAS, EEC and CIPAC numbers</td></tr>
<tr><td>CAS</td><td>57966-95-7 (E isomer) [1]
166900-80-7 [2]
<s>93195-85-8 (Z isomer)</s>[2]</td></tr>
<tr><td>EEC</td><td>261-043-0</td></tr>
<tr><td>CIPAC</td><td>419</td></tr>
<tr><td colspan="2">1.3.5 Molecular and structural formula, molecular mass</td></tr>
<tr><td>Molecular formula</td><td>$C_7H_{10}N_4O_3$</td></tr>
<tr><td>Structural formula</td><td> 
Cymoxnil isomers (E/Z), E/Z ratio min.99:1
 
E isomer

 
Z isomer</td></tr>
<tr><td>Molecular mass</td><td>198.2 g/mol</td></tr>
<tr><td>1.3.6 Method of manufacture (synthesis pathway) of the active substance</td><td>Confidential information – data provided in Volume 4.</td></tr>
<tr><td>1.3.7 Specification of purity of the active substance in g/kg</td><td>Task Force
Min. 970 g/kg (E:Z isomer ratio: min 99: max 1).

<u>Du Pont</u>
min. 970 g/kg (DAR, reference source)
min. 970 g/kg (Renewal)

<u>Oxon</u>
min. 970 g/kg (DAR, reference source)
min. 970 g/kg and 975 g/kg (Renewal)

<u>Indofil</u>
min. 980 g/kg (Renewal)</td></tr>
</table>

[2] Based on ECHA SID Team conclusion, 93195-85-8 is a deleted CAS no. associated to CAS 57966-95-7.

	UPL min. 970 g/kg and 990 g/kg (Renewal) Belchim not applicable, not a manufacture of cymoxanil for AIR 4 **Agria SA** min. 985 g/kg **SFP** min. 980 g/kg
1.3.8 Identity and content of additives (such as stabilisers) and impurities **Task Force** Cymoxanil technical does not contain any impurities of toxicological, ecotoxicological or environmental relevance.	
1.3.8.1 Additives	**1.3.8.2 CONFIDENTIAL information – see Volume 4.**
1.3.8.3 Significant impurities	**1.3.8.4 CONFIDENTIAL information – see Volume 4.**
1.3.8.5 Relevant impurities	**Task Force** *Based on cimoxanil Annex I (91/414/ EEC) inclusion:* Cymoxanil technical does not contain any impurities of toxicological, ecotoxicological or environmental relevance. **Agria SA:** assessment is ongoing **SFP:** assessment is ongoing
1.3.9 Analytical profile of batches	CONFIDENTIAL information – see Volume 4.

1.4 INFORMATION ON THE PLANT PROTECTION PRODUCT

1.4.1 Applicant	**Task Force (CTF)** Members: Belchim, Du Pont, Indofil, Oxon, UPL C/o Battelle UK Limited Avenue des Morgines, 12 1213 Petit-Lancy Geneva Switzerland **<confidential>** <confidential> <confidential> <confidential> **<confidential>** <confidential> <confidential> **Agria SA** Agria SA Asenovogradsko Shose

	Plovdic 4009, Bulgaria Contact: <confidential> <confidential> <confidential> **SFP** Société Financière de Pontarlier 11, Boulevard de la Grande Thumine Parc d'Ariane – Bât B F-13090 Aix en Provence
1.4.2 Producer of the plant protection product	Producer of Cymbal 45, (code no.BCP308) Task Force formulation: Belchim Producer of Rival Duo: <confidential> Producer of Dauphin 45 (code number FDJ03): SFP Manufacturing sites addresses: CONFIDENTIAL information – see Volume 4.
1.4.3 Trade name or proposed trade name and producer's development code number of the plant protection product	**Cymbal 45**, Cymoxanil 45 % WG, code number **BCP308** (Task Force) **Rival Duo**; code number: none (Agria SA) The formulation of cymoxanil (50 g/L) and propamocarb hydrochloride 400 g/L) **Dauphin 45,** code number **FDJ03** (SFP)
1.4.4 Detailed quantitative and qualitative information on the composition of the plant protection product	

1.4.4.1 Composition of the plant protection product

Cymbal 45, Cymoxanil 45 % WG (BCP308)

Active substance	g/kg	% (w /w)
cymoxanil, pure	450	45.0

Rival Duo

Active substances	g/L	% (w /w)*
cymoxanil, pure	50	4.57
propamocarb,pure	400	36.46

* based on the density 1.097 gL

1.4.4.2 Dauphin 45 (FDJ03)

Active substance	g/kg	% (w /w)
cymoxanil, pure	450	45.0
cymoxanil, technical*	459	45.9

* at a minimum declared purity (98 %)

Detailed composition of formulation is given in CONFIDENTIAL information – see Volume 4.

1.4.4.3 Information on the active substances

All formulations contain active substance cymoxanil

Cymoxanil	**Name/ Code number**
ISO common name	Cymoxanil
CAS No,	57966-95-7
EC no.	261-043-0
CIPAC No.	419
Salt, ester, anion or cation present	-

Rival Duo contains second active substance propamocarb:

Propamocarb	**Name/ Code number**
ISO common name	Propamocarb hydrochloride
IUPAC name	propyl 3-(dimethylamino) propylcarbamate hydrochloride
CAS No,	25606-41-1
EC no.	247-155-9
CIPAC No.	
Salt, ester, anion or cation present	hydrochloride

1.4.4.4 Information on safeners, synergists and co-formulants

CONFIDENTIAL information – see Volume 4.

1.4.5 Type and code of the plant protection product

Formulation	Type/Code[3]
Cymbal 45 (BCP308)	WG (water dispersible granules)
Rival Duo	SC (soluble concentrate)
Dauphin 45 (FDJ03)	WG (water dispersible granules)

[3] Croplife Technical Monograph no.2, 7th edition, revised March 2017

1.4.6 Function	fungicide
1.4.7 Field of use envisaged	Cymbal 45 WG : Grape, tomatoe, potatoe Rival Duo : potato: Late blight *(Phytophtora infestants)* control Dauphin 45 : Tomatoes, potatoes, grapes
1.4.8 Effects on harmful organisms	Protective and curative mode of action. Cymoxanil prevents spore germination, haustorial formation and mycelial growth of the target pathogens. Control of fungal plant pathogens belonging to Peronosporales - *Phytophthora*, *Plasmopara*, and *Peronospora* spp., causing downy mildew and blight in crops, e.g. *Plasmopara viticola* (downy mildew in grapes) and *Phytophthora infestans* (late blight in potatoes/tomatoes).

1.5 DETAILED USES OF THE PLANT PROTECTION PRODUCT

1.5.1 Details of representative uses

Table 1.5.1-1: The GAP table of representative uses by the Cymoxanil Task Force (CTF) for the product "Cymoxanil 45 WG"

Crop and/or situation (a)	**Member State or Country**	**Product name**	**F G or I** (b)	**Pests or Group of pests controlled** (c)	**Preparation**		**Application**				**Application rate per treatment**			**PHI (days)** (m)	**Remarks**
					Type (d-f)	Conc. a.s. (i)	method kind (f-h)	range of growth stages & season (j)	number min-max (k)	Interval between application (min)	kg a.s /hL min-max (l)	Water L/ha min-max	kg a.s./ha min-max (l)		
Grapes	C EU, S EU	'Cymoxanil 45 WG'	F	Foliar fungi e.g. *Plasmopara viticola*	WG	450 g/kg	Foliar spray	BBCH 11 – 89	5	7 days	0.01-0.08	150 – 1000*	0.12	28	
Tomatoes	EU all zones	'Cymoxanil 45 WG'	G	Foliar fungi e.g. *Phytophthora infestans*	WG	450 g/kg	Foliar spray	BBCH 12 – 89	5	7 days	0.01-0.025	600 – 1500	0.15	3	
Tomatoes	S EU	'Cymoxanil 45 WG'	F	Foliar fungi e.g. *Phytophthora infestans*	WG	450 g/kg	Foliar spray	BBCH 12 – 89	5	7 days	0.015-0.1	150 – 1000	0.15	3	
Potatoes	EU all zones	'Cymoxanil 45 WG'	F	Foliar fungi e.g. *Phytophthora infestans*	WG	450 g/kg	Foliar spray	BBCH 10 – 95	5	5 days	0.015-0.1	150 – 1000	0.15	7	

* - the volume of water shown in table was changed by the applicant a week before the submission of the application to EFSA, an initial volume was 150 – 1200 L/ha

Table 1.5.1-2: The GAP table of representative uses by the Agria S. A. for the product "Rival Duo"

Crop and/or situation (a)	**Member State or Country**	**Product name**	**F G or I** (b)	**Pests or Group of pests controlled** (c)	**Preparation**		**Application**				**Application rate per treatment**			**PHI (days)** (m)	**Remarks**
					Type (d-f)	Conc. a.s. (i)	method kind (f-h)	range of growth stages & season (j)	number min-max (k)	Interval between application (min)	kg a.s /hL min-max (l)	Water L/ha min-max	kg a.s./ha min-max (l)		
Potatoes	C EU, S EU	'Rival Duo'	F	Late blight *Phytophthora infestans* PHYTIN	SC	50 g/l Cym 400 g/l Prop	Foliar spray	BBCH 20 – 95	6	7 days	0.025-0.04 Cym 0.2-0.33 Prop	300 – 500	0.125 Cym 1.0 Prop	14	

Table 1.5.1-3: The GAP table of representative uses by the Société Financière de Pontarlier (SFP) for the product "Dauphin 45"

Crop and/or situation (a)	Member State or Country	Product name	F G or I (b)	Pests or Group of pests controlled (c)	Preparation		Application				Application rate per treatment			PHI (days) (m)	Remarks
					Type (d-f)	Conc. a.s. (i)	method kind (f-h)	range of growth stages & season (j)	number min-max (k)	Interval between application (min)	kg a.s /hL min-max (l)	Water L/ha min-max	kg a.s./ha min-max (l)		
Grapes	S EU	'Dauphin 45 / FDJ03'	F	Downy mildew (*Plasmopara viticola*)	WG	450 g/kg	Foliar spray	BBCH 13 – 85	4	7-10 days	0.01-0.06	200 – 1000	0.12	28	
Tomatoes	S EU	'Dauphin 45 / FDJ03'	F	Late blight (*Phytophtora infestans*)	WG	450 g/kg	Foliar spray	BBCH 13 – 87	5	7 days	0.01-0.06	200 – 1000	0.12	3	Tomatoes for fresh consumption
Tomatoes	S EU	'Dauphin 45 / FDJ03'	F	Late blight (*Phytophtora infestans*)	WG	450 g/kg	Foliar spray	BBCH 13 – 87	5	7 days	0.01-0.06	200 – 1000	0.12	10	Industrial tomatoes
Potatoes	CEU	'Dauphin 45 / FDJ03'	F	Late blight (*Phytophtora infestans*)	WG	450 g/kg	Foliar spray	BBCH 21 – 95	8	7-10 days	0.02-0.1	100 – 600	0.10	7	
Potatoes	SEU	'Dauphin 45 / FDJ03'	F	Late blight (*Phytophtora infestans*)	WG	450 g/kg	Foliar spray	BBCH 21 – 95	8	7-10 days	0.01-0.06	200 – 1000	0.12	7	

(a) For crops, the EU and Codex classification (both) should be taken into account; where relevant, the use situation should be described (e.g. fumigation of a structure)
(b) Outdoor or field use (F), greenhouse application (G) or indoor application (I)
(c) *e.g.* biting and suckling insects, soil born insects, foliar fungi, weeds
(d) *e.g.* wettable powder (WP), emulsifiable concentrate (EC), granule (GR), dispersible granule (WG)
(e) GCPF Codes – GIFAP Technical Monograph N° 2, 1989
(f) All abbreviations used must be explained
(g) Method, e.g. high volume spraying, low volume spraying, spreading, dusting, drench
(h) Kind, e.g. overall, broadcast, aerial spraying, row, individual plant, between the plant – type of equipment used must be indicated
(i) g/kg or g/L. Normally the rate should be given for the active substance (according to ISO) and not for the variant in order to compare the rate for same active substances used in different variants (e.g. fluoroxypyr). **In certain cases, where only one variant synthesised, it is more appropriate to give the rate for the variant (e.g. benthiavalicarb-isopropyl).**
(j) Growth stage at last treatment (BBCH Monograph, Growth Stages of Plants, 1997, Blackwell, ISBN 3-8263-3152-4), including where relevant, information on season at time of application
(k) Indicate the minimum and maximum number of application possible under practical conditions of use
(l) The values should be given in g or kg whatever gives the more manageable number (e.g. 200 kg/ha instead of 200 000 g/ha or 12.5 g/ha instead of 0.0125 kg/ha
(m) PHI - minimum pre-harvest interval

1.5.2 Further information on representative uses

The product "Cymoxanil 45 WG"
The product "Cymoxanil 45 WG" is foliar fungicide intended to control late blight on potatoes and tomatoes. Besides, this product is used to control *Plasmopara viticola* on grapes.

Method of application:
The product is applied as a foliar spray.

Maximum number of applications and their timings:
A maximum of five applications may be made to all supported crops with a seven-days interval.
Application can occur throughout the season when the grapes are at growth stages from BBCH 11 to BBCH 89, tomatoes are at the growth stages BBCH 12-89, potatoes are at the growth stages BBCH 10-95.

Minimum waiting periods or other precautions between last application and sowing or planting succeeding crops:
There are no waiting periods before planting succeeding crops.

Proposed instruction for use:
For full details, please refer to product label at document C.

The product "Rival Duo"
The product "Rival Duo" is foliar fungicide intended to control late blight on potatoes.

Method of application:
The product is applied as a foliar spray.

Maximum number of applications and their timings:
Potato: 6 applications at a minimum of 7 day intervals, BBCH 20-95

Duration of protection afforded by each application:
The level of protection afforded by a single application depends on climatic conditions e.g. rainfall post application and crop growth.
As an example potatoes treated for late blight control under dry conditions when the crop is mature will require re-application at approximately 14 day intervals, whereas if the crop is growing rapidly under similar climatic conditions in order to protect the new foliage treatments will be applied at either 7 or 10 day intervals. In the worst-case situation when the crop is growing actively and the risk of infection from late blight is high applications are recommended every seven days.

Minimum waiting periods or other precautions between last application and sowing or planting succeeding crops:
Propamocarb hydrochloride 400 g/L + Cymoxanil 50 g/L SC has no negative effect on the rotational crops.

Limitations on choice of succeeding crops:
There is no limitation on the choice of succeeding crops.

Proposed instruction for use:
For full details, please refer to product label at document C.

The product "Dauphin 45"
The product "Dauphin 45" is foliar fungicide intended to control late blight on potatoes and tomatoes. Besides, this product is used to control downy mildew on grapes.

Method of application:
The product is applied as a foliar spray.

Maximum number of applications and their timings:

Potatoes (at growth stages – BBCH 21-95) – maximum 8 applications with 7 to 10 days' interval, grapes (at growth stages – BBCH 13-85) – maximum 4 applications with 7 to 10 days' interval and tomatoes (at growth stages – BBCH 13-87) – maximum 5 applications with 7 days' interval.

Duration of protection afforded by each application and the maximum number of applications:
Cymoxanil is mostly used in combination with protective fungicides (e.g. Mancozeb), either as a tank mix or in alternating applications, in order to improve its protectant action that can thus last more than 10 days on the treated organs. This property is strongly dependent on the cymoxanil which acts by synergy with the associated products.

Minimum waiting periods or other precautions between last application and sowing or planting succeeding crops:
In general, it is not necessary to specify a minimum waiting or other precaution between the last application and sowing or planting a succeeding crop, as the active substance has fungicidal activity.

Limitations on choice of succeeding crops:
There are no limitations on the choice of succeeding crops as a consequence of using FDJ03.

Proposed instruction for use:
For full details, please refer to product label at document C.

1.5.3 Details of other uses applied for to support the setting of MRLs for uses beyond the representative uses

No uses beyond the representative ones were applied for in order to support the setting of MRLs.

1.5.4 Overview on authorisations in EU Member States

The product "Cymoxanil 45 WG" is registered in Italy, Malta, Greece, Cyprus, Bulgaria, Serbia, Belgium, Luxembourg, The Netherlands, Austria, Germany, Slovakia, Czech Republic, Hungary, United Kingdom, Ireland, Slovenia, Poland, Romania, Denmark, Finland, Sweden.

The plant protection product Cymoxanil 50 g/L + propamocarb 400g/L SC ("Rival Duo") is registered in Czech Republic (CZ), Germany (DE), Romania (RO), Slovakia (SK), United Kingdom (UK), Bulgaria (BU), Austria (AT), Hungary (HU), Slovenia.

The product "Dauphin 45" is registered in United Kingdom, Poland, Ireland, France, Greece, Italy, Belgium, Austria.

Level 2

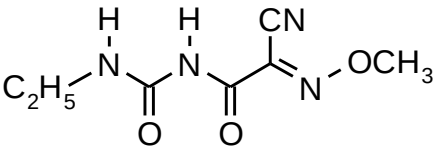
CYMOXANIL

2 SUMMARY OF ACTIVE SUBSTANCE HAZARD AND OF PRODUCT RISK ASSESSMENT

2.1 IDENTITY

2.1.1 Summary of identity

Table 1: Summary of identity of the active substance

Name(s) in the IUPAC nomenclature or other international chemical name(s)	1-[*(E/Z)* -2-cyano-2-methoxyiminoacetyl]-3-ethylurea
Other names (usual name, trade name, abbreviation)	cymoxanil
ISO common name (if available and appropriate)	cymoxanil
EC number (if available and appropriate)	261-043-0
EC name (if available and appropriate)	2-cyano-N-[(ethylamino)carbonyl]-2-(methoxyimino) acetamide [1]; (2*E*)-2-cyano-N-[(ethylamino)carbonyl]-2-(methoxyimino)acetamide; [2][4]
CAS number (if available)	57966-95-7 [1]; 166900-80-7 [2]
Other identity code (if available)	CIPAC: 419
Molecular formula	$C_7H_{10}N_4O_3$
Structural formula	
Molecular weight or molecular weight range	198.2 g/mol
Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	E:Z isomer ratio 99:1
Description of the manufacturing process and identity of the source (for UVCB substances only)	Not a UVCB substance Cymoxanil is manufactured at large scale production
Degree of purity (%) (if relevant for the entry in Annex VI)	Minimum 97 % w/w

[4] Based on ECHA SID Team conclusion. A multi-constituent substance cymoxanil described by its main constituent name.

2.2 PHYSICAL AND CHEMICAL PROPERTIES [EQUIVALENT TO SECTION 7 OF THE CLH REPORT TEMPLATE]

2.2.1 Summary of physical and chemical properties of the active substance

Table 2: Summary of physicochemical properties of the active substance

Property	Value	Reference	Comment (e.g. measured or estimated)
Physical state at 20°C and 101,3 kPa	Solid	Moore 2003	Measured
Melting/freezing point	160-163 ºC	Huntley, 2000 Subbaiah, 2013a van der Baan-Treur, 2003 Betteley, 1995a Garofani, 2009a Schmuckler & LeSieur, 1993 Weissenfeld, 2008	Measured
Boiling point	215 ºC	Subbaiah, 2013b Weissenfeld, 2008	Measured
Relative density	Not applicable	-	Cymoxanil is not a liquid
Vapour pressure	1.50×10^{-4} Pa at 20ºC	Schmuckler & Cooke, 1993 Betteley, 1995a Ganesh, 2013 Garofani S, 2009b	Measured
Water solubility	[g/L]: pH 5: pH 7: 10ºC: 0.7 0.6 20ºC: 0.9 0.8 30ºC: 1.2 1.0	Hansen, 2000 Kousalya S., 2013 Betteley, 1995a Garofani S, 2009d	Measured
Flash point	Not applicable	-	Cymoxanil is not a liquid
Flammability	Not flammable	Gravell, 1996 Naveetha, 2013 Garofani, 2009h Betteley, 1995b	Measured
Explosive properties	Not explosive	Gravell, 1996 Betteley, 1995b Cage, 2009	Measured
Self-ignition temperature	Not flammable	Gravell, 1996 Naveetha, 2013 Garofani, 2009h Betteley, 1995b	Measured
Oxidising properties	Not oxidising	Gravell, 1996 Smeykal, 2008 Hatzenbeler, 2009 Garofani, 2009j Habeck, 2009c Betteley, 1995b Jackson, 2007	Measured
Dissociation	pKA 8.9 – 9.7	Schmuckler and	Measured

Property	Value	Reference	Comment (e.g. measured or estimated)
constant		Moore, 1993 Betteley, 1995a Garofani 2009g	

2.2.1.1 Evaluation of physical hazards [equivalent to section 8 of the CLH report template]

2.2.1.1.1 Explosives *[equivalent to section 8.1 of the CLH report template]*

Table 3: Summary table of studies on explosive properties

Method	Results	Remarks	Reference
EEC A.14	Not explosive		Gravell, 1996
EEC A.14	Not explosive		Betteley, 1995
EEC A.14	Not explosive		Cage, 2009

2.2.1.1.1.1 Short summary and overall relevance of the provided information on explosive properties

Under the condition of the test, cymoxanil is not explosive. Cymoxanil does not belong to the additional hazard class 'desensitised explosives' in terms of criteria of the 12th ATP and the UN Recommendations on the Transport of Dangerous Goods, Manual of tests and criteria.

Information on tests performed and their results provided in Cymoxanil RAR Vol.3 B.2 (AS) Physical and chemical properties.

2.2.1.1.1.2 Comparison with the CLP criteria

Cymoxanil is not classified as explosive nor a desensitised explosive based on results of the three studies performed as refernced above.

Based on the screening procedure according to Reg (EU) 1272/2008 Annex I 2.1.4.2 and 2.1.4.3, the substance is not classified as explosive if there are no chemical groups associated with explosive properties present in the molecule as given in the Table A6.1 in Appendix 6 of the UN recommendations.

Chemical groups indicating explosive properties in organic materials:

Structural feature	Examples
C-C unsaturation	Acetylenes, acetylides, 1,2-dienes
C-Metal, N-Metal	Grignard reagents, organo-lithium compounds
Contiguous nitrogen atoms	Azides, aliphatic azo compounds, diazonium salts, hydrazines, sulphonylhydrazides
Contiguous oxygen atoms	Peroxides, ozonides
N-O	Hydroxylamines, nitrates, nitro compounds, nitroso compounds, N-oxides, 1,2-oxazoles
N-halogen	Chloramines, fluoroamines
O-halogen	Chlorates, perchlorates, iodosyl compounds

None of these groups associated with explosive properties in the molecule are present in cymoxanil.

In regards to classification procedure for desentisised explosives with reference to 12th ATP and the Reg. (EU) 2019/521, the procedure does not apply if the substances or mixtures contain no explosives according to criteria of section 2.1 of the CLP Reg. (EU) 1272/2008.

Cymoxanil contains no explosives therefore the classidication procedure for desensitised explosives does not apply to cymoxanil.

2.2.1.1.1.3 Conclusion on classification and labelling for explosive properties

Not classified.

2.2.1.1.2 Flammable gases (including chemically unstable gases) *[equivalent to section 8.2 of the CLH report template]*

Not relevant, cymoxanil is a solid.

Table 4: Summary table of studies on flammable gases (including chemically unstable gases)

Method	Results	Remarks	Reference
Not relevant			

2.2.1.1.2.1 Short summary and overall relevance of the provided information on flammable gases (including chemically unstable gases)

Not relevant, cymoxanil is a solid.

2.2.1.1.2.2 Comparison with the CLP criteria

Not relevant, cymoxanil is a solid.

2.2.1.1.2.3 Conclusion on classification and labelling for flammable gases

Not relevant, cymoxanil is a solid.

2.2.1.1.3 Oxidising gases *[equivalent to section 8.3 of the CLH report template]*

Not relevant, cymoxanil is a solid.

Table 5: Summary table of studies on oxidising gases

Method	Results	Remarks	Reference
Not relevant			

2.2.1.1.3.1 Short summary and overall relevance of the provided information on oxidising gases

Not relevant, cymoxanil is a solid.

2.2.1.1.3.2 Comparison with the CLP criteria

Not relevant, cymoxanil is a solid.

2.2.1.1.3.3 Conclusion on classification and labelling for oxidising gases

Not relevant, cymoxanil is a solid.

2.2.1.1.4 Gases under pressure *[equivalent to section 8.4 of the CLH report template]*

Not relevant, cymoxanil is a solid.

Table 6: Summary table of studies on gases under pressure

Method	Results	Remarks	Reference
Not relevant			

2.2.1.1.4.1 Short summary and overall relevance of the provided information on gases under pressure

Not relevant, cymoxanil is a solid.

2.2.1.1.4.2 Comparison with the CLP criteria

Not relevant, cymoxanil is a solid.

2.2.1.1.4.3 Conclusion on classification and labelling for gases under pressure

Not relevant, cymoxanil is a solid.

2.2.1.1.5 Flammable liquids *[equivalent to section 8.5 of the CLH report template]*

Not relevant, cymoxanil is a solid.

Table 7: Summary table of studies on flammable liquids

Method	Results	Remarks	Reference
not relevant			

2.2.1.1.5.1 Short summary and overall relevance of the provided information on flammable liquids

Not relevant, cymoxanil is a solid.

2.2.1.1.5.2 Comparison with the CLP criteria

Not relevant, cymoxanil is a solid.

2.2.1.1.5.3 Conclusion on classification and labelling for flammable liquids

Not relevant, cymoxanil is a solid.

2.2.1.1.6 Flammable solids *[equivalent to section 8.6 of the CLH report template]*

Table 8: Summary table of studies on flammable solids

Method	Results	Remarks	Reference
EEC A.10	Not flammable		Gravell, 1996
EEC A.10	Not flammable		Naveetha, 2013
EEC A.10	Not flammable		Garofani, 2009
EEC A.10	Not flammable		Betteley, 1995

2.2.1.1.6.1 Short summary and overall relevance of the provided information on flammable solids

Under the condition of the test, cymoxanil is not flammable.

Information on tests performed and their results provided in Cymoxanil RAR Vol.3 B.2 (AS) Physical and chemical properties.

2.2.1.1.6.2 Comparison with the CLP criteria

Cymoxanil is not a flammable solid.
Available data from the A10 test method with the result that cymoxanil is not highly flammable indicate that a classification as a flammable solid does not apply, therefore no more testing is necessary. It is not necessary to determine the influence of the wetted zone as described in the UN Test N.1.

2.2.1.1.6.3 Conclusion on classification and labelling for flammable solids

Not classified.

2.2.1.1.7 Self-reactive substances *[equivalent to section 8.7 of the CLH report template]*

Not relevant, cymoxanil is not a self reactive substance.

Table 9: Summary table of studies on self-reactivity

Method	Results	Remarks	Reference
Not relevant			

2.2.1.1.7.1 Short summary and overall relevance of the provided information on self-reactive substances

The following information has been available from the applicant:

Using decision logic 2.8 for self-reating substances (see Guidance on the application of the CLP criteria), cymoxanil would be categorised as a Type G substance. Therefore, cymoxanil is not classified as a self reactive substance.

Substaces of Type G have no hazard communication elements assigned.

2.2.1.1.7.2 Comparison with the CLP criteria

Cymoxanil molecule does not have any chemical groups associated with the explosive properties (see 2.2.1.1.2 above). Additionally, in the three separate tests for explosive properties, cymoxanil returned negative results (see 2.2.1.1.1 above).

Examples of chemical groups indicating self reactive properties in organic materials as given in in the Table A6.3 in Appendix 6 of the UN recommendations incorporated below:

Structural feature	Examples
Mutually reactive groups	Aminonitriles, haloanilines, organic salts of oxidizing acids
S=O	Sulphonyl halides, sulphonyl cyanides, sulphonyl hydrazides
P-O	Phosphites
Strained rings	Epoxides, aziridines
Unsaturation	Olefins, cyanates

Cymoxanil does contain an amino group (as a secondary amine) and a nitrile group and although the presence of aminonitriles implies that the substance may possess self-reactive properties according to the UN RTDG (see table above) the presence of these two moieties does not confirm self-reactive properties to cymoxanil since it is not an α- or β-aminonitrile and in three self-heating studies (see 2.2.1.1.10 below), cymoxanil did not exhibit any exothermic events at temperatures of up to 450ºC.

In the three self heating studies (see 2.2.1.1.10 below) cymoxanil did not exhibit any exothermic events at temperatures of up to 450ºC.

In flammability tests (see 2.2.1.1.6 above) cymoxanil was determined to be not a flammable solid.

According to the notifier, *using decision logic 2.8 for self-reacting substances (see Guidance on the application of the CLP criteria), cymoxanil would be categorised as a Type G substance. Therefore cymoxanil is not classified as a self-reactive substance.*

According to the part II of the UN RTDG Manual of Tests and Criteria the types of self-reactive substance range from type A, which may not be accepted for transport in the packaging in which it is tested to type G, which is not subject to the provisions for self reactive substances of Division 4.1.

2.2.1.1.7.3 Conclusion on classification and labelling for self-reactive substances

The results of decision logic 2.8 would not be applicable to classify cymoxanil as a self reactive type G substance. Decision logic 2.8 is applicable when the test methods A-H part II of the UN RTDG Manual of Tests and criteria have been performed.

Cymoxanil is not classified as a self - reactive substance.

2.2.1.1.8 Pyrophoric liquids *[equivalent to section 8.8 of the CLH report template]*

Not relevant, cymoxanil is a solid.

Table 10: Summary table of studies on pyrophoric liquids

Method	Results	Remarks	Reference
Not relevant			

2.2.1.1.8.1 Short summary and overall relevance of the provided information on pyrophoric liquids

Not relevant, cymoxanil is a solid.

2.2.1.1.8.2 Comparison with the CLP criteria

Not relevant, cymoxanil is a solid.

2.2.1.1.8.3 Conclusion on classification and labelling for pyrophoric liquids

Not relevant, cymoxanil is a solid.

2.2.1.1.9 Pyrophoric solids *[equivalent to section 8.9 of the CLH report template]*

Table 11: Summary table of studies on pyrophoric liquids

Method	Results	Remarks	Reference
Not relevant			

2.2.1.1.9.1 Short summary and overall relevance of the provided information on pyrophoric solids

No tests have been conducted but safe long-term use of cymoxanil demonstarates that it is not a pyrophoric solid. Cymoxanil does not spontaneously ignite in air.

2.2.1.1.9.2 Comparison with the CLP criteria

No tests have been conducted but safe long-tem use is evidence that cymoxanil even in small quantities is not liable to ignite within five minutes after coming into contact with air.

Based on provisions of the Reg. (EU) 1272/2008, and the consideration of criteria for pyrophoric solids (see Annex I part 2, 2.10.4.1), the classification procedure for pyrophoric solids need not be applied when experience in manufacture or handling shows that the substance or mixture does not ignite spontaneously on coming into contact with air at normal temperatures (i.e. the substance is known to be stable at room rtemperature for prolonged periods of time (days)).

2.2.1.1.9.3 Conclusion on classification and labelling for pyrophoric solids

Not classified. Cymoxanil is not a pyrophoric solid.

2.2.1.1.10 Self-heating substances *[equivalent to section 8.10 of the CLH report template]*

Table 12: Summary table of studies on self-heating substances

Method	Results	Remarks	Reference
EEC A.16	Not auto-flammable up to 140ºC		Gravell, 1996
EEC A.16	Not auto-flammable up to 450ºC		Betteley, 1995
OECD 113	Not auto-flammable up to 250ºC		Schmukler & LeSieur, 1993

2.2.1.1.10.1 Short summary and overall relevance of the provided information on self-heating substances

Under the condition of the tests, cymoxanil is not auto-flammbe up to 450ºC.

Information on tests performed and their results provided in Cymoxanil RAR Vol.3 B.2 (AS) Physical and chemical properties.

2.2.1.1.10.2 Comparison with the CLP criteria

Based on provisions of the Reg. (EU) 1272/2008 on the classification criteria for the self-heating substances Annex I part 2, 2.11.2.1, self-heating properties are tested using methods given in UN Test N4 Part III, sub-section 33.3.1.6 of the UN RTGD. Therefore the results from test method EC A.16 (Studies 2 and 3) would not be sufficient to conclude on this hazard class. They would be regarded as important but additional information on the safety properties of cymoxanil.

The 1st study summarised above and its results (*Gravel,1996*) based on the modified Bowes Cameron Cage test, as per United Nations Transport of Dangerous Goods Recommendations that was used to determine this safety property of cymoxanil would sufficiently meet the criteria of the CLP to demonstrate the substance is not self-heating. A negative result was obtained in a test using 100 mm cube sample at 140ºC.

2.2.1.1.10.3 Conclusion on classification and labelling for self-heating substances

Cymoxanil is not classified as a self-heating substance.

2.2.1.1.11 Substances which in contact with water emit flammable gases *[equivalent to section 8.11 of the CLH report template]*

Table 13: Summary table of studies on substances which in contact with water emit flammable gases

Method	Results	Remarks	Reference
Not relevant			

2.2.1.1.11.1 Short summary and overall relevance of the provided information on substances which in contact with water emit flammable gases

No tests have been conducted but safe long-term use of cymoxanil demonstarates that it does not a emit a gas (flammable or otherwise) when in contact with water.

2.2.1.1.11.2 Comparison with the CLP criteria

Based on provisions of the Reg. (EU) 1272/2008 on the classification criteria for the substances which in contact with water emit flammable gases (Annex I part 2, 2.12.4.1) the classification procedure to cymoxanil for this class does not need to be applied based on the following:

- the chemical structure of cymoxanil does not contain metals of metalloids;
- experience in cymoxanil production and handling demonstrates cymoxanil does not react with water;
- cymoxanil is soluble in water to form a stable mixture.

2.2.1.1.11.3 Conclusion on classification and labelling for substances which in contact with water emit flammable gases

Not classified. Cymoxanil does not emit flammable gases in contact with water.

2.2.1.1.12 Oxidising liquids *[equivalent to section 8.12 of the CLH report template]*

Not relevant, cymoxanil is a solid.

Table 14: Summary table of studies on oxidising liquids

Method	Results	Remarks	Reference
Not relevant			

2.2.1.1.12.1 Short summary and overall relevance of the provided information on oxidising liquids

Not relevant, cymoxanil is a solid.

2.2.1.1.12.2 Comparison with the CLP criteria

Not relevant, cymoxanil is a solid.

2.2.1.1.12.3 Conclusion on classification and labelling for oxidising liquids

Not relevant.

2.2.1.1.13 Oxidising solids *[equivalent to section 8.13 of the CLH report template]*

Table 15: Summary table of studies on oxidising solids

Method	Results	Remarks	Reference
EEC A.17	Oxidising	See below	Gravell, 1996
EEC A.17	Not oxidising		Betteley, 1995
EEC A.17	Not oxidising		Smeykal, 2008
EEC A.17	Not oxidising		Garofani, 2009
EEC A.17	Not oxidising		Habeck, 2009c
EEC A.17	Not oxidising		Jackson, 2007

2.2.1.1.13.1 Short summary and overall relevance of the provided information on oxidising solids

The 1st study by Gravell (1996) is considered unreliable as the composition of the mixtures used were not reported and no test for a false-positive, using kieselguhr, was conducted. The other 5 studies are considered more reliable and since all 5 report that under the conditions of the test, cymoxanil is not oxidising, the results of the Gravell study are disregarded.

Information on tests performed and their results provided in Cymoxanil RAR Vol.3 B.2 (AS) Physical and chemical properties.

2.2.1.1.13.2 Comparison with the CLP criteria

According to CLP Regulation, oxidising properties are tested using UN test O.1 or O.3 see Annex I, 2.14.2.1. Results from test method EC A.17 are not sufficient to conclude on this hazard class. The test UN O.1 is not equivalent to test EC A17.
Basen on results of studies by EC A17 performed cymoxanil was not regarded to be an oxidizing substance. As long as test UN O.1 has not been performed non-oxidizing properties of cymoxanil in terms of CPL could not be confirmed.

Based on provisions of the Reg. (EU) 1272/2008 Annex I, 2.14.2.1, an oxidising solid shall be classified in one of the three categories for this class using test O.1 in part III sub-section 34.4.1 of the UN Recommendations on the Transport of Dangerous Goods, Manual of Tests and Criteria in accordance with the Table 2.14.1 of the CLP Reg.(EU) 1272/2008 incorporated below:

Category	Criteria
1	Any substance or mixture which, in the 4:1 or 1:1 sample-to-cellulose ratio (by mass) tested, exhibits a mean burning time less than the mean burning time of a 3:2 mixture, by mass, of potassium bromate and cellulose.
2	Any substance or mixture which, in the 4:1 or 1:1 sample-to-cellulose ratio (by mass) tested, exhibits a mean burning time equal to or less than the mean burning time of a 2:3 mixture (by mass) of potassium bromate and cellulose and the criteria for Category 1 are not met.
3	Any substance or mixture which, in the 4:1 or 1:1 sample-to-cellulose ratio (by mass) tested, exhibits a mean burning time equal to or less than the mean burning time of a 3:7 mixture (by mass) of potassium bromate and cellulose and the criteria for Categories 1 and 2 are not met.

According to Additional Classification Considerations (2.14.4.1), for organic substances the classification procedure for this class shall not apply if:

(a) the substance or mixture does not contain oxygen, fluorine or chlorine ; or
(b) the substance or mixture contains oxygen, fluorine or chlorine and these elements are chemicaly bounded only to carbon or hydrogen.

Based on the chemical structure of cymoxanil, it contains one oxygen atom in methoxyimino acetyl group which is chemically bonded to nitrogen. Therefore, Additional Classification Considerations (2.14.4.1 (a) and (b)) would not seem to be applicable.

2.2.1.1.13.3 Conclusion on classification and labelling for oxidising solids

Not classified based on results of tests EC A17, cymoxanil is not an oxidising substance.
Based on criteria of the CLP when the Additional Classification Considerations, procedure 2.14.4.1 would not apply, it could be further disscussed whether the oxygen-nitogen bond would have any influence on possibility of oxidative properties of cymoxanil.

2.2.1.1.14 Organic peroxides *[equivalent to section 8.14 of the CLH report template]*

Table 16: Summary table of studies on organic peroxides

Method	Results	Remarks	Reference
Not relevant, cymoxanil does not contain the peroxide moiety			

2.2.1.1.14.1 Short summary and overall relevance of the provided information on organic peroxides

Not relevant.

2.2.1.1.14.2 Comparison with the CLP criteria

Based on provisions of the Reg. 1272/2008 and definition of organic peroxides (Annex I, 2.15.1.1), cymoxanil is not the s substance that contains the bivalent –O-O- structure and therefore cannot be considered a derivative of hydrogen peroxide.

2.2.1.1.14.3 Conclusion on classification and labelling for organic peroxides

Not relevant.

2.2.1.1.15 Corrosive to metals *[equivalent to section 8.15 of the CLH report template]*

2.2.1.1.15.1 Short summary and overall relevance of the provided information on the hazard class corrosive to metals

Cymoxanil is a solid with a melting point of 162ºC. It has no strongly acidic moieties, a 1% solution of cymoxanil has a pH ~6. It does not contain a halogen nor form complexes with metals.

Therefore cymaoxanil is unlikely to be corrosive to metals and a classification is unwarranted.

2.2.1.1.15.2 Comparison with the CLP criteria

Based on criteria of the Reg. (EU) 1272/2008 and the CLP guidance 2.16.4.1, cymoxanil is not the substance to be consided for classification of this class, i.e. solids corrosive to metals.
Cymoxanil is a solid with a high melting point, therefore is not a solid that may become a liquid (during transport); the substance is not acidic and its molecule does not contain halogen nor form complexes with metals.

2.2.1.1.15.3 Conclusion on classification and labelling for corrosive to metals

Cymoxanil is not a solid corrosive to metals.

2.2.2 Summary of physical and chemical properties of the plant protection product

Information on the physical and chemical properties of the plant protection product is not considered to be of relevance for the CLP process conducted by ECHA.

Considering the overall assessment and the results of tests performed as well as information on cymoxanil practical handling and storage cymoxanil is not classified based on its physical and chemical properties. However its properties as of non oxidising solid based on criteria of the CLP Regulation (EU) 1272/2008, Additional considerations 2.14.4.1 (b) could be further disscussed. None of cymoxanil formulations - Cymbal 45, Dauphin 45 and Rival Duo are classified based on their physical and chemical properties. For the detailed information on the properties of these formulations please refer to their parts of the draft assessment reports (Volumes 3 B2 (CP)).

RAC evaluation of physical hazards

Summary of the Dossier Submitter's proposal

The DS proposed no classification for all physical hazards, based on test results and the results of the screening procedure relevant for each hazard class.

Comments received during consultation

One comment was received from a Member State Competent Authority (MSCA) about typos, an obsolete CAS number and a request to state the purity of the test substance.

Assessment and comparison with the classification criteria

As cymoxanil is a solid, hazard classes for gases and liquids do not apply.

Three tests according to EEC method A.14 showed cymoxanil not to be explosive. However, these are not sufficient for classification according to the CLP Regulation, which requires the use of the relevant UN RTDG test methods. Cymoxanil does not contain structural features related to explosive properties as laid out in table A6.1 of Annex 6 of UN RTDG.

Four tests according to EEC method A.10 were negative for flammability. When negative, these tests are equivalent to UN RTDG N.1 tests.

Cymoxanil does not contain any molecular structures associated with self-reactive properties and no peroxide or acidic moieties. Thus, it does not fulfil criteria for self-reactive substances, organic peroxides, and corrosive to metals.

According to two EEC A.16 and one OECD TG 113 tests, cymoxanil is not a self-heating

substance.

Based on long-term handling experience, cymoxanil does not emit flammable gases upon contact and does not react with water.

Five EEC method A.17 tests concluded that cymoxanil was not an oxidising solid. One test according to this method showing oxidising properties was deemed unreliable, since no information were provided regarding composition of the mixtures used. The results from these tests are not sufficient for classification. Cymoxanil contains one methoxyimino group, thus an oxygen atom that is bound to nitrogen (and not exclusively to carbon or hydrogen as is required by the CLP Regulation for substances that are considered not oxidising). However, there are no indications that cymoxanil has oxidising properties.

Thus, RAC agrees in line with its previous opinion (RAC, 2012) and with the assessment of the DS and therefore proposes **no classification** for the physical hazards.

2.3 DATA ON APPLICATION AND EFFICACY

There were 3 applicants (AGRIA S. A., Société Financière de Pontarlier, Belchim Crop Protection NV/SA). However, the information on evaluation of efficacy was submitted by only one (AGRIA S.A.) of three applicants. Therefore, this section presents only a summary of information regarding the efficacy of the plant protection product "Rival Duo".

2.3.1 Summary of effectiveness

A total of 13 efficacy trials (2NL, 2 UK, 2 DE, 2 CZ, 5 HU) were carried out to evaluate the efficacy of Propamocarb hydrochloride 400 g/l + Cymoxanil 50 g/l SC for the control of *Phytophthora infestans* in potatoes. All trials were conducted according to GEP and followed the appropriate EPPO standards by officially recognized testing organizations. Due to the absence of infection (*P. infestans*) on potatoes, 1 trial conducted in Hungary was excluded from summary.

The level of efficacy of Propamocarb hydrochloride 400 g/l + Cymoxanil 50 g/l SC applied at the minimum effective dose previously determined for Austria, Czech Republic, Germany, United Kindom, Hungary (2.5 l/ha) was investigated on the basis of 7 valid trials (1 DE, 2CZ, 4 HU). The data package clearly demonstrates the ability of Propamocarb hydrochloride 400g/l + Cymoxanil 50g/l SC applied at 2.5 l/ha (1000 g a. i./ha of Propamocarb + 125 g a. i./ha of Cymoxanil) to control *Phytophthora infestans* over the entire potato crop cycle. Considering that the test product and standard reference provided consistently a fully equivalent control of the disease under each step of the infection development, it is considered that the use of Propamocarb hydrochloride 400 g/l + Cymoxanil 50 g/l SC applied at 2.5 l/ha (1000 g a. i./ha of Propamocarb + 125 g a. i./ha of Cymoxanil) to control potato late blight in Austria, Czech Republic, Germany, United Kingdom and Hungary is justified.

The level of efficacy of Propamocarb hydrochloride 400 g/l + Cymoxanil 50 g/l SC applied at the minimum effective dose previously determined for the Netherlands (2 l/ha) was investigated on the basis of 8 valid trials (2 NL, 2 UK, 2 DE, 2CZ). The data package clearly demonstrates the ability of Propamocarb hydrochloride 400 g/l + Cymoxanil 50 g/l SC applied at 2 l/ha (800 g a. i./ha of Propamocarb + 100 g a. i./ha of Cymoxanil) to control *Phytophthora infestans* over the entire potato crop cycle. Considering that the test product and standard reference provided an equivalent or better control of the disease under each step of the infection development, it is considered that the use of Propamocarb hydrochloride 400 g/l + Cymoxanil 50 g/l SC applied at 2 l/ha (800 g a. i./ha of Propamocarb + 100g a. i./ha of Cymoxanil) to control potato late blight in the Netherlands is justified.

The level of efficacy of Propamocarb hydrochloride 400 g/l + Cymoxanil 50 g/l SC applied at the minimum effective dose previously determined for the Hungary (2,5 l/ha) was investigated on the basis of 4 valid trials. The data package demonstrates the ability of Propamocarb hydrochloride 400 g/l + Cymoxanil 50 g/l SC applied at 2.5 l/ha (1000 g a. i./ha of Propamocarb + 125 g a. i./ha of Cymoxanil) to control *Phytophthora infestans* over the entire potato crop cycle. Considering that the test product and standard references provided consistently an equivalent control of the disease for several steps of the infection development, it is considered that the use of Propamocarb hydrochloride 400g/l + Cymoxanil 50g/l SC applied at 2.5 l/ha (1000 g a. i./ha of Propamocarb + 125 g a. i./ha of Cymoxanil) to control potato late blight in Hungary is justified.

Refer to Section B.3.9 in Volume 3CP Rival Duo.

2.3.2 Summary of information on the development of resistance

Propamocarb hydrochloride 400 g/l + Cymoxanil 50 g/l SC plant protection product is formulated as a suspension concentrate (SC) containing 2 different active substances: propamocarb hydrochloride (400 g/l) and cymoxanil (50 g/l).

The active substance propamocarb belongs to the family of carbamates (FRAC code 28). This active substance acts on lipid synthesis pathway and alternate membrane integrity of the pathogen cells. Due to its mode of action, propamocarb presents a low to medium resistance risk and resistance management strategies are required.

The active substance cymoxanil belongs to the family of cyanoacetamide-oxime (FRAC code 27). Cymoxanil acts like both contact and systemic fungicides. The preventive activity of cymoxanil is short-live (2-4 days) and it's quickly metabolized in plant. Greater protection may be achieved by curative application (247-48 hours after infection by the fungus). It inhibits sporulation, but the specific mode of action on fungi is still unknown. The risk of resistance inherent to this active substance is low to medium.

According to the FRAC (Pathogen risk list – December 2013), *Phytophthora infestans* is classified as a medium risk pathogen for the development of resistence.

The FRAC established a combined risk diagram based on inherent fungicide risk and inherent pathogen risk. In conclusion, the combined resistance risk factor based on inherent fungicide risk and inherent pathogen risk is classified as medium (4).

General rules to delay the risk of resistance development has to be followed:
- Strictly follow the label recommendations (dose rates, timing of applications, number of recommended applications);
- Apply in accordance of the regional disease forecasts: Apply as soon as there is a risk of infection or an official overwintering warning has been issued;
- Crop rotations: they can reduce the resistant populations over time and also reduce the selection pressures by not having to apply pesticides onto consecutive crops which are common hosts;
- Resistant crop varieties: they can be used to reduce infestations and therefore, to reduce the quantity of pesticide amount applied in the field;
- Cultural measures: adjusting planting dates, providing an environment that favors beneficial insects;
- Cultivation (Ploughing...);
- Good crop hygiene: destroying the crop residues that can contain overwintering fungus;
- Make sequential applications with other active substance with different mode of action.

Refer to Section B.3.10 in Volume 3CP Rival Duo

2.3.3 Summary of adverse effects on treated crops

Phytotoxicity to host crop
The phytotoxicity was evaluated in a total of 13 efficacy trials. No phytotoxicity symptoms caused by Propamocarb hydrochloride 400g/l + Cymoxanil 50g/l SC at all dose rates from 1.33 to 4l/ha were recorded in any of the 13 trials conducted.

Effect on the yield of treated plants and plant products
Effects of Propamocarb hydrochloride 400g/l + Cymoxanil 50g/l SC on the yield of the treated crop was investigated in presence of *Phytophthora infestans* in a total of 3 different efficacy trials. The results confirm that Propamocarb hydrochloride 400g/l + Cymoxanil 50g/l SC does not have adverse effects on yield of treated crop.

Impact on the quality of plants and plant products
No specific study investigated the effect of Propamocarb hydrochloride 400g/l + Cymoxanil 50g/l SC applications on the quality of the harvested tubers.

Effect on the processing procedure
No specific study investigated the effect of Propamocarb hydrochloride 400g/l + Cymoxanil 50g/l SC applications on transformation processes.

Impact on treated plants or plant products to be used for propagation

No specific study investigated the effect of Propamocarb hydrochloride 400g/l + Cymoxanil 50g/l SC applications on impact on treated plants or plant products to be used for propagation.

Refer to Section B.3.11 in Volume 3CP Rival Duo.

2.3.4 Summary of observations on other undesirable or unintended side-effects

Impact on succeeding crops
No specific trials were carried out on potato to assess the possible impact of Propamocarb hydrochloride 400g/l + Cymoxanil 50g/l SC applications on succeeding crops.

Impact on other plants including adjacent crops
No study was led about the impact of Propamocarb hydrochloride (400 g/l) + Cymoxanil (50 g/l) on other plants. Nevertheless, the phytotoxicity to non-target terrestrial plants was determined separately for those two active ingredients. The data justifies the recommendation of no restrictions on adjacent crops after the application of propamocarb HCl (400 g/l) and cymoxanil (50 g/l) separately and thus supposedly on Propamocarb hydrochloride 400g/l + Cymoxanil 50g/l SC.

Refer to Section B.3.12 in Volume 3CP Rival Duo.

2.4 FURTHER INFORMATION

2.4.1 Summary of methods and precautions concerning handling, storage, transport or fire

Active substance – cymoxanil

Handling
Technical and organizational measures recommendations: Use process enclosures, local exhaust ventilation and other suitable engineering controls to keep airborne levels below recommended exposure limits. If user operations generate dust, fume or mist, use ventilation to keep exposure to airborne contaminants below the exposure limit.
Advice on general occupational hygiene: Do not eat, drink or smoke when handling the product. In case of contamination change the work clothing. Do not breathe dust and avoid contact with eyes and skin. Do not handle this product without wearing the recommended personal protective clothing and equipment.

Storage
Keep in unopened original packing. Keep in cool, dry, well-ventilated place far from sources of ignition. Keep container tightly closed. Prevent static electricity generation. Do not allow accumulation of dust in significant concentrations. Keep out of reach of children. Keep away from: medicinal products, food, forage, fertilizers and seed, hazardous infectious substances, radioactive substances, explosive substances, highly reactive oxidizing substances.

Fire
Extinguishing media
Suitable extinguishing media: dry powder, carbon dioxide, water spray, foam.
Special hazards arising from the substance or mixture
In case of fire, along with other products of combustion, the toxic fumes may be released.
Advice for firefighters
Full impervious coverall clothing. Self-containing breathing apparatus.

Transport
Danger code (Kemler): 90
UN number :3077
UN proper shipping name: Environmentally hazardous substance, solid, n.o.s (cymoxanil)
Transport hazard class(es): 9
Packing group: III
Environmental hazards:
Environmentally hazardous substance indication (ADR/RID/ IMDG-Code/ ICAO-TI/ IATA-DGR): yes
Marine pollutant (IMDG): yes

The product "Cymoxanil 45 WG"

Handling

Additional hazards when proceeded: avoid all eye and skin contact and do not breathe vapour and mist
Precautions for safe handling: Ensure good ventilation of the work station. Obtain special instructions before use. Do not handle until all safety precautions have been read and understood. Wear personal protective equipment. Do not breathe dust/fume/gas/mist/vapours/spray. Avoid contact with skin and eyes.
Hygiene measures: Contaminated work clothing should not be allowed out of the workplace. Wash contaminated clothing before reuse. Do not eat, drink or smoke when using this product. Always wash hands after handling the product

Storage
Store locked up. Store in a well-ventilated place. Keep cool.

Fire
Extinguishing media
Suitable extinguishing media: water spray, dry powder, foam.
Unsuitable extinguishing media: do not use extinguishing media containing water.
Special hazards arising from the substance or mixture
Hazardous decomposition products in case of fire- toxic fumes may be released.
Advice for firefighters
Cool closed containers exposed to fire with water spray. Contain the extinguishing fluids by bunding (the product is hazardous for the environment)

Transport
Danger code (Kemler): 90
UN-Number: 3077
Packaging group: III
Transport hazard class (es): 9
Description of goods: 3077 Environmentally hazardous substances, solid, N.O.S. (2-cyano-N-[(ethylamino)carbonyl]-2-(methoxyimino) acetamide)
Environmental hazards: Dangerous for the environment: Yes (ADR, IMDG, IATA, AND, RID), Marine pollutant – IMDG
Transport by sea and air transport: no data available

The product "Rival Duo"

Handling
Precautionary measures: use process enclosures, local exhaust ventilation and other suitable engineering controls.
Measures to prevent fire: If user operations generate aerosol, fume or mist, use ventilation.
Measures to prevent aerosol and dust: Regularly clean the premises and facilities wearing personal protective equipment and using professional fire-safe cleaning tools.
Advice on general occupational hygiene: Do not eat, drink or smoke when handling the product. In case of contamination change the work clothing. Avoid inhalation, ingestion, contact with eyes and skin. Wear the recommended personal protective clothing and equipment.

Storage
Keep in dry, cool, well-ventilated place far from sources of ignition. Prevent static electricity generation. Keep out of reach of children. Keep in unopened original packing. Keep away from medical products, food, forage, fertilizers, seed, hazardous infectious substances highly reactive oxidizing substances.

Fire
Extinguishing media
Suitable extinguishing media: soft stream water fog, foam, carbon dioxide, dry chemical.
Unsuitable extinguishing media: water jet.
Special hazards arising from the substance or mixture
Hazardous combustion products: If involved in a fire, may evolve oxides of nitrogen, HCl, carbon dioxide, carbon monoxide. Do not breathe fumes.
Advice for firefighters
Full protective clothing and self-contained breathing apparatus.

Transport
UN-No. (ADR): not applicable
UN proper shipping name: not applicable
Transport hazard class (es): not applicable

Packing group: not applicable
Environmental hazards: not applicable
Marking: not applicable

The product "Dauphin 45"

Handling
Information for safe handling: Ensure good ventilation of the work station. Avoid contact with skin and eyes. Wear recommended personal protective equipment. Do not breathe dust. Limit quantities of product at the minimum necessary for handling and limit the number of exposed workers. If an exposure to the product is possible, delimit concerned areas and use suitable warning and safety signals, including "no smoking" signals. Do not expose pregnant or breastfeeding women.
Information about fire and explosion protection: Isolate fire area. Evacuate downwind. Do not breathe fumes. Contain the extinguishing fluids by bunding. Use water spray or fog for cooling exposed containers. Do not attempt to take action without suitable protective equipment. Self-contained breathing apparatus. Complete protective clothing.

Storage
Store in a cool, well-ventilated place. Keep container tightly closed. Keep in original packaging.

Fire
Extinguishing media
Suitable extinguishing media: powder, carbon dioxide (CO_2), water spray, foam.
Special hazards arising from the substance or mixture
Fire hazard: Presents no particular fire or explosion hazard.
Hazardous decomposition products in case of fire: On combustion or on thermal decomposition (pyrolysis) releases.
Advice for firefighters
Firefighting instructions: Isolate fire area. Evacuate downwind. Do not breathe fumes. Contain the extinguishing fluids by bunding. Use water spray or fog for cooling exposed containers.
Protection during firefighting: Do not attempt to take action without suitable protective equipment. Self-contained breathing apparatus. Complete protective clothing.

Transport
Land transport ADR/RID (cross-border):
ADR/RID Class: 9 (M7)
Danger code (Kemler): 90
UN-Number: 3077
Packaging group: III
Hazard label: 9
Description of goods: 3077 Environmentally hazardous substance, solid, N.O.S. (2-cyano-N-[(ethylamino)carbonyl]-2-(methoxyimino) acetamide)
Limited quantities (LQ): LQ27
Transport category: 3
Tunnel restriction code: E
Maritime transport IMDG:
IMDP Class: 9 packaging group: III. Dangerous for the environment: Yes. Marine pollutant: yes
Air transport ICAO-TI and IATA-DGR:
ICAO/IATA Class: 9 packaging group: III. Dangerous for the environment: Yes

2.4.2 Summary of procedures for destruction or decontamination

Active substance – cymoxanil

Neutralisation procedures
For containment: Collect spillage. Absorb with an inert material – sand, zeolite.
Methods for cleaning up: Use vacuum cleaning. Collect into an appropriate, labelled tightly sealed waste container. Store the container at an appropriate place for further treatment or disposal according to the national legislation.

Controlled incineration

A specific study on the thermal decomposition has not been carried out. Current practice is to incinerate at a temperature greater than 900°C with a residence time of 24 secs in the chamber. Oxygen supply should be adjusted to generate <100 ppm CO in the stack. Consideration of the content of halogens is not relevant.

Waste treatment method
Waste from residues /unused products: In accordance with local and national regulations. Must be incinerated in a suitable incineration plant holding a permit delivered by the competent authorities. Do not allow material to contaminate ground water systems.
Contaminated packaging: Do not re-use empty containers. Do not contaminate ponds, waterways or ditches with chemical or used container.

The product "Cymoxanil 45 WG"

Neutralisation procedures
A neutralisation procedure is not relevant as the product is not strongly acidic or alkaline.

Controlled incineration
Incineration must be done under controlled conditions according to the Directives 94/67/EC and 2000/76/EC:
- Residence time greater than 2 seconds;
- Presence of more than 6 % of oxygen.

Since the active substance and the formulants in Cymoxanil 45 WG contain no halogens, the following criteria are considered suitable:
- Temperature above 850 °C.

Waste treatment methods
Dispose of contents/container in accordance with licensed collector's sorting instructions.

The product "Rival Duo"

Neutralisation procedures
Collect spillage. Absorb with an inert material – sand, zeolite. Use vacuum cleaning.

Controlled incineration
Recommended treatment method: burning in appropriately licensed incinerations.

Waste treatment methods
Disposal must be carried out in accordance with the provisions of the national legislation. The small product quantities should be stored in solid waste containers. The container should be clearly labelled. Store in well-ventilated areas. The water used for contaminated surface washing should be collected for further treatment.

The product "Dauphin 45"

Neutralisation procedures
For containment: Sweep up or vacuum up the product.
Methods for cleaning up: Cautiously neutralize remainder. Wash contaminated area with large amounts of water.
Other information: Dispose of contaminated materials in accordance with current regulations.

Controlled incineration
Not applicable.

Waste treatment methods
After cleaning, recycle or dispose of at an authorised site. Do not allow into drains or water courses.
Product/Packaging disposal recommendations: Dispose of in accordance with relevant local regulations.

2.4.3 Summary of emergency measures in case of an accident

Active substance – cymoxanil

Description of first aid measures
General information: When symptoms persist, seek medical attention.
Inhalation: Remove from exposure area to fresh air. Provide artificial breathing if the breathing has stopped. Seek medical attention immediately.

Ingestion: Never give anything by mouth to an unconscious person. Seek medical attention immediately, Don't induce vomiting. If the person is conscious, rinse the mouth thoroughly and have the patient drink a glass of water
Contact with skin and clothing: Remove contaminated clothing and footwear. Was affected area with plenty of water. Seek medical attention if irritation persists.
Contact with eyes: Immediately rinse for at least 15 minutes with large quantity of drinking water while holding eyes open. Immediately seek qualified medical advice.
Most important symptoms and effects, both acute and delayed: Possible manifestation of allergic symptoms such as urticarial, allergic edema. Possible changes in catarrhal mucous membrane of eyes and upper respiratory tract.
Indication of any immediate medical attention and special treatment needed: Treat symptomatically.

Accidental release measures
Personal precautions, protective equipment and emergency procedures
Mark out the contaminated area with signs and prevent access to unauthorized personnel. Clear the danger area. Provide local and general exhaust ventilation. Do not breathe dust. Avoid contact with skin and eyes. Use protective clothing and gloves, respiratory mask with an effective particulate filter, chemical goggles for eye protection.

Environmental precautions
Avoid release into environment. In case of accidental release take precautions to protect the surface and underground water, soil and sewage from contamination. Remove the sources of heat and flames. In case of spill into the sewage, surface water, ground water or soil notify the competent authorities immediately.

Methods and material for containment and cleaning up
For containment: Collect spillage. Absorb with an inert material – sand, zeolite.
Methods for cleaning up: Sweep up and vacuum up the product. Collect into an appropriate, labelled tightly sealed waste container. Store the container at an appropriate place for further treatment or disposal according to the national legislation.

The product "Cymoxanil 45 WG"

Description of first aid measures
First-aid measures general: Remove contaminated clothes. If you feel unwell, seek medical advice (show the label where possible).
Iinhalation: Remove person to fresh air and keep comfortable for breathing.
Skin contact: Wash skin with plenty of water. Take off contaminated clothing. If skin irritation or rash occurs: Get medical advice/attention.
Eye contact: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. If eye irritation persists: Get medical advice/attention.
Ingestion: Call a poison center or a doctor if you feel unwell. Do not induce vomiting. Do not ingest.
Most important symptoms and effects, both acute and delayed:
Symptoms/effects after skin contact: May cause an allergic skin reaction.
Symptoms/effects after eye contact: Eye irritation.
Indication of any immediate medical attention and special treatment needed: Treat symptomatically.

Accidental release measures
Personal precautions, protective equipment and emergency procedures
For non-emergency personnel: Ventilate spillage area. Do not breathe dust/fume/gas/mist vapours/spray. Avoid contact with skin and eyes.
For emergency responders: Do not attempt to take action without suitable protective equipment.

Environmental precautions
Avoid release to the environment. Do not allow into drain or water courses.

Methods and material for containment and cleaning up
For containment: Collect spillage. Contain the spilled material by bunding.
Methods for cleaning up: Mechanically recover the product. Notify authorities if product enters sewers or public waters. Dispose of materials or solid residues at an authorized site.

The product "Rival Duo"

Description of first aid measures
Inhalation: Remove person to fresh air and rest. Seek medical advice if breathing is difficult.

Skin contact: Wash all contaminated clothing before the use. Wash immediately with plenty of soap and water. If irritation persists, consult a doctor.
Eye contact: Rinse immediately and thoroughly for at least 15 minutes. Consult an ophthalmologist if irritation persists.
Ingestion: Do not induce vomiting. Get medical advice.
Most important symptoms and effects, both acute and delayed: Not known
Indication of any immediate medical attention and special treatment needed: Treat symptomatically.

Accidental release measures
Personal precautions, protective equipment and emergency procedures
For those staff which does not meet for emergency: Keep unnecessary personnel away
For the persons responsible for emergency: Eliminate all ignition sources (flame and spark). Provide local and general exhaust ventilation. Use protective clothing and gloves, respiratory mask, chemical goggles for eye protection.

Environmental precautions
In case of accidental release take precautions to protect the surface and underground water, soil, sewage from contamination. Remove sources of heat and flames. In case of spill into the sewage, surface water, ground water or soil notify the competent authorities immediately.

Methods and materials for containment and cleaning
Absorb with an inert material – sand, zeolite. Use vacuum cleaning. Do not dispose the product and contaminated materials into the sewage systems, water sources or water bodies. Collect into an appropriate, labelled tightly sealed waste container. Store the container at an appropriate place for further treatment or disposal according to the national legislation.

The product "Dauphin 45"

Description of first aid measures
General information: In all cases of doubt, or when symptoms persist, seek medical attention.
Inhalation: Remove person to fresh air and keep comfortable for breathing. Get medical advice and attention if you feel unwell.
Skin contact: Remove all contaminated clothing and footwear. Wash immediately with plenty of soap and water. If irritation persists, consult a doctor.
Eye contact: Rinse immediately and thoroughly, pulling the eyelids well away from the eye (15 minutes minimum). Consult an ophthalmologist if irritation persists.
Ingestion: Rinse mouth out with water. Call a physician immediately.
Most important symptoms and effects, both acute and delayed: No additional information available
Indication of any immediate medical attention and special treatment needed: Treat symptomatically.

Accidental release measures
Personal precautions, protective equipment and emergency procedures
For non-emergency personnel: Mark out the contaminated area with signs and prevent access to unauthorized personnel. Clear the danger area. Avoid contact with skin and eyes. Do not attempt to take action without suitable protective equipment.
For emergency responders: Do not attempt to take action without suitable protective equipment.

Measures for environmental protection
Do not discharge into drains or rivers. Contain the spilled material by bunding.

Measures for cleaning/collecting
For containment: Sweep up or vacuum up the product.
Methods for cleaning up: Cautiously neutralize remainder. Wash contaminated area with large amounts of water.
Other information: Dispose of contaminated materials in accordance with current regulations.

2.5 METHODS OF ANALYSIS

2.5.1 Methods used for the generation of pre-authorisation data

Methods for the analysis of the active substance as manufactured

Information on methods of analysis to determine cymoxanil and its impurities in technical grade active substance and formulations have been assessed according to the criteria of the guidance document SANCO/3030/99 rev.4.

Validated HPLC-UV methods are available for the determination of cymoxanil and its both isomers E and Z in the technical active substance and its formulations. Most of the methods are based on the CIPAC method 419 /TC/M/3 or modified version of it.

Validated analytical methods are available to determine impurities in the technical grade cymoxanil (ref. dRAR Vol.4 Annexes C).

Methods for the determination of relevant impurities in the technical active substance and its formulations have not been required as long as the revised reference specification of the active has not been confirmed and the assessment of the impurities relevance or non-relevance accomplished.

The methods used for the risk assessment

Information on methods of analysis have been submitted to support studies carried out in different areas of the risk assessment and therefore the criteria of the guidance document SANCO/3029/99 rev.4 have been applied.

Numerous methods have been available and therefore submitted as methods for the generation of pre-approval data required for the risk assessment.

The information on validation of the methods that were regarded by the applicants as relevant for the assessment please refer to the Vol 3 B5.1.2. It should be noted that the outcome on the acceptability of the whole study reports would also rely on the assessment of the relevant area of expertise from which the method comes.

2.5.2 Methods for post control and monitoring purposes

The criteria of SANCO/825/00 rev.8.1 were used for the assessment of the analytical methods proposed for monitoring purposes. Therefore, the following analytical methods listed below have been considered as suitable for monitoring and the enforcement purposes.

2.5.2.1 Methods for residue determination in food/feed of plant origin

The validated LC-MS/MS multiresidue methods and their independent laboratory validation superseding the previous methods assessed in the DAR for cymoxanil Annex I inclusion have been available.

For plant commodities, validated LC-MS/MS methods enabling the determination of cymoxanil according to the residue definition for monitoring have been presented for all commodity groups (see the Table below). The HPLC–MS/MS methods monitoring two *m/z* transitions are highly specific and therefore confirmatory methods are not required. For methods for residue determination in food/feed of plant origin independent laboratory validation is required; this data requirement for some methods has not been fulfilled as marked in the table below.

Table 2.5.2-1: Methods for cymoxanil determination in food of plant origin (AIR 4)

Commodity *(matrix group*)*	Method	LOQ	Reference	EU review
wheat grain (1) tomato (2) oilseed rape (3) grapes (4)	HPLC-MS/MS (DFG S19) monitoring two mass transitions	0.01 mg/kg	*Lakaschus, Gizler, 2013;* *Cermak, 2013* *(ILV)*	New data Renewal

Commodity *(matrix group*)*	Method	LOQ	Reference	EU review
potato, lettuce (2) hazelnut (3) grapes (4)	LC-MS/MS (QuEChERS) monitoring two mass transitions	0.02 mg/kg	*Richter, 2009*	New data[1] Renewal
potato (2) grapes (4)		0.02 mg/kg	*Wolf, 2009* *(ILV)*	
potato, lettuce (2) hazelnut (3) grapes (4)		0.01 mg/kg	*Tussetschlager, Jooss, 2018,* *(supplemental validation, lower LOQ)*	
potato (2) grapes (4)		0.01 mg/kg	*Airs, 2018* *(ILV, lower LOQ)*	
tomatoes (2) grapes (4)	LC-MS/MS monitoring two mass transitions	0.01 mg/kg	*Howard, 2013*[2]	New data Renewal
tomatoes (2)	LC-MS/MS (QuEChERS) monitoring two mass transitions	0.01 mg/kg	*Perny, 2014*[2]	
potatoes (2)	LC-MS/MS (QuEChERS) monitoring two mass transitions	0.01 mg/kg	*Stouvent, 2017*[3]	

*according to SANCO 825/00 rev.8.1- Commodities and matrix groups:
1) dry commodities (high protein/high starch content)
2) commodities with high water content;
3) high oil content
4) high acid content commodities.

As seen from the summary table above, for the cymoxanil renewal, a new fully validated HPLC-MS/MS method based on the multiresidue method DFG S19 has been fully validated for all matrix groups according to the SANCO/825/00 rev. 8.1 and with the extraction efficiency of the method considered (*Position paper to cover the extraction efficiency of the Multi-Residue Method DFG S19*). The method was fully and independently validated and therefore can be recommended for the enforcement/monitoring purposes.

2.5.2.2 Methods for residue determination in food/feed of animal origin

The methods considered to be not required as long as the residue definition has not been established. For the assessment in regards to non-relevance of the residue definition in animal matrices for monitoring based on animal metabolism studies please refer to the residue part of assessment Vol.3 CA B7.

2.5.2.3 Methods for the determination of the active substance and/or metabolites in soil

For the proposed residue definition in soil for the enforcement, please refer to Vol.3_CA-B8.

The summary of the new methods superseding the previous methods for cymoxanil residues determination in soil is given in the table below.

[1] Data/information on extractionn efficiency not available and to be further considered.
[2,2,3] ILV not available

Table 2.5.2.3: Methods for cymoxanil residue determination in soil

Matrix	Analyte	Method principle ***LOQ***	Reference	EU review
soil	cymoxanil	HPLC-MS/MS monitoring two mass transitions *0.01 mg/kg*	*Garofani, 2009 (CH-285/2008, primary method)* *Garofani, 2009 (CH-377/2013, confirmatory method)* *Nichetti, 2017 (CH-199/2016, linearity range)*	New data Renewal
soil	cymoxanil	HPLC-MS/MS (QuEChERS) monitoring two mass transitions *0.05 mg/kg*	*Kreidler, 2016b*	New data Renewal

For the purpose of renewal, the new LC-MS/MS methods with two mass transitions validated per analyte have been available. The methods were fully validated and therefore can be recommended for the enforcement/monitoring purposes for the determination of cymoxanil in soil with the LOQ of 0.01 mg/kg and 0.05 mg/kg.

2.5.2.4 Methods for the determination of the active substance and/or metabolites in water

For the proposed residue definition in surface and ground water for the enforcement please refer to Vol. 3CA B-8.

The summary of the new methods that supersede the previous methods of residue analysis in water submitted for Annex I inclusion is given in the table below.

Table 2.5.2-4: Methods for cymoxanil residues determination in water

Matrix	Analyte	Method principle ***LOQ***	Reference	EU review
drinking and surface water	cymoxanil IN-KQ960	HPLC-MS/MS *0.1 µg/L* *0.1 µg/L* monitoring two mass transitions	*Leak, 2010* *(primary method)* *Cermak, 2013b* *(confirmatory method, ILV)*	New data Renewal
surface water drinking water	cymoxanil IN-KQ960	HPLC-MS/MS monitoring two mass transitions *0.1 µg/L* *0.1 µg/L*	*Mewis, 2017*	New data Renewal
drinking water	IN-KQ960	*0.1 µg/L*	*Lakaschus, Gizler, Fritz, 2017b* *(ILV)*	

For the purpose of renewal the new LC-MS/MS methods with two mass transitions validated per analyte have been available. The methods were fully validated and therefore can be recommended for the enforcement/monitoring

purposes for the analytes – cymoxanil and its metabolite IN-KQ960 determination in surface and drinking water with the LOQ of 0.1 µg/L per analyte. The second method (*Mewis, 2017*) has not been independently validated for cymoxanil determination in drinking water which is a data requirement according to the Reg. (EU) 283/2013.

5.2.5 Methods for the determination of the active substance and/or metabolites in air

The proposed residue definition in air for monitoring is cymoxanil (ref. DRAR Vol. 3 CA B-8).

The summary on the new methods that supersede the previous methods of residue analysis in air submitted for Annex I inclusion is given in the table below.

Table 2.5.2-5: Method for trinexapac ethyl residue determination in air

Matrix	Analyte	Method principle ***LOQ***	Reference	EU review
air	cymoxanil	HPLC-UV (DAD) *0.17 µg/m^3*	*Garofani, 2009* *Nicheti, 2017b (linearity range)*	New data Renewal
air	cymoxanil	HPLC-MS/MS monitoring two mass transitions *0.3 µg/m^3*	*Wolf, 2009*	New data Renewal

The methods are fully validated and therefore can be recommended for the enforcement/monitoring purposes for cymoxanil determination in air with the LOQ of 0.17 µg/m^3 and 0.3 µg/m^3.

2.5.2.6 Analytical methods (residue) for body fluids and tissues (Annex IIA 4.2.5; Annex IIIA 5.2)

Based on the data requirements enforced by the provisions of the Reg. (EU) 283/2013 Part A Section 4, *4.2 (d) Methods, with a full description , shall be submitted for:*

(d) the analysis in body fluids and tissues for active substances and relevant metabolites.

The methods for cymoxanil determination in body fluids and tissues have been available and summarised in the table below.
It could be noted that the residue definition for body fluids and tissues has not been available therefore the parent compound cymoxanil was aanalysed.

Table 2.5.2-6: Methods for cymoxanil residues determination in body fluids and tissues

Matrix	Analyte	Method principle ***LOQ***	Reference	EU review
animal muscle	cymoxanil	HPLC-MS/MS 0.01 mg/kg monitoring two mass transitions	*<confidential> 2016* *<confidential> 2016 (ILV)*	New data Renewal
body fluid (plasma)	cymoxanil	HPLC-MS/MS monitoring two mass transitions 0.1 mg/L	*<confidential> 2019*	New data Renewal

animal muscle	cymoxanil	HPLC-MS/MS 0.01 mg/kg monitoring two mass transitions	<*confidential*> *2015* <*confidential*> *2018* *(ILV)*	New data Renewal

2.6 EFFECTS ON HUMAN AND ANIMAL HEALTH

The data on active substance cymoxanil were evaluated during the first Annex I review of cymoxanil and were presented in the monograph (Vol.3, Annex B, Section B6, Point B.6.3, June 2007) and addendum to the monograph (April 2008). Proposal for Harmonised Classification and Labelling (CLH report, version number 2, 16.05.2011) submitted to ECHA was based mainly on the information presented in this assessment of cymoxanil.

A harmonised classification and labelling for cymoxanil has been adopted by the ECHA Committee for Risk Assessment (RAC) in 14 September 2012 (ECHA/RAC/CLH-O-0000002970-73-01/F). All the human health hazard classes (except respiratory sensitisation, aspiration hazard and adverse effects on or via lactation as well as endocrine disruption properties) were reviewed by the ECHA RAC. The resulting classification is available in Commission Regulation (EU) No 605/2014 (6th adaptation to technical and scientific progress of Regulation (EC) No 1272/2008) and the classification for human health is the following:

Acute Tox., 4 H302;

Skin Sens. 1, H317;

STOT RE 2, H373 (blood, thymus);

Repr. 2, H361fd

In addition to the above studies evaluated during the first Annex I review of cymoxanil, the various applicants involved in the process of renewal submitted a wide range of new studies on active substance cymoxanil: three *in vitro* comparative metabolism studies, three acute (oral, dermal and eye irritation) toxicity studies, two phototoxicity assays, eight *in vitro* and *in vivo* genotoxicity studies, one generation reproduction toxicity study, and two QSAR studies. Additionally twenty studies (acute toxicity, genotoxicity and QSAR) on different metabolites of cymoxanil have been submitted for the purpose of renewal to meet the data requirement for such investigations in Regulation 283/2013. Re-evaluation of all old and new studies has been performed by the RMS. Based on re-evaluation of all old and new studies, some change of the harmonised classification of cymoxanil is proposed by the RMS:

Acute Tox. 4, H302;

Skin Sens. 1A, H317;

STOT RE 2, H373 (blood, thymus, eye);

Repr. 2, H361fd

More detailed results of all studies are presented in Volume 3, section B.6.

2.6.1 Summary of absorption, distribution, metabolism and excretion in mammals

Table 17: Summary table of toxicokinetic studies

Method	Results	Remarks	Reference
	***In vivo* rat studies**		
The absorption, distribution, metabolism and excretion of [2-^{14}C]-DPX-T3217 in the rat US EPA Pesticide Assessment Guidelines, Subdivision F, 85-1 (1982) GLP Acceptable	**Plasma kinetics**, low dose: C_{max} 4.3 - 5.5 µg/ml T_{max} 2.7 – 3.3 h $T_{½}$ 23.1 – 24.1 h AUC 79.6-81.6 µg equiv/mL·h Plasma kinetics, high dose: C_{max} 135 -143 µg/ml T_{max} 3.2 – 5.2 h $T_{1/2}$ 18.7 – 19.5 h AUC 3224 - 3252 µg equiv/mL·h The plasma / whole blood ratios exceeded 1 up to 24 h **Distribution**: Rapid widely distributed in tissues, the highest residues in kidneys and liver. **Metabolism**: Metabolised extensively.	**Test substance**: DPX-T3217 (cymoxanil) Non-labelled: purity 99% Radiochemical: purity 98% Oral route/Dose (average mg/kg bw): Single low dose 2.5 Single high dose 120 Repeated dose 2.5 (14 daily doses of non-radiolabelled cymoxanil prior to the radiolabelled dose) Rat: Crl:CD / BR (Sprague-Dawley) Group size: 3-8/sex/dose No bile excretion measured. Carcass and expired air not investigated. For plasma/blood kinetics studies only 3 animals per sex were used. The total mean recovery was low	RAR B.6.1.1.1., 1995

Method	Results	Remarks	Reference
	Primary metabolites in the excreta: polar material consisting mainly of glycine and IN-W3595. Trace amounts of IN-U3204 in urine (high dose). **Excretion** after 96 hours: *Urinary*: 63.8 – 68.3% (single low dose), 72.8 – 74.4% (single high dose) and 67.4 – 74.8% (multiple low doses) *Faecal:* 17.3 – 23.6% (single low dose), 16.7 – 17.4% (single high dose) and 15.7 – 20.3% (multiple low doses) >85% was eliminated in the excreta within the 48 hours	(< 90%) in excretion study (single low dose). Compounds in excreta (urine) which comprised greater than 5 % of the administered dose were not identified (Repeated dose, ♀).	
Biliary excretion of [^{14}C]-cymoxanil in the rat US EPA Pesticide Assessment Guidelines, Subdivision F, 85-1 (1984) GLP Acceptable	**Excretion** after 48 hours: *Urinary*: 62.6 – 65.0% (low dose), 70.2% (♂, high dose) *Bile*: 6.2 – 8.3% (low dose), 9.6% (♂, high dose) *Faecal:* 14.3 – 14.5% (low dose), 9.48% (♂, high dose) >78% was eliminated in the excreta within the 48 hours **Metabolism**: *Low dose:* Metabolised extensively to polar material consisting mainly of glycine (49 - 54% of the administrated radioactivity) and IN-W3595 (6 - 10%) in bile and urine (combined). Metabolite A (8 - 10%) could be detected in urine only. *High dose* (Supplement No.1): Metabolised extensively to IN-W3595 and polar material. Metabolite A was identified and accounted for 0.3 – 3.0% of the administrated radioactivity in urine. Trace amounts of IN-U3204 in urine.	**Test substance**: DPX-T3217 (cymoxanil) Non-labelled: purity 99% Radiochemical: purity 98% Oral route/Dose (average mg/kg bw): Single low dose 2.5 Single high dose 120 (Supplement No1) Rat: Crl:CD / BR (Sprague-Dawley) Group size: 5/sex/low dose; 3 males/high dose Main study: identification of metabolite profile by co-chromatography was obtained using only one HPLC. Supplement No1: only three bile-duct cannulated male rats were used; carcass not investigated.	RAR B.6.1.1.2., 1995 and 1997 (supplement)
^{14}C-cymoxanil: Pharmacokinetics in the rat after single oral administrations at the doses of 10 and 100 mg/kg Not stated. Equivalent to OECD 417 (1984) GLP Acceptable	**Plasma kinetics**, low dose: C_{max} 23.6 µg/ml T_{max} 0.5 h $T_{½}$ 12.2 h $AUC_{0\text{-}inf}$ 353.141 µg equiv/mL·h Plasma kinetics, high dose: C_{max} 189.4 µg/ml T_{max} 0.5 h $T_{½}$ 11.7 h $AUC_{0\text{-}inf}$ 2265.851 µg equiv/mL·h The whole blood / plasma ratios were about 0.7 up to 24 hours **Distribution**: Rapid widely distributed in tissues, the highest residues in kidneys (♀), stomach wall (♂) and liver (♂)	**Test substance**: Cymoxanil Non-labelled (002/98): purity not reported Radiochemical (batch CFQ 9873): purity >98% Oral route/Dose (average mg/kg bw): Single low dose 10 Single high dose 100 Rat: Crl:CD / BR (Sprague-Dawley) Group size: 4/sex/dose; 6 males/bile sampling dose group Bile excretion measured only in	RAR B.6.1.1.3., 1999

Method	Results	Remarks	Reference
	Excretion after 48 hours: *Urinary*: 59.0 – 72.5% (low and high dose) *Faecal:* 14.6 – 29.3% (low dose and high dose) ≥ 90% was eliminated in the excreta within the 48 hours *Bile*: 6.2 – 8.3 % (low and high dose, 8 hours after administration)	males in separate bile-cannulated groups. Urine, faeces, cage wash, expired air and carcass were measured in non-bile-cannulated animal groups.	
^{14}C-cymoxanil: Pharmacokinetics in the rat after repeated oral administrations GLP Acceptable	**Plasma kinetics**, male: C_{max} 84.8 µg/ml T_{max} 2.0 h $T_{½}$ 31.7 h AUC 1461.4 µg/mL·h AUC_{24} 930.2 µg eq/mL·h Plasma kinetics, female: C_{max} 87.7 µg/ml T_{max} 3.0 h $T_{½}$ 30.1 h AUC 1635.2 µg/mL·h AUC_{24} 1036.7 µg eq/mL·h Plasma kinetics, mean: C_{max} 84.6 µg/ml T_{max} 2.0 h $T_{½}$ 30.8 h AUC 1548.2 µg/mL·h AUC_{24} 983.5 µg eq/mL·h The whole blood/plasma ratios were about 0.9 up to 8 hours **Distribution**: Rapid widely distributed in tissues, the highest residues in stomach wall and kidneys up to 4 hour after dosing. The radioactivity increased in fat 168 hours after the last administration. **Excretion** 168 hours after the last administration: *Urinary*: 61.6 % (mean), *Faecal:* 15.1% (mean) > 80% was eliminated in the excreta (168 hours after the last administration)	**Test substance**: Cymoxanil Non-labelled (002/98): purity >98% Radiochemical (batch CFQ 9873): purity >98% Oral route/Dose (average mg/kg bw): repeated oral dose 50 for 7 days Rat: Crl:CD / BR (Sprague-Dawley) Group size: 4/sex/dose; Measurement of test substance in bile and expired air was not performed. Low total mean recovery (< 85%) in excretion study.	RAR B.6.1.1.4., 2000
Chromatographic investigation of samples obtained from a metabolism study in rat with cymoxanil GLP Acceptable in assessing metabolism	**Metabolism**: Metabolised extensively to polar material consisting mainly of glycine, IN-W3595, IN-R3274 and hippuric acid in urine and faeces. Collection intervals after the last administration: urine samples 0 – 8/8 – 24 hours, faeces samples 0 – 24 hours	**Test substance**: Cymoxanil Non-labelled (002/98): purity >99% Radiochemical (batch 3304.265): purity >99% Oral route/Dose (average mg/kg bw): repeated oral dose 50 for 7 days Rat: Crl:CD / BR (Sprague-Dawley) Group size: 4/sex/dose; Only tentative assessment of	RAR B.6.1.1.5., 2001 *(is considered as part of RAR B.6.1.1.4., 2000)*

Method	Results	Remarks	Reference
		cymoxanil metabolism: Compounds in excreta (urine) which comprised greater than 5 % (up to 19.1%) of the administered dose were not identified. Identification by co-chromatography was obtained using only one analytically independent system (TLC).	
Metabolism of ^{14}C-cymoxanil in the Sprague-Dawley rat after a single oral dose GLP Acceptable in assessing metabolism	**Metabolism**: Metabolised extensively to polar material consisting mainly of glycine, IN-W3595, Metabolite 4 and hippuric acid in urine and faeces. **Excretion** after 24 hours: *Urinary*: 38.3 – 65.2% *Faecal:* 1.8 – 7.7% 40.1 – 72.9% was eliminated in the excreta within the 24 hours.	**Test substance**: Cymoxanil Unlabelled (19800042): purity 99.2% Radiochemical (batch 3362-236) : purity >97.4% Oral route/Dose (average mg/kg bw): single oral dose 100 Rat: Crl:CD / BR (Sprague-Dawley) Group size: 2/sex; Only 2 animals per sex were used. Study is only as supplementary in assessing excretion.	RAR B.6.1.1.6., 2003
***In vitro* Comparative Metabolism**			
^{14}C-cymoxanil: Comparative metabolism using mouse, rat, rabbit, dog and human liver microsomes GLP Acceptable	Quite consistent pattern of cymoxanil breakdown products and metabolism among all species. The major metabolite is IN-W3595 (plus the metabolite IN M2417-016) making up app 30% of total radioactivity in humans, app 50 - 60% in the rat and dog, and app 70 - 80% in mice and rabbits following 120 min incubation in liver microsomes in the presence of NADPH. Twelve components were detected in human microsomes incubation samples by HPLC. In species investigated metabolite IN-JX915-003 and component M4 accounted for up to 8% of the sample radioactivity. There are some qualitative metabolites differences (>3 fold) in comparison human with other species. There is no unique human metabolite.	**Test substance**: Radiolabelled [^{14}C]-Cymoxanil purity 100% **Final concentrations:** 20 µM **Positive control:** [^{3}H]-testosterone **With/without enzyme cofactor:** β-NADPH Final concentration 1 mM Samples **analysed** by HPLC Metabolites **investigated by LC-MS** **Recovery of AR:** 84-98% **Liver microsomes:** **Human**: mixed gender, pooled from 150 (75♂ and 75♀) donors, **Mouse**: CD-1, mixed gender, pooled of 1220♂ and 25♀ **Rat**: Sprague-Dawley, mixed gender, pooled of 454♂ and 200♀ **Dog**: Beagle, mixed gender, pooled of 8♂ and 12♀ **Rabbit**: New Zealand White, female, pooled of 4♀	RAR B.6.1.3.1., 2018
Comparative *in vitro* metabolism study of [^{14}C[cymoxanil in primary rat hepatocytes and human, rat, mouse, dog and rabbit microsomes	*Rat hepatocytes:* Transformation to one major metabolite IN-W3595 (>60%) and others IN-KQ960 (<5%), IN-U3204 (<10%) *Microsomes* Quite similar pattern of cymoxanil breakdown products and metabolism among all species.	**Test substance**: Radiolabelled [acetyl 2-^{14}C]cymoxanil, purity >98% **Final concentration:** Hepatocytes: 8.38 µM Microsomes: 9.2-9.8 µM **Positive control:** Testosterone, purity ≥99.0%	RAR B.6.1.3.2., 2018

Method	Results	Remarks	Reference
GLP Acceptable	The major metabolite is IN-W3595 making up app 30% of total radioactivity in humans (mix and male), 48% in the rat, 53% in dog, 56% in mice and 60% in rabbits following 60 min incubation in liver microsomes in the presence of NADPH. Eight components were detected in human microsomes incubation samples by HPLC. Four metabolites IN-U3204, IN-R3274, IN-KQ960 and IN-KP533 accounted for 2-8% of the sample radioactivity in species investigated There are some qualitative metabolites differences (about 3 fold) in comparison human with other species. There is no unique human metabolite.	**With enzyme cofactor (NADPH) in microsomes:** Final concentration 20 mM **Analysed** by LSC and HPLC, Metabolites identity confirmed by TLC **Recovery of AR:** Hepatocytes: 94% Microsomes: 93.4-99.0 % for all species **Hepatocytes:** **Rat**: Wistar (one male donor) **Liver microsomes:** **Human 1**: pooled mixed gender from 34 donors **Human 2**: pooled male gender from 20 donors **Rat**: Sprague-Dawley, male, pooled from 34 donors **Mouse**: CD-1, male, pooled from 100 donors **Dog**: Beagle, male, pooled of 10 donors **Rabbit**: New Zealand White, male, pooled of 2 donors	
Comparative *in vitro* metabolism of [^{14}C]-Cymoxanil using rat, dog and human liver microsomes GLP Acceptable	Metabolism of Cymoxanil is identical across all tested species, and no unique human metabolite was detected. Seven metabolites were detected in rat, dog and human microsomes with cofactor incubation samples. Metabolites have not been identified in this study.	**Test substance**: Radiolabelled [^{14}C]-Cymoxanil, purity >97% **Final concentration:** 10 (9.83)µM **Positive control:** [^{14}C]- -testosterone, Radiochemical purity > 97 % 200 µM **With/without enzyme cofactor:** NADPH Final concentration 3 mM **Analysed** by HPLC **Recovery of AR:** ≥98.6 % for all species **Liver microsomes:** **Human**: mixed gender, pooled from 50 (30♂ and 20♀) donors **Rat**: Wistar, mixed gender, pooled 1:1 of 200♂ and 100♀ **Dog**: Beagle, mixed gender, pooled 1:1 of 8♂ and 12♀	RAR B.6.1.3.3., 2018

2.6.1.1 2.6.1.1 Short summary and overall relevance of the provided toxicokinetic information on the proposed classification(s)

The available data regarding absorption, distribution, metabolism and excretion of Cymoxanil include six studies in the rats that were evaluated during the original EU review. Six studies investigated low (2.5, 10) and/or high (50, 100 and 120) doses by oral route after single and/or multiple dosing (for 7 or 14 days).

Three new *in vitro* comparative metabolism studies (one each by a different applicant: CTF, Agria and SFP) have

been submitted for the purpose of renewal to meet the data requirement for such investigations in Regulation 283/2013.

Absorption

Based on all studies submitted (single oral low and high dose; multiple dosing) the radiolabelled test substance is rapidly but incompletely absorbed. With respect to T_{max}, a rapid absorption is evident: T_{max} values for whole blood and plasma are in the range of 0.5 – 3.3 hours following single low dose (2.5 and 10 mg/kg), 0.5 – 5.2 hours following single high dose (100 and 120 mg/kg) and 2.0 hours following repeated dose (50 mg/kg for 7 days).

The urinary excretion (including cage wash) accounted for 63.7 – 79.5 % of the administered radioactivity, biliary excretion was in the range of 6.2 – 9.6 % of administered dose within 48 - 120 h, irrespective of dose and sex. Four biliary excretion studies were undertaken in which a single dose of the test substance (2.5, 10, 100 and 120 mg/kg bw/day) was administered; however bile excretion was measures in both sexes of rats only in one assay (RAR B.6.1.1.2., 1995 and 1997 (supplement)). It is noteworthy that considering all relevant ADME studies, some difference in sex with respect to excretion (in urine and faeces) cannot be excluded. The enteral resorption after oral administration in respective studies investigating separately excretion via urine (including cage wash) and in some studies via bile can therefore be quantified to be in the range of 65.4 – 86.5 % for males and 69.8 – 76.9% for females, 48 – 120 hours after administration, irrespective of dosing. Taken into account the deviations of some studies from the current regulatory guidance, the Study 2 (RAR B.6.1.1.2., 1995 and 1997 (supplement)) is considered as the most reliable assay in assessing absorption. The enteral absorption was estimated 76.0% for the both sex (amount of radioactivity excreted via urine including radioactivity detected in cage wash, bile and carcass), at 48 h after low dose (2.5 mg/kg) administration. Overall, the oral absorption value 76% for cymoxanil is considered appropriate for AOEL calculation.

Pharmacokinetic parameter

As regards the plasma kinetic, the absorption of radioactivity is rapid but T_{max} values for whole blood and plasma are in quite wide range 0.5 – 5.2 hours following single low and high dose. After the last 7th dose the peak ^{14}C-residue concentrations were reached within 3 hours (T_{max}). It is noteworthy that unlike the animals of the repeated dose study, the animals of both two single dose studies (RAR B.6.1.1.1., 1995 and RAR B.6.1.1.3., 1999) were fasted for overnight 12 hours before dosing (and additionally 4 hour after dosing in RAR B.6.1.1.3., 1999).
The elimination half live ($T_{1/2}$) was shown to be 11.7 – 24.1 hours after single oral dosing; a slightly increase could be observed for animals administered multiple daily doses ($T_{1/2}$ of 30.8 – 31.7 hours).
The plasma / whole blood ratios exceeded 1 during the initial phase up to 24 h and declined during the elimination phase due to a possible re-incorporation of ^{14}C residues (glycine and/or other amino acids) into the erythrocytes.

Distribution

Tissue/blood ratios did not indicate a selective accumulation of ^{14}C-residues in any organ/tissue investigated (following single low/high and repeated oral dose) with the exception of kidneys and liver as the main metabolism/excretion organs showing higher residue levels when compared to the whole blood. The half-lives for the elimination of radioactivity from the mentioned organs are in the range of 23.4 – 32.9 hours (kidneys) and 28.3 – 37.9 hours (liver) after single low and high dose administration. Additionally, some indication of slightly higher residues than observed in whole blood was found in thyroid of females (single low dose) and in skin (single high dose) of both sexes at 96 hour sampling. A rapid decline of ^{14}C cymoxanil equivalents in all tissues and organs was observed with time after treatment (<1% of AD at the 96 h sampling).

Some indication of slightly higher residues than observed in plasma was found in the kidneys, the liver and in the stomach wall at different hours after dosing after administration of single low / high or repeated doses. 120 or 168 hours after administration of single low / high or repeated doses, tissue/plasma ratios increase above 1 for nearly all organs/tissues investigated. The increase of tissue/plasma ratios is conclusive, since the ^{14}C-residues are incorporated into red blood cells. The residual radioactivity of all organs/tissues declines with time after treatment.

A certain difference (not seen in the singe oral administration studies) between the plasma and tissue elimination kinetics at 24 and 168 hours should be indicated in repeated dose 50 mg/kg for 7 days study: the tissue/plasma ratios increased above unity and upwards for nearly all organs/tissues investigated (*RAR B.6.1.1.4., 2000*). The residual radioactivity in all organs declines with time after treatment with the exception of fat, in which the radioactivity increased, though the increase of radioactivity in fat tissue was not considered statistically significant (t-test; $p < 0.05$). 24 hours after the last administration ^{14}C-residues in fat were found to be 11.52 – 15.72 µg/g and 18.05 – 25.35 µg/g 168 hours after the last administration. The increase of the radioactive residues in fat maybe could be explained by the extensive metabolism of cymoxanil in rats (mainly to glycine and other amino acids) indicating re-incorporation of the ^{14}C labelled carbon atom. It can be concluded, that no

potential of bioaccumulation can be assumed.

Excretion

After oral application of radioactive labelled cymoxanil (all dose levels tested), the major route of excretion was via urine (63.7 – 79.5 % of administered radioactivity including cage wash); faeces contained 14.3 – 29.9 % and less than 3% was eliminated in expired air within 48 – 168 hours, irrespective of dose regimen or sex. Biliary excretion accounted for 2.0 – 9.6 %. > 80 % of the applied radioactivity could be excreted within 48 hours. 81.5 – 96.3 % of administered radioactivity was shown to be excreted via urine, faeces or was found in bile, cage wash and in expired air at the termination of the studies submitted (i.e. 48 – 168 hours after administration), irrespective of dose regimen or sex. Repeated dosing did not show any impact on the rate and extent of excretion. Some differences in sex with respect to excretion were observed in one study (out of four): for females the excretion in faeces was higher and faster than for males, whereas urinary excretion was lower following single low and high dose 120 hours after administration.

Metabolism

Cymoxanil was shown to be extensively metabolised: no parent compound (or only a trace level) could be detected in any samples investigated (faeces, urine, bile). For faeces only about 30 – 70 % of the recovered radioactivity could be extracted. The main portion of the urine (50-83% of urine radioactivity recovered), faecal (50 - 63%) and bile (<66%) radioactivity could be attributed to a polar fraction containing mainly bound glycine (conjugated with endogenous substances), irrespective of dose regimen or sex. One further metabolite 2-cyano-2-methoxyiminoacetic acid (IN-W3595) was detected in urine (10-45% of urine radioactivity recovered), faeces (<7%) and bile (<36%) too, irrespective of dose regimen or sex. It should be noted that an apparently larger amount of this metabolite was detected in the female urine following single low dose. Further degradation product like Metabolite A could be detected in urine (<16 % of recovered radioactivity) but not in bile samples. The metabolite IN-U3204, hippuric acid and the oxime (Metabolite 4/INT-3204) were only found in urine but in very low amounts. All metabolites identified are intermediates leading to the formation of glycine used for incorporation and conjugation. The proposed metabolic pathway is shown below.

Comparative *in vitro* metabolism

According to Regulation (EU) No. 283/2013, an *in vitro* comparative metabolism study on animal species used in pivotal studies and human is required. Three new *in vitro* comparative metabolism studies (one each by a different applicant: CTF, Agria and SFP) have been submitted for the purpose of renewal to meet the data requirement for such investigations in Regulation 283/2013. The RMS considers the studies to be acceptable to meet the data requirement. It should be noted that when three studies were conducted, two important documents were published: *'EFSA Workshop on in vitro comparative metabolism studies in regulatory pesticide risk assessment' (EFSA Supporting publication 2019: EN-1618, 16 pp.)* and the *OECD Guidance Document 'Good In vitro Method Practices (GIVIMP)' (OECD, 2018).* The EFSA Event Report focuses on the minimum amount of information that should be provided to satisfy the data requirement and should be included in the study protocols, the key elements to be considered for the interpretation of the studies outcome. Therefore, some RMS comments are based on this document in the absence of OECD test guideline for this kind of studies.

In Study 1 (RAR B.6.1.3.1., 2018) and Study 2 (RAR B.6.1.3.2., 2018), the *in vitro* metabolism of cymoxanil by rat, mouse, dog, rabbit and human liver microsomes has been compared. In Study 3 (RAR B.6.1.3.3., 2018), the *in vitro* metabolism of cymoxanil by rat, dog and human liver microsomes has been compared. Additionally, comparative *in vitro* metabolism of [^{14}C[cymoxanil in primary rat hepatocytes was analysed in one assay (RAR B.6.1.3.2., 2018).

Based on the results, it could be concluded that cymoxanil is extensively metabolised in liver microsomes of all investigated species (rat, mouse, dog, rabbit and human) but to a lesser extent in human incubates and in the following order: metabolism rabbit > metabolism mouse > metabolism rat > metabolism dog > metabolism human. A pattern of cymoxanil breakdown products and metabolism among all investigated species (mouse, rat, rabbit, dog and human) is quite consistent; however, some quantitative differences were observed. The major metabolite IN-W3595 was the same in all investigated species and making up ≥30% of total radioactivity in liver microsomes in the presence of cofactor. No unique human metabolites were detected.

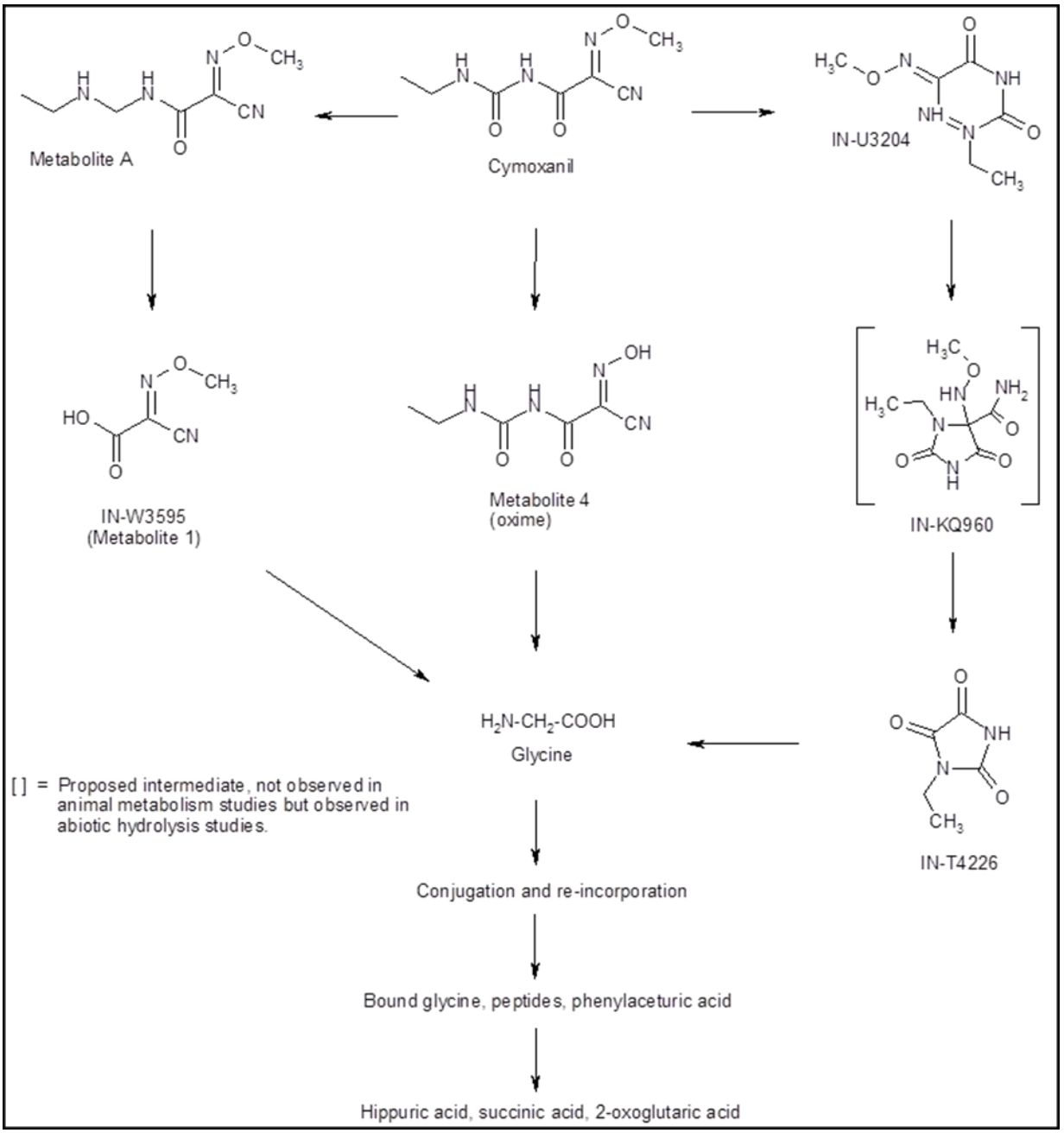
More than twelve components/metabolites, in addition to parent cymoxanil were detected in mouse, rat, rabbit, dog and human microsomes incubation samples by HPLC, however, not all metabolites were identified in the studies submitted and/or were not confirmed by another dissimilar, analytically independent system. In general, each of these components/metabolites did not exceed 10% level or exceeded rarely. Three metabolites (IN-JX915-003, IN-T4226-001 and IN-R3273-006) were detected in mouse, rat, dog and human microsomes incubate (with cofactor) by HPLC, confirmed by LC-MS and accounted 1-7% of the sample radioactivity.

It is noteworthy that the main breakdown product IN-W3595 of cymoxanil and metabolite IN-U3204 in both *in vitro* and *in vivo* rat studies were the same. In addition, three rat metabolites were identified by HPLC and

confirmed by LC-MS analysis: IN-JX915-003, IN-T4226-001 and IN-R3273-006 following 120 min incubation in rat microsomes incubation samples in the presence/absence of cofactor. Other three metabolites (IN KQ960-004, IN-KP533-003 and IN-U3204-010) were identified by HPLC and confirmed by LC-MS analysis only in the absent of cofactor.

Since some metabolites have not been identified in comparative *in vitro* metabolism studies, no thorough comparison between *in vitro* and *in vivo* rat studies data can be performed.

Proposed metabolic pathway of Cymoxanil in the rat



2.6.2 Summary of acute toxicity

2.6.2.1 Acute toxicity - oral route

Table 18: Summary table of animal studies on acute oral toxicity

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance (Batch No; purity)	Dose levels, duration of exposure	Value LD_{50}	Reference
OECD 401 (1987) GLP Acceptable.	Rat: Sprague-Dawley, Crl:CD/BR strain 10/sex/dose	Cymoxanil IN T3217-113 or DPX-T3217-113 97.8%	Doses: males: 250, 500, 1000, 3000 mg/kg bw females: 500, 1000, 2000, 3000 mg/kg bw Exposure: once by gavage	760 mg/kg (♂) 1200 mg/kg (♀) 960 mg/kg (combined)	RAR B.6.2.1.1., 1992
OECD 401 (1987) GLP Acceptable.	Rat: Sprague-Dawley CD 5/sex/dose	Cymoxanil Batch no/ Purity is not available	Doses: 300, 387, 500 mg/kg bw Exposure: once by gavage	538 mg/kg (♂) 356 mg/kg (♀) 485 mg/kg (combined)	RAR B.6.2.1.2., 1992a

No human data on acute oral toxicity are available. No other studies relevant for acute oral toxicity are available.

2.6.2.1.1 Short summary and overall relevance of the provided information on acute oral toxicity

Two acute oral studies with cymoxanil are available. In two experiments the test species was the rat.

The calculated oral LD_{50} of cymoxanil for male rats was 760 mg/kg body weight (95% confidence interval of 360 - 1700 mg/kg), for female rats was 1200 mg/kg body weight (95% confidence interval of 720 - 2000 mg/kg) and for the sexes combined was 960 mg/kg body weight (95% confidence interval of 610-1500 mg/kg) (RAR B.6.2.1.1., 1992). Deaths occurred up to 2 days after dosing. Clinical signs of toxicity observed in males and female rats were: lethargic behaviour, low posture, hunched posture, prostrate posture, dry red ocular / nasal discharge, low/high carriage and incoordination. These signs persisted up to 1 to 6 days after treatment in males and 1 – 7 days after treatment in females. Complete recovery was then recorded for all survivors until the end of the observation period. There was no evidence from gross pathological examination of specific organ toxicity.

The calculated oral LD_{50} of cymoxanil for male rats was 538 mg/kg body weight (95% confidence interval of 326 - 888 mg/kg), for female rats was 356 mg/kg body weight (95% confidence interval of 177 - 714 mg/kg) and for the sexes combined was 485 mg/kg body weight (95% confidence interval of 316 - 744 mg/kg) (RAR B.6.2.1.2., 1992a). Deaths occurred at each dose level, from 2 hours to one day after treatment. Toxicity signs were noted in all treated groups: lethargy and ataxia with signs of decreased respiratory rate and laboured transpiration. Isolated incidents of toxicity noted were exophthalmos, occasional body tremors and loss of righting reflex. Common abnormalities noted at necropsy of animals that died during the study were haemorrhagic lungs, dark liver, dark kidneys and haemorrhage or sloughing of the gastric mucosa. Surviving animals appeared normal one to four days after dosing.

2.6.2.1.2 Comparison with the CLP criteria regarding acute oral toxicity

Classification for acute oral toxicity under Regulation (EC) No 1272/2008 (Section 3.1) is required for substances with an acute oral LD_{50} value (or estimated LD_{50} value) of ≤2000 mg/kg bw. The lowest acute oral LD_{50} was 356 mg/kg bw for female rats as reported by the results of the second study (RAR B.6.2.1.2., 1992a). Since the oral studies in rats consistently revealed 300< LD_{50} values <2000 mg/kg bw, classification for acute oral toxicity according to CLP regulation is required. Cymoxanil is classified as Acute Tox. 4; H302 according to CLP Regulation (EC) No. 1272/2008.

2.6.2.1.3 Conclusion on classification and labelling for acute oral toxicity

Based on the available data, classification as Acute Tox. 4; H302 is required for acute oral toxicity according to Regulation (EC) No 1272/2008. For acute oral toxicity the Acute Toxicity Estimate (ATE) value of 356 mg/kg bw is proposed, based on the results of the second study (RAR B.6.2.1.2 Study 2, 1988) , which gives the lowest ATE value.

2.6.2.2 Acute toxicity - dermal route

Table 19: Summary table of animal studies on acute dermal toxicity

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance (Batch No; purity)	Dose levels, duration of exposure	Value LD_{50}	Reference
OECD 402 (1987) Limit test GLP Acceptable.	Rat: Sprague-Dawley, Hsd/Ola strain 5/sex/dose	Cymoxanil 793 97.6%	Dose: 2000 mg/kg bw on at least 10% of the body surface Exposure: 24 hours (semi-occlusive)	>2000 mg/kg	RAR B.6.2.2.1., 1994a
OECD 402 (1981) Limit test GLP Acceptable.	Rat: Sprague-Dawley CD 5/sex/dose	Cymoxanil Batch no/ Purity is not available	Dose: 2000 mg/kg bw on at least 10% of the body surface Exposure: 24 hours (semi-occlusive)	>2000 mg/kg	RAR B.6.2.2.2., 1992b

No human data on acute dermal toxicity are available. No other studies relevant for acute dermal toxicity are available.

2.6.2.2.1 Short summary and overall relevance of the provided information on acute dermal toxicity

Two acute dermal studies with cymoxanil are available. In two experiments the test species was the rat.

No deaths occurred in two acute dermal toxicity studies at the limit dose of 2000 mg/kg body weight. No sign of systemic toxicity or skin irritation were noted; however, slightly reduced body weight gain was recorded for males and females in both studies. Gross necropsy did not reveal any abnormalities. The acute dermal LD_{50} of cymoxanil in the rat was therefore found to be >2000 mg/kg body weight under the conditions of this study.

2.6.2.2.2 Comparison with the CLP criteria regarding acute dermal toxicity

Classification for acute dermal toxicity under Regulation (EC) No 1272/2008 (Section 3.1) is required for substances with an acute dermal LD_{50} value of ≤2000 mg/kg bw. Cymoxanil is reported to have an acute dermal LD_{50} of >2000 mg/kg bw; therefore classification is not required for acute dermal toxicity.

2.6.2.2.3 Conclusion on classification and labelling for acute dermal toxicity

Based on the available data, no classification is required for acute dermal toxicity according to Regulation (EC) No 1272/2008.

2.6.2.3 Acute toxicity - inhalation route

Table 20: Summary table of animal studies on acute inhalation toxicity

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance (Batch No; purity), form and particle size (MMAD)	Dose levels, duration of exposure	Value LC_{50}	Reference
OECD 403 (1981) GLP Acceptable.	Rat: Sprague-Dawley, Crl:CD BR strain 5/sex/dose	Cymoxanil DPX-T3217-115 (milled from T3217-113) 98.2% Milled solid formulation MMAD = 2.6; 2.8; 3.1 µm	Doses (mg/L): 3.21 ± 0.48 4.98 ± 1.14 5.06 ± 0.40 Exposure: 4 hours (nose only)	>5.06 mg/L air	RAR B.6.2.3., 1992

No human data on acute inhalation toxicity are available. No other studies relevant for acute inhalation toxicity are available.

2.6.2.3.1 Short summary and overall relevance of the provided information on acute inhalation toxicity

In the acute inhalation toxicity study, one male rat died during exposure at 4.98 mg/L. Clinical signs observed immediately after exposure were abnormal gait or mobility, lethargy, irregular respiration, ocular/nasal/oral discharges, vocalizations, hunched posture, diarrhoea and stained fur. One male showed tremors on days 7-15 at 5.06 mg/mL. There was initial body weight loss of up to 18%, but after day 6 the animals generally started to regain weight. At necropsy, one male and one female at 5.06 mg/L had discolouration of one liver lobe and another male had an enlarged lymph node, both of which findings were considered incidental. There was no evidence of specific organ toxicity.

2.6.2.3.2 Comparison with the CLP criteria regarding acute inhalation toxicity

Classification for acute inhalation toxicity under Regulation (EC) No 1272/2008 (Section 3.1 of Annex I) is required for substances (dusts and mists) with an acute inhalation LC50 value of ≤5 mg/L. Cymoxanil is reported to have an acute inhalation LC50 of >5.06 mg/L; therefore classification is not required for acute inhalation toxicity.

2.6.2.3.3 Conclusion on classification and labelling for acute inhalation toxicity

Based on the available data, no classification is required for acute inhalation toxicity according to Regulation (EC) No 1272/2008.

RAC evaluation of acute toxicity

Summary of the Dossier Submitter's proposal

No cases of acute intoxication with cymoxanil have been reported in workers of three manufacturing plants.

Oral

The DS summarised two acute oral toxicity studies. Both were performed in rats according to OECD TG 401 and following GLP standards. In one, the combined LD_{50} for both sexes was 960 mg/kg bw (760 mg/kg bw for males and 1200 mg/kg bw for females). In the other study, the combined LD_{50} was 485 mg/kg bw (538 mg/kg bw for males and 356 mg/kg bw for females).

Clinical signs comprised lethargy, ataxia, low, hunched, or prostrate posture, loss of righting reflex, incoordination, laboured respiration and decreased respiratory rate, dry red nasal and/or ocular discharge, and isolated incidents of exophthalmos and tremors. Clinical signs resolved within one to seven days after exposure. No gross pathological findings were observed in the first study. In the second study, haemorrhagic lungs and gastric mucosa, dark liver and kidneys were reported in animals that died during the study.

Dermal

The DS summarised two acute dermal toxicity studies performed in rats according to OECD TG 402 (limit test) and under GLP conditions. No mortalities occurred at the dose of 2000 mg/kg bw. No dermal irritation or clinical signs, and no gross pathological abnormalities were observed.

Inhalation

One acute inhalation toxicity in rats was available. It was conducted according to OECD TG 403 (1981) and GLP standards. Concentrations of 3.21, 4.98, and 5.06 mg/L dust

containing 98.2% of the pure substance were applied nose only for four hours.

One male rat died in the mid dose group. No other mortalities occurred. Therefore, the LC_{50} for this study was > 5.06 mg/L. One male from the high dose group showed tremors on day 7-15 of the observation period. Clinical signs observed immediately after exposure were abnormal gait, lethargy, irregular respiration, ocular, nasal, or oral discharges, vocalization, hunched posture, diarrhoea, and stained fur.

Conclusion on classification

Based on two oral acute toxicity studies that yielded LD_{50} values in the range for Cat. 4 (300 < ATE ≤ 2000 mg/kg bw) the DS proposed to classify cymoxanil as Acute Tox. 4 (H302), using the lowest LD_{50} value reported for female rats (356 mg/kg bw) as the ATE.

For acute dermal and inhalation toxicity, based on the fact that no treatment related mortalities were observed above the cut-off values for Category 4 (2000 mg/kg bw for acute dermal toxicity, and 5 mg/L for acute inhalation toxicity), the DS proposed no classification for these two hazard classes.

Comments received during consultation

One MSCA commented on this hazard class and supported the proposed classification as Acute Tox. 4, H302 with an ATE of 356 mg/kg bw.

Assessment and comparison with the classification criteria

Two reliable **acute oral toxicity** studies performed according to the version of OECD TG 401 current at the time both yielded LD_{50} values that were within the boundaries for Cat. 4 categorisation for acute oral toxicity. Therefore, RAC concurs with the DS that classification for cymoxanil as **Acute Tox 4, H302** is warranted. However, RAC proposes to round the ATE value based on the most sensitive species (female rats) to 360 mg/kg bw.

RAC also concurs with the DS that **no classification for acute dermal toxicity** is warranted based on guideline and GLP compliant studies presented in the CLH dossier, in which no mortalities were observed at the cut-off value for classification.

For **acute inhalation toxicity**, one guideline and GLP compliant study using milled pure substance with (guideline compliant) mean mass aerodynamic diameters of 2.6 to 3.1 µm and a 4-hour nose only exposure in rats was presented. One death occurred in the mid dose group but none in the high dose group. Thus, the LC_{50} for this study was above the highest concentration tested (5.06 mg/L) and above the cut-off value for classification (5 mg/L). Therefore, RAC concurs with the DS that **no classification** for this hazard class is warranted.

These recommendations are in line with RAC's previous evaluation (RAC, 2012) of Cymoxanil.

2.6.2.4 Skin corrosion/irritation

Table 21: Summary table of animal studies on skin corrosion/irritation

Method,	Species,	Test	Dose	Results	Reference

<table>
<tr><th>guideline, deviations if any</th><th>strain, sex, no/group</th><th>substance (Batch No; purity)</th><th>levels, duration of exposure</th><th>- Observations and time point of onset
- Mean scores/animal
- Reversibility</th><th></th></tr>
<tr><td>OECD 404 (1981)
GLP
Acceptable</td><td>Rabbit: New Zealand White
3 females</td><td>Cymoxanil
793
97.6%</td><td>Dose:
0.5 g on a skin area 6.25 cm^2
Exposure:
4 hours (semi-occlusive)</td><td>
<table>
<tr><th>Scores observed after</th><th>1 hour</th><th>24 hours</th><th>48 hours</th><th>72 hours</th></tr>
<tr><td>Erythema</td><td>0, 0, 0</td><td>0, 0, 0,</td><td>0, 0, 0,</td><td>0, 0, 0,</td></tr>
<tr><td>Oedema</td><td>0, 0, 0,</td><td>0, 0, 0,</td><td>0, 0, 0,</td><td>0, 0, 0,</td></tr>
</table>
</td><td>RAR B.6.2.4., 1994b</td></tr>
<tr><td></td><td></td><td></td><td></td><td></td><td></td></tr>
</table>

No human data on skin corrosion/irritation are available. No other studies relevant for skin corrosion/irritation are available.

2.6.2.4.1 Short summary and overall relevance of the provided information on skin corrosion/irritation

One skin irritation study in the rabbit is available. 3 female New Zealand White rabbits were exposed to cymoxanil (0.5 g moistened with distilled water, exposure area of 6.25 cm^2) with a gauze pad, secured with elastic adhesive semi-occlusive dressing. After an exposure period of 4 h, the test sites were washed with warm water. The animals were observed daily for clinical signs, and examinations of the skin sites were conducted at 1, 24, 48 and 72 h after removal of the dressings: erythema, eschar formation and oedema were assessed according to the Draize criteria. The test material didn't produce clinical signs of toxicity or dermal responses. Based on the available data, cymoxanil does not meet the criteria for classification as a skin irritant according to Regulation (EC) No 1272/2008.

2.6.2.4.2 Comparison with the CLP criteria regarding skin corrosion/irritation

Skin irritation is defined as the production of reversible damage to the skin following the application of a test substance for up to 4 hours (Section 3.2.1.1 of Annex I of the CLP Regulation). Classification of a substance for skin irritation (Category 2) is required on the basis of an animal study showing a mean value of ≥2.3 - ≤4.0 for erythema/eschar or for oedema in at least 2 of 3 tested animals from gradings at 24, 48 and 72 hours after patch removal or, if reactions are delayed, from three consecutive days after the onset of skin reactions. Classification is also required for inflammation that persists to the end of the observation period (normally 14 days) in at least 2 animals, particularly taking into account findings such as alopecia, hyperkeratosis, hyperplasia, and scaling. Classification may also be required in some cases where there is pronounced variability of response among animals, with very definite positive effects related to exposure in a single animal but less than the criteria listed above.

In the single study available, no signs of toxicity, nor any dermal responses, were recorded during the observation period, resulting in a primary irritation score of zero for all animals.

2.6.2.4.3 Conclusion on classification and labelling for skin corrosion/irritation

Based on the available data, cymoxanil does not meet the criteria for classification as a skin irritant according to Regulation (EC) No 1272/2008.

RAC evaluation of skin corrosion/irritation

Summary of the Dossier Submitter's proposal

No human data are available on skin corrosion or irritation.

The DS summarised one OECD TG 404 (1981) study conducted according to GLP standards with cymoxanil at a dose of 0.5 g on an area of 6.25 cm2. No signs of erythema or oedema were observed in any of the three rabbits at any of the observation

points. No clinical signs were reported.

Conclusion on classification

Since no signs of toxicity or dermal irritation were observed in a reliable OECD TG 404 study, the DS concluded that no classification was warranted for skin corrosion/irritation.

Comments received during consultation

No comments were received.

Assessment and comparison with the classification criteria

One OECD TG 404 study was summarised in the CLH report. Cymoxanil technical grade (purity 97.6%) moistened with water was applied to the clipped backs of three female New Zealand White (NZW) rabbits at a dose of 0.5 g on an area of 6.25 cm2 (equating to a concentration of 0.08 g/cm2) under semi-occlusive dressing for 4 hours. No irritation or corrosion responses were observed at any of the readings post-exposure. Mean scores for erythema and oedema were 0 for all animals.

Thus, RAC concurs with the DS that in accordance with the Guidance on the Application of the CLP Criteria (CLP guidance) **no classification** for skin corrosion/irritation is warranted.

This recommendation is in line with RAC's previous evaluation (RAC, 2012) of Cymoxanil.

2.6.2.5 Serious eye damage/eye irritation

Table 22: Summary table of animal studies on serious eye damage/eye irritation

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance (Batch No; purity)	Dose levels duration of exposure	Results - Observations and time point of onset - Mean scores/animal - Reversibility	Reference
OECD 405 (1987) GLP Acceptable	Rabbit: New Zealand White 4 females	Cymoxanil 793 97.6%	Dose: 12 mg (1 rabbit) and 60 mg (the weight occupying a volume of 0.1 ml) (3 rabbits) Exposure: single instillation in the everted lower eyelid of one eye	Slight conjunctival irritation was observed: Redness score 1 in 4/4 rabbits and Chemosis score 1 in 2/4 rabbits (in one animal at 12 mg and one at 60 mg) after 1h. All findings resolved within 24 h and no effects on conjunctivae, cornea or iris were detected.	RAR B.6.2.5.1., 1994c
OECD 405 (1987) GLP Acceptable **Deviations:** Purity and homogeneity of test materials not	Rabbit: New Zealand White 1 male and 2 females	Cymoxanil	Dose: 0.1 ml Exposure: single instillation in conjunctival sac of the left eye The treated eyes were not washed after instillation of the test substance.	Slight to moderate conjunctival irritation was observed in all treated eyes one hour after treatment: Redness score 1 in 1/3 rabbits, score 2 in 2/3 rabbits and Chemosis score 1 in 1/3 rabbits, score 2 in 2/3 rabbits including discharge in all rabbits. The test substance generated mean score of corneal opacity 0-0-0, iritis 0-0-0, and conjunctival redness 0.33-	RAR B.6.2.5.2., 1992c

<table>
<tr>
<td>determined.

Lot/Batch number is not available.</td>
<td></td>
<td></td>
<td></td>
<td>0-0 and of oedema (chemosis) 0.33-0-0 of 3 tested animals under the conditions tested at 24, 48 and 72 hours after installation of the test material.
<table>
<tr><th>Scores observed after</th><th>24 hours</th><th>48 hours</th><th>72 hours</th></tr>
<tr><td>Conjunctival redness</td><td>1, 0, 0</td><td>0, 0, 0</td><td>0, 0, 0</td></tr>
<tr><td>Conjunctival chemosis</td><td>1, 0, 0</td><td>0, 0, 0</td><td>0, 0, 0</td></tr>
<tr><td>Iritis</td><td>0, 0, 0</td><td>0, 0, 0</td><td>0, 0, 0</td></tr>
<tr><td>Corneal opacity</td><td>0, 0, 0</td><td>0, 0, 0</td><td>0, 0, 0</td></tr>
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<td></td>
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</table>

No human data on serious eye damage/eye irritation are available. No other studies relevant for serious eye damage/eye irritation are available.

2.6.2.5.1 Short summary and overall relevance of the provided information on serious eye damage/eye irritation

Two eye irritation studies in the rabbits are available.

In one guideline compliant GLP eye irritation assay Cymoxanil technical (97.6% purity), 12 mg, was placed directly into the everted lower eyelid of one eye of a single New Zealand White rabbit, to check initially for any severe response (RAR B.6.2.5.1., 1994c). A second animal then received 60 mg (the weight occupying a volume of 0.1 mL) in the same way, before the remaining two animals were similarly treated, the material occupying a volume of 0.1 mL. The contralateral eye of each animal served as control. The rabbits were observed daily for any signs of toxicity. Examination of the eyes was made after 1, 24, 48 and 72 h after treatment, then again at 4 and 7 days. There were no general clinical signs of toxicity throughout. Slight conjunctival irritation was observed: Redness (some blood vessels hyperaemic) score 1 in 4/4 rabbits and Chemosis score 1 in one animal at 12 mg and one at 60 mg after 1h. All findings resolved within 24 h and no effects on conjunctivae, cornea or iris were detected, i.e. the overall mean score for each parameter was zero.

In the other assay 3 rabbits (1 male and 2 female) received 46 mg (the weight occupying a volume of 0.1 mL) of undiluted cymoxanil into the everted lower lid RAR B.6.2.5.2., 1992c). Slight to moderate conjunctival irritation was observed in all treated eyes one hour after treatment: Redness score 1 in 1/3 rabbits, score 2 in 2/3 rabbits and Chemosis score 1 in 1/3 rabbits, score 2 in 2/3 rabbits including discharge in all rabbits. All treated eyes appeared normal 48 hours after treatment. On the basis of this study, cymoxanil generated mean score of corneal opacity 0.0-0.0-0.0, iritis 0.0-0.0-0.0, and conjunctival redness 0.33-0.0-0.0 and of chemosis 0.33-0.0-0.0 of 3 tested animals under the conditions tested at 24, 48 and 72 hours after installation of the test material.

2.6.2.5.2 Comparison with the CLP criteria regarding serious eye damage/eye irritation

Serious eye damage (Category 1) is defined as the production of tissue damage in the eye, or serious physical decay of vision, following application of a substance to the anterior surface of the eye, which is not fully reversible within 21 days of application (Section 3.3.1.1 of Annex I of the CLP Regulation).

Eye irritation (Category 2) is defined as the production of changes in the eye following the application of test substance to the anterior surface of the eye, which are fully reversible within 21 days of application (Section 3.3.1.1 of Annex I of the CLP Regulation).

Classification in Category 1 is required for substances producing (in at least in one animal) effects on the cornea, iris or conjunctivae that are not expected to reverse or have not fully reversed within the observation period normally 21 days. Classification is also required where (in at least 2 of 3 animals) mean scores of ≥3 for corneal opacity or >1.5 for iritis are attained following grading at 24, 48 and 72 hours after installation of the test material.

Classification in Category 2 is required for substances producing (in at least 2 of 3 animals) mean scores of ≥1 for corneal opacity, ≥1 for iritis, ≥2 for conjunctival redness (erythema) and/or ≥2 for oedema (chemosis) following grading at 24, 48 and 72 hours after installation of the test material.

In the two studies available, findings were limited to slight / moderate conjunctival redness and slight chemosis at 1 hour after application and cymoxanil generated mean score 0.33 of conjunctival redness / chemosis only for one animal in one study. All findings resolved either within 24 h or 48 h, no effects on cornea or iris were detected. Cymoxanil does therefore not require classification for serious eye damage (Category 1) or for eye irritation (Category 2) according to Regulation (EC) No 1272/2008.

2.6.2.5.3 Conclusion on classification and labelling for serious eye damage/eye irritation

Cymoxanil is not classified for eye irritation according to Regulation (EC) No 1272/2008 on the basis of the available data.

RAC evaluation of serious eye damage/irritation

Summary of the Dossier Submitter's proposal

No human data are available on eye irritation or damage.

The DS summarised two OECD TG 405 (1987) studies that were conducted according to GLP standards.

The first study was already evaluated in the first DAR for cymoxanil (2007) and (presumably) used to evaluate the eye irritation hazard in the 2012 RAC opinion on cymoxanil. In this study, slight redness (grade 1) of the eye was observed in all animals one hour after exposure (1 animal was applied 12 mg of the substance and 3 animals were applied 60 mg of the substance). Chemosis (grade 1) was observed in the animal receiving the lower dose and one animal of the high dose group at the one-hour reading. All effects were resolved by the 24-hour reading. Thus, mean values for conjunctival redness, conjunctival chemosis, iritis, and corneal opacity were 0 in this study.

In the second study, three NZW rabbits (one male, two females) were instilled with 46 mg of cymoxanil to the conjunctival sac of one eye. Grade 1 or 2 redness, discharge, chemosis, and iritis was observed in all animals at the one-hour reading. At the 24-hour reading, effects had resolved in all but the male animal which showed grade 1 redness and chemosis. Thus, mean grades for conjunctival redness and conjunctival chemosis were 0.33 for 1/3 animals, and 0 for iritis and corneal opacity in all animals.

Conclusion on classification

Since mean scores for conjunctival redness, conjunctival chemosis, iritis, and corneal opacity were below guidance values in both OECD TG 405 studies presented, the DS concluded that no classification was warranted for eye damage/eye irritation.

Comments received during consultation

No comments were received.

Assessment and comparison with the classification criteria

Two OECD TG 405 studies were summarised in the CLH report. Based on mean scores of 0 in the first study, RAC in 2012 proposed no classification for eye damage/eye irritation for cymoxanil.

In the second study presented in the CLH report that was submitted by the applicant in the current renewal process for cymoxanil a lower dose of the substance was instilled into one eye of three rabbits. Only one of these animals (the only male) showed slight redness and slight chemosis (both grade 1) at the 24-hour observation yielding a mean score for these eye irritation endpoints of 0.33 for this animal. No such effects were observed in

the remaining two rabbits at the 24, 48, and 72-hour readings.

According to CLP guidance, a substance is placed in category 2 for eye irritation when it produces in at least in 2 of 3 tested animals, a positive response of: (a) corneal opacity ≥ 1 and/or (b) iritis ≥ 1, and/or (c) conjunctival redness ≥ 2 and/or (d) conjunctival oedema (chemosis) ≥ 2. None of these criteria were fulfilled for cymoxanil in either study.

Thus, RAC concurs with the DS that in accordance with CLP guidance **no classification** for eye damage/eye irritation is warranted.

This recommendation is in line with RAC's previous evaluation (RAC, 2012) of Cymoxanil.

2.6.2.6 Respiratory sensitisation

No animal studies on respiratory sensitisation are available.
No human data on respiratory sensitisation are available. No other studies relevant for respiratory sensitisation are available.

2.6.2.6.1 Short summary and overall relevance of the provided information on respiratory sensitisation

No data are available on the potential of cymoxanil to cause respiratory sensitisation.

2.6.2.6.2 Comparison with the CLP criteria regarding respiratory sensitisation

A respiratory sensitiser is described as a substance that will lead to hypersensitivity of the airways following inhalation of the substance (Section 3.4.1.1 of Annex I of the CLP Regulation). Respiratory sensitisers are allocated into Sub-category 1A (strong sensitisers) or Sub-category 1B (other sensitisers), based on a weight of evidence from reliable and good quality evidence from human cases or epidemiological studies and/or observations from appropriate studies in experimental animals. Substances are classified as Category 1 respiratory sensitisers where data are not sufficient for sub-categorisation, if there is evidence in humans that the substance can lead to specific respiratory hypersensitivity, and/or if there are positive results from an appropriate animal test. Substances are classified as Sub-category 1A respiratory sensitisers where there is evidence of a high frequency of occurrence in humans, or a probability of occurrence of a high sensitisation rate in humans based on animal or other tests. Substances are classified as Sub-category 1B respiratory sensitisers where there is evidence of a low to moderate frequency of occurrence in humans, or a probability of occurrence of a low to moderate sensitisation rate in humans based on animal or other tests.

In the absence of relevant human or non-human data, cymoxanil is not classified as a respiratory sensitiser.

2.6.2.6.3 Conclusion on classification and labelling for respiratory sensitisation

In the absence of any data, cymoxanil does not require classification for respiratory sensitisation according to Regulation (EC) No 1272/2008.

RAC evaluation of respiratory sensitisation

Summary of the Dossier Submitter's proposal

No animal or human data are available on respiratory sensitisation for cymoxanil.

Therefore, the DS concluded that **no classification** was warranted for respiratory sensitisation due to lack of data.

Comments received during consultation

No comments were received.

Assessment and comparison with the classification criteria

Since no data on this endpoint are available, RAC concurs with the DS that **no classification** of cymoxanil for respiratory sensitisation is possible.

2.6.2.7 Skin sensitisation

Table 23: Summary table of animal studies on skin sensitisation

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels duration of exposure	Results	Reference
OECD 406 (1981) *Magnusson and Kligman Maximisation* test GLP Acceptable	Guinea pig: Dunkin-Hartley 2/sex intradermal induction dose range 2/sex topical dose range 13/sex vehicle controls, 10/sex test animals 3/sex positive control	Cymoxanil DPX-T3217-113 97.8%	**Preliminary study:** *Intradermal injections:* 0.5%, 1.5%, 3.0% and 5.0% w/v *Topical application:* 1.0%, 5%, 10% and 25% w/v. 25% (w/v) topical concentration did not give rise to irritating effects. **Induction:** 0.1 ml 3.0% w/v suspension in saline (intradermal); 10% SLS in petrolatum 0.3 ml 25% w/v aliquots of suspension in petrolatum (topical) for 48 h; **Challenge:** 0.2 ml 25% w/v suspension in petrolatum (occlusive, 24 h)	**Non-sensitiser** **Preliminary study:** 25% (w/v) topical concentration in petrolatum did not give rise to irritating effects. **Test:** None of the animals showed any skin responses in challenge phase **Negative control**: no responses were observed **Positive control:** intradermal induction dose 0.1 ml 0.1 % w/v suspension of 1-chloro-2,4-dinitrobenzene, DNCB) 100% response was observed 24 and 48 h in challenge phase. Data confirmed the sensitivity of the test system *The topical induction and the challenge concentration 25% used in this study was apparently too low*	RAR B.6.2.6.1., 1992
OECD 406 (1992) *Magnusson and Kligman Maximisation* test GLP Acceptable.	Guinea pig: Albino Hartley 2+2 males intradermal induction dose range 4 males topical dose range 5 males vehicle controls, 10 males test animals 5 males	Cymoxanil 29800123 99.4%	**Preliminary study:** *Intradermal injections: 0.5% and 1.0% w/v* *Topical application*: 15% and 25% w/v. 25% (w/v) topical concentration was the max practical concentration that could be prepared; it did not give rise to irritating effects. **Induction:**	**Non-sensitiser** **Preliminary study:** 25% (w/v) topical concentration in paraffin oil was the max practical concentration that could be achieved; it did not give rise to irritating effects. **Test:** None of the animals showed any skin responses in challenge phase **Negative control**: no responses were observed **Positive control:** intradermal	RAR B.6.2.6.2., 2003

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels duration of exposure	Results	Reference
	positive control		0.1 ml 1.0% w/v solution of cymoxanil in 0.5% carboxymethylcellulose (CMC) (intradermal); 0.5 ml 10% SLS in mineral oil 0.5 ml 25% w/v solution in paraffin oil (topical) for 48 h; **Challenge:** 0.5 ml 25% w/v solution in paraffin oil (occlusive, 24 h)	induction dose 0.5 ml 1 % w/v alcoholic solution of 1-chloro-2,4-dinitrobenzene (DNCB); 100% response was observed 24 and 48 h in challenge phase. Data confirmed the sensitivity of the test system. *The topical induction and the challenge concentration 25% w/v used in this study was apparently too low*	
OECD 406 (1981) *Magnusson and Kligman Maximisation* test GLP Acceptable.	Guinea pig: Dunkin-Hartley 2 females intradermal induction dose range 4 females topical dose range 5 females vehicle controls, 10 females test animals	Cymoxanil 793 97.6%	**Preliminary study:** *Intradermal injections:* 0.1%, 0.25%, 0.5%, 1.0%, 2.5% and 5% w/v *Topical application*: 10%, 20%, 30% and 40% w/v. 40% (w/v) topical concentration was the max practical concentration that could be prepared; it did not give rise to irritating effects. **Induction:** 0.1 ml 1.0% w/v solution of cymoxanil in Alembicol-D (triglycerides from coconut oil) (intradermal); 0.2 ml 10% SLS in petrolatum 0.4 ml 40% w/v solution in Alembicol-D (topical) for 48 h; **Challenge:** 0.2 ml 20 and 40% w/v solution in Alembicol-D (occlusive, 24 h)	**Sensitiser** **Test:** 90% and 100% of the test animals were positive at both sites exposed to 20 and 40% w/v of cymoxanil, respectively, during the observation period after challenge(24, 48 and 72 h). **Negative control**: 20% showed slight erythema at the site with 20% cymoxanil concentration 24h after challenge. **Positive control:** positive control data submitted (1987 – 1992 year); intradermal induction dose 0.1 % w/v of formalin (aqueous dilutions); 80 - 100% response was observed in challenge phase. Data confirmed the sensitivity of the test system.	RAR B.6.2.6.3., 1994

No human data on skin sensitisation are available. No other studies relevant for skin sensitisation are available.

2.6.2.7.1 Short summary and overall relevance of the provided information on skin sensitisation

Three studies for skin sensitisation according to Guinea Pig Maximisation Test by Magnusson & Kligman

(GPMT) are available. These studies have been conducted according to OECD Test Guideline 406 (1981 and 1992) and meet the GLP criteria; regarding the study design, the studies are comparable and differ in the vehicle and doses used. Only in one GPMT for skin sensitisation cymoxanil proved positive (RAR B.6.2.6.3., 1994).

In one assay Cymoxanil technical, dosed as a 3.0% (w/v) suspension in saline (intradermal induction), a 25% (w/v) suspension in petrolatum (topical induction) and the same concentration for challenge phase, was tested for dermal contact sensitivity in 10/sex guinea pigs (*RAR B.6.2.6.1., 1992*). None of the animals treated with cymoxanil showed any skin responses in challenge phase. 1-chloro-2,4-dinitrobenzene (DNCB) was used as the positive control (3/sex animals) and 100% response was observed 24 and 48 h in challenge phase. Vehicle (petrolatum) was administered to 13/sex guinea pigs (control group) and no responses were observed in the vehicle control animals. Cymoxanil technical was not a skin sensitiser under the conditions of this test. It should be noted that the topical induction and the challenge concentrations used in this study were apparently too low according to TG. Therefore, the reliability of the negative results obtained in this study is questionable.

Based on other maximisation assay (*RAR B.6.2.6.2., 2003*) Cymoxanil technical, dosed as a 1.0% (w/v) solution of cymoxanil in 0.5% carboxymethylcellulose (intradermal induction), a 25% (w/v) solution in paraffin oil (topical induction) and the same concentration for challenge phase, was tested for dermal contact sensitivity in 10 male guinea pigs. None of the animals treated with cymoxanil showed any skin responses in challenge phase. Vehicle (carboxymethylcellulose) was administered to 5 male guinea pigs (negative control group) and there were no skin responses in any animal. 1-chloro-2,4-dinitrobenzene (DNCB) was used as the positive control (5 males) and its data confirmed the sensitivity of the test system. Cymoxanil technical was not a skin sensitiser under the conditions of the test. It should be noted that the topical induction and the challenge concentrations used in this study were apparently too low according to TG. However, it was highlighted in the amendment to the study report that 25% (w/v) was the highest concentration in paraffin oil which was achieved. Therefore, the choice of vehicle and the reliability of the negative results obtained in this study are questionable.

In the other reliable study Cymoxanil technical, dosed as a 1.0% (w/v) solution of cymoxanil in Alembicol-D (triglycerides from coconut oil) (intradermal induction), a 40% (w/v) solution in Alembicol-D (topical induction) and the 40 and 20% (w/v) concentrations for topical challenge phase, was tested for dermal contact sensitivity in 10 female guinea pigs (*RAR B.6.2.6.3., 1994*). Following the challenge phase, 90% and 100% animals in the test group showed slight to moderate erythema at both sites 20% and 40%, respectively, during the observation period (24, 48 and 72 h after removal of the patches). All but one animal showed slight to well-defined oedema during the observation period (24, 48 and 72 h after removal of the patches). Overall, 90% and 100% of the test animals was considered to show positive evidence at both sites exposed to 20 and 40% w/v of cymoxanil, respectively, during the observation period after removal of the patches. It should be noted that after the challenge application, necrosis was additionally observed at sites of 3 test animals exposed to 40% w/v of cymoxanil. Vehicle (Alembicol-D) was administered to 5 female guinea pigs (negative control group). Only one of the 5 vehicle controls (20%) showed slight erythema at the site challenged with 20% cymoxanil concentration 24h after challenge. Although no positive control was included in the study, sensitivity of the test system was checked separately twice yearly using known sensitizer formalin. Based on the data submitted (1987 – 1992 year), 80 - 100% of the animals of the positive control group at 0.1 % intradermal induction dose of formalin (aqueous dilutions) reacted upon challenge. Cymoxanil technical (purity 97.6%) was considered to be a contact dermal sensitiser.

Based on RAC Opinion (*14 September 2012*), "*two of the three skin sensitisation studies were negative and no major differences between the studies could be identified which could explain the different results. No data on humans regarding skin sensitisation is available for cymoxanil. Since the data on skin sensitisation in guinea pigs following exposure to cymoxanil is considered not to be sufficient for a sub-categorisation, cymoxanil should be classified according to the criteria under CLP as Skin Sens. 1, H317".*

According to OECD TG 406 (1992), the concentration used for topical induction should be the highest to cause mild-to-moderate skin irritation and for the challenge exposure should be the highest non-irritant dose. However, the choice of vehicle (petrolatum/ paraffin oil) was doubtful and/or the topical induction/challenge concentrations used were apparently too low (25% w/v) in two studies because no signs of irritation were observed in any of the animals dosed topically at this concentrations in a preliminary tests. Therefore, the reliability of the negative results obtained in the first two studies is questionable. No signs of irritation were induced even by 40% (w/v) concentration used in a preliminary test of the thirst study. However, following topical induction with 40% (w/v) concentration and topical challenge phase with 20% (w/v) concentrations, 90% animals showed positive evidence (slight to moderate erythema and slight to well-defined oedema) as well as necrosis was additionally observed at sites of 3 test animals exposed to 40% w/v of cymoxanil Therefore, the third study (*RAR B.6.2.6.3., 1994*) might be considered as the most reliable assay in assessing skin sensitisation. Based on it cymoxanil technical meets the criteria to be classified as a skin sensitizer (H317), sub-category 1A in accordance with Regulation (EC) No 1272/2008 on the basis of the available data.

2.6.2.7.2 Comparison with the CLP criteria regarding skin sensitisation

A skin sensitiser is defined as a substance that will lead to an allergic response following skin contact (Section 3.4.1.2 of Annex I of the CLP Regulation). Skin sensitisers are allocated into sub-category 1A (strong sensitisers) or sub-category 1B (other sensitisers), based on a weight of evidence of reliable and good quality evidence from human cases or epidemiological studies and/or observations from appropriate studies in experimental animals.

Substances are classified as Category 1 skin sensitisers where data are not sufficient for sub-categorisation, if there is evidence in humans that the substance can lead to sensitisation by skin contact in a substantial number of persons, or if there are positive results from an appropriate animal test. High potency is determined according to the results from the animal studies as given in CLP Annex I, Table 3.4.3 and low to moderate potency is determined according to the results from the animal studies as given in CLP Annex I, Table 3.4.4.

Substances are classified as sub-category 1A skin sensitisers where there is evidence of a high frequency of occurrence in humans and/or a high potency in animals. For the Guinea pig maximisation test, substances are allocated to sub-category 1A where a response of ≥30% is seen at intradermal induction concentrations of ≤0.1%; or where a response of ≥60% is seen at intradermal induction concentrations of >0.1% to ≤1%.

Substances are classified as sub-category 1B skin sensitisers where there is evidence of a low to moderate frequency of occurrence in humans and/or a low to moderate potency in animals. For the Guinea pig maximisation test, substances are allocated to sub-category 1B where a response of ≥30% to <60% is seen at intradermal induction concentrations of >0.1% to ≤1%; or where a response of ≥30% is seen at intradermal induction concentrations of >1%.

Cymoxanil technical (purity 97.6%) gave a positive result (>60%) in the Guinea pig maximisation test at intradermal induction concentrations of 1%. Since the incidence sensitised guinea pigs is above the cut-off of 60% at 1% intradermal induction dose in the most reliable assay, the substance is considered to be a strong skin sensitiser, and should be classified as a Category 1 (Sub-category 1A) skin sensitiser (*ECHA, 2017: Guidance on the Application of the CLP Criteria, Version 5.0*). Additionally, for determination of potency category for the sensitiser the *Table 3.7 'Potency on basis of the Guinea Pig Maximisation Test'* from the *Guidance on the Application of the CLP Criteria section 3.4.2.2.5.* '*Setting of specific concentration limits'* should be used. Based on the most reliable assay, cymoxanil has a strong potency of skin sensitisation, therefore, the generic concentration limit should be applied.

2.6.2.7.3 Conclusion on classification and labelling for skin sensitisation

Cymoxanil does warrant classification for skin sensitisation Skin Sens. 1 H317 (Sub-category 1A) in accordance with Regulation (EC) No 1272/2008 on the basis of the available data.

RAC evaluation of skin sensitisation

Summary of the Dossier Submitter's proposal

No human data are available on skin sensitisation.

The DS summarised three OECD TG 406 guinea pig maximisation tests (GPMT, also known as the Magnusson and Kligman test), two of which followed the test guideline as adopted 1981, and one that followed the amended guideline from 1992. All three studies were conducted according to GLP standards and used cymoxanil of purities between 97.6% and 99.4%. In two of the studies using intradermal induction concentrations of 1% in 0.5% carboxymethylcellulose and 3% in 0.9% saline, cymoxanil was deemed non-sensitising, when 25% cymoxanil in paraffin oil or petrolatum, respectively, was employed for the topical challenge. The remaining study with an induction concentration of 1% in Alembicol-D yielded positive results in 90% (challenge with 20% cymoxanil) and 100% (challenge with 40% cymoxanil) of the tested animals.

Conclusion on classification

In the 2012 RAC opinion, cymoxanil was classified as Skin Sens. 1, H317 without sub-categorisation. RAC argued that all three GPMT studies were of similar reliability and no factors could have been identified that may have explained the different results.

Therefore, the data were not sufficient for sub-categorisation. In contrast to this, the DS argued that the topical induction concentration in the two negative studies was too low (since it didn't induce mild irritation as required by the test guideline), and the choice of vehicles was not sufficiently justified. Therefore, the DS deemed the third study most reliable and based their classification proposal on the positive result yielded in this study (> 60% animals reacting to an intradermal induction concentration of 1%). Thus, they concluded that **Skin Sens. 1A, H317** was warranted.

The DS further concluded that according to the CLP Guidance, Table 3.7, and the results of this last GPMT, cymoxanil is a strong sensitiser (more than 60% of animals reacting to a 1% intradermal induction concentration). Therefore, the GCL for the strong potency group (0.1%) applies.

Comments received during consultation

Two MSCAs commented during consultation. Both supported Skin Sens. 1A classification. One MSCA supported the DS in their assessment of the reliability of the studies but pointed out that no irritation was observed also in the third study using 40% cymoxanil in Alembicol-D for topical induction. The DS responded that technically the third study also failed to meet the requirements of OECD TG 406.

Assessment and comparison with the classification criteria

Three OECD TG 406 studies were summarised in the CLH report.

Concerning the reliability of results, RAC notes that there are several points to consider:

a) Two of the studies gave negative results with *none* of the ten treated animals reacting, and one gave positive results with *all* of the treated animals reacting. However, this was also the study using the lowest test substance purity.
b) The two studies with negative results used 25% in the respective vehicle as topical induction and challenge concentrations. The study with positive results used 40% cymoxanil for topical induction and 20% or 40% for challenge. Nine or ten of the 10 treated animals in each group reacted to both of the challenge concentrations, respectively, with generally stronger reactions with the higher concentration. Some of the animals in the high concentration group even showed necrotic patches at the treatment site.
c) None of the topical induction concentrations used in any of the studies induced irritation. However, all three of the studies did indeed follow the guideline in employing a topical treatment with 10% SLS prior to topical induction to create skin irritation as is required by point 19 of the TG (OECD TG 406) for non-irritating test substances.
d) Concerning the vehicle used, the guideline requires a "suitable vehicle" and a justification for its choice. In the study summaries available to RAC no justification for the choice of the respective vehicle was provided for any of the three studies. However, all three chosen vehicles (paraffin oil, petrolatum, and Alembicol-D [triglycerides from coconut oil]) are lipophilic mixtures of several organic compounds. While paraffin oil and petrolatum contain long-chain hydrocarbons, Alembicol-D consists of triglycerides. It seems unlikely that vehicle choice could have influenced the outcome of the studies.
e) Nevertheless, it should be noted that slight irritative effects were reported in control animals after topical induction with the vehicle Alembicol-D. No such findings were

reported for the other two vehicles but summaries of the study results were less detailed.

f) The two negative studies included concurrent positive controls, this was not the case for the third (positive) study (sensitivity of the test system was tested periodically using formalin as the test substance).

g) According to the CLP Regulation, substances should be placed in Cat. 1A for skin sensitisation when ≥ 60% of animals are responding at > 0.1% to ≤ 1% intradermal induction dose. While two studies (one negative, one positive) used 1% cymoxanil as intradermal induction concentration, one of the studies used 3% cymoxanil and still yielded negative results.

Overall, RAC considers all of the presented studies to be of similar reliability. Taking into account all of the above and based on the uncertainties surrounding the discrepancy in the results of the three studies, RAC does not consider any amendment to their previous assessment to be warranted. Thus, in contrast to the DS, RAC proposes to retain the existing classification of cymoxanil as **Skin Sens. 1, H317 without sub-categorisation**.

RAC concurs with the DS that no concern regarding a potential extreme sensitisation potency for cymoxanil could be identified and no SCL needs to be set.

These recommendations are in line with RAC's previous evaluation (RAC, 2012) of Cymoxanil.

2.6.2.8 Phototoxicity

Table 24: Summary table of studies on phototoxicity

Method, guideline, deviations if any	Test substance (Batch No/ purity)	Dose levels duration of exposure	Results	Reference
OECD 432 (2004) GLP With deviations: *light source emits wavelengths only in the range of UVA (supposedly315 to 400 nm) and others* Supplementary	Cymoxanil 211 99.3%	**Doses**: 0.05, 0.14, 0.41, 1.23, 3.7. 11.1, 33.3 and 100 µg/mL negative control (PBS), and positive control (Chlorpromazine) **Exposure:** BALB/3T3 mouse fibroblast cell were treated for 1 h with different concentrations of the test solution and further 50 min in absence and in presence of a nontoxic dose of UVA light (not specified)	No EC_{50} values could be calculated due to the complete absence of cytotoxicity at all dose levels PIF was assigned a value 1.0 and the MPE was not calculated No evidence of phototoxicity above 315 nm under the conditions of this study but test material absorbs mainly at wavelengths <325nm	RAR B.6.2.7.1., 2017
OECD 432 (2004) GLP With deviation: *light source emits wavelengths only in the range of 320 to 400 nm* Supplementary	Cymoxanil 2060129 98.5%	**Doses**: 1000; 500; 250; 125; 62.5; 31.25; 15.62 and 7.81 µg/mL negative control (HBSS), and positive control (Chlorpromazine) **Exposure:** BALB/3T3 mouse fibroblast cell were treated for 1 h with different concentrations of the test solution and further	62% death cells (with irradiation) at the highest dose. 57% death cells in absence of UVA light. PIF determined by the software « Phototox » version 2 is 1.302 and the MPE is 0.095. Cymoxanil technical can be assigned as "not phototoxic" above 320 nm but test material absorbs mainly at	RAR B.6.2.7.2., 2018

		50 min in absence and in presence of a nontoxic dose of UVA light (320 to 400 nm)	wavelengths <325nm	

No human data on phototoxicity are available. No other studies relevant for phototoxicity are available.

2.6.2.9 Aspiration hazard

No data are available on evidence for aspiration hazard.

2.6.2.9.1 Short summary and overall relevance of the provided information on aspiration hazard

'Aspiration' is defined as the entry of a liquid or solid substance or mixture directly through the oral or nasal cavity, or indirectly from vomiting, into the trachea and lower respiratory system (Section 3.10.1.2 of Annex I of the CLP Regulation). Aspiration toxicity includes severe acute effects such as chemical pneumonia, varying degrees of pulmonary injury or death following aspiration. Substances are classified as hazard Category 1 for aspiration toxicity if they meet the following criteria: substances known to cause human aspiration toxicity hazards or to be regarded as if they cause human aspiration toxicity hazard; a classification is based on reliable and good quality human evidence or if it is a hydrocarbon and has a kinematic viscosity of 20,5 mm^2 /s or less, measured at 40° C.

2.6.2.9.2 Comparison with the CLP criteria regarding aspiration hazard

In the absence of any relevant human data and whereas cymoxanil is not hydrocarbon, cymoxanil is not classified as a respiratory sensitiser.

2.6.2.9.3 Conclusion on classification and labelling for aspiration hazard

Whereas cymoxanil is not hydrocarbon and in the absence of any relevant human data, cymoxanil does not require classification for aspiration hazard according to Regulation (EC) No 1272/2008.

RAC evaluation of aspiration toxicity

Summary of the Dossier Submitter's proposal

According to CLP guidance "a classification is based on reliable and good quality human evidence or if the substance is a hydrocarbon and has a kinematic viscosity of 20.5 mm^2/s or less, measured at 40 °C". No animal or human data are available on a potential aspiration hazard for cymoxanil and the substance is not a hydrocarbon.

Therefore, the DS concluded that **no classification** was warranted for Aspiration Hazard.

Comments received during consultation

No comments were received.

Assessment and comparison with the classification criteria

Since no data on this endpoint are available and cymoxanil is not a hydrocarbon (thus, does not fulfil classification criteria), RAC concurs with the DS that **no classification** of cymoxanil for Aspiration Hazard is warranted.

2.6.2.10 Specific target organ toxicity-single exposure (STOT SE) [equivalent to section 10.11 of the CLH report template]

Table 25: Summary table of animal studies on STOT SE (specific target organ toxicity-single exposure)

Method, guideline, deviations[1] if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results** **- NOAEL/LOAEL** **- target tissue/organ** **- critical effects at the LOAEL**	**Reference**
Please refer to Sections 2.6.2			

No human data on STOT SE (specific target organ toxicity-single exposure) are available. No other studies relevant for STOT SE are available.

2.6.2.10.1 Short summary and overall relevance of the provided information on specific target organ toxicity – single exposure (STOT SE)

No new information regarding STOT–SE was received for renewal. No specific target organ toxicity was observed after a single dose/exposure concentration of cymoxanil.

2.6.2.10.2 Comparison with the CLP criteria regarding STOT SE (specific target organ toxicity-single exposure)

Classification in STOT SE Category 1 is required for substances that have produced significant toxicity in humans or that, on the basis of studies in experimental animals, can be presumed to have the potential to produce significant toxicity in humans following a single exposure. Substances are classified in Category 1 on the basis of reliable and good quality evidence from human cases, or observations from animal studies in which significant and/or severe effects of relevance to human health were produced at generally low exposure concentrations. Exposure levels relevant to classification in Category 1 are defined (Section 3.8.2.1.9.3 of Annex I of the CLP Regulation) as ≤300 mg/kg bw (oral route, rat); ≤1000 mg/kg bw (dermal route, rat) and ≤1 mg/L (inhalation route, rat, dust/mist/fume).

Classification in STOT SE Category 2 is required for substances showing significant toxic effects of relevance to humans, in studies in experimental animals and at generally moderate exposure levels. Exposure levels relevant to classification in Category 1 are defined (Section 3.8.2.1.9.3 of Annex I of the CLP Regulation) as 2 000 ≥ C > 300 mg/kg bw (oral route, rat); 2 000 ≥ C > 1 000mg/kg bw (dermal route, rat) and 5.0 ≥ C > 1.0 mg/L (inhalation route, rat, dust/mist/fume).

Classification in STOT-SE Category 3 is reserved for transient target organ effects and is limited to substances that have narcotic effects or cause respiratory tract irritation. These are effects which adversely alter human function for a short duration after exposure and from which humans may recover in a reasonable period without leaving significant alteration of structure or function.

In the absence of human data and in the absence of any effects (clinical signs or pathology) considered to constitute significant or severe effects in the acute oral, dermal or inhalation toxicity studies, classification of cymoxanil in Category 1 or Category 2 for STOT SE is not required.

With regard to Category 3 for STOT SE, signs following inhalation exposure to cymoxanil were indicative of non-specific, general toxicity. As there was no evidence of specific toxic effects on a target organ or tissue, no signs of respiratory tract irritation or narcotic effects, no classification for specific target organ toxicity (single exposure) is proposed.

2.6.2.10.3 Conclusion on classification and labelling for STOT SE (specific target organ toxicity-single exposure)

No effects observed in acute toxicity studies would trigger criteria for classification and labelling for STOT-SE according to Regulation (EC) No 1272/2008.

RAC evaluation of specific target organ toxicity – single exposure (STOT SE)

Summary of the Dossier Submitter's proposal

In the absence of human data and in the absence of any effects (clinical signs or pathology) considered to constitute significant or severe effects in the acute oral, dermal or inhalation toxicity studies, the DS stated that classification of cymoxanil in Category 1 or 2 for STOT SE is not required. With regard to Category 3, the DS argues that clinical signs following inhalation exposure to cymoxanil were indicative of non-specific, general toxicity.

As there was no evidence of toxic effects on a specific target organ or tissue, and since there were no signs of respiratory tract irritation or narcotic effects, no classification for specific target organ toxicity (single exposure) was proposed by the DS.

Comments received during consultation

No comments were received.

Assessment and comparison with the classification criteria

There are two studies on acute oral toxicity, which lead to a classification as Acute Tox. 4, H302, two studies on acute dermal toxicity and one study on acute inhalation toxicity (see Acute Toxicity). No human data on STOT SE are available. No new information regarding STOT SE was received for renewal.

As no specific target organ toxicity was observed in acute toxicity studies with cymoxanil, RAC agrees with the DS that no classification for STOT SE 1 or 2 is warranted.

However, some clinical signs were observed in three acute toxicity studies with oral and inhalation exposure, in one in vivo micronucleus test in mice (1993) within 1 hour after gavage dosing and in one bone marrow cytogenetic assay (1982) with a single oral gavage dose. These may be relevant for STOT SE 3 (narcotic effects) classification: lethargic behaviour, low, depressed or prostrate posture, incoordination, abnormal gait, irregular respiration, tremors, ataxia with signs of decreased respiratory rate, and isolated incidents of loss of righting reflex (oral exposure), and abnormal gait or mobility, low gait, and lethargy (exposure via inhalation). RAC notes that these signs occurred in all dose groups but that in the oral toxicity studies these doses (from 250 mg/kg bw in one study with mortality in 2/10 males, and from 300 mg/kg bw in the second study with mortality in 0/5 males and 2/5 females) were also associated with mortality. Nevertheless, only one male of the mid dose group (4.98 mg/L) died in the inhalation toxicity study (none in the low and high dose groups at 3.21 mg/L or 5.06 mg/L, respectively), and clinical signs were also observed in surviving animals of the two oral toxicity studies. In these animals, effects resolved by day 7 at the latest. In the micronucleus test, transient lethargy and/or abnormal gait was observed also in the mid dose group (225 mg/kg bw/d) along with ruffled fur but no other clinical signs. In this study, although 6/18 females died in the high dose group (450 mg/kg bw/d for males and 350 mg/kg bw/d for females), none of the males died but the males exhibited the same

clinical signs as females. In the cytogenetic assay, some animals of the low and mid doses (50 and 100 mg/kg bw/d, respectively) were described as slightly depressed but there was no mortality . This effect in itself is not relevant for classification but adds to the overall picture.

Overall, in light of the proposed ATE of 360 mg/kg bw for acute oral toxicity, and the overall non-specific clinical signs, RAC considers these effects do not warrant classification.

This recommendation is in line with RAC's previous evaluation (RAC, 2012) of Cymoxanil.

2.6.3 Summary of repeated dose toxicity (short-term and long-term toxicity) [section 10.12 of the CLH report]

2.6.3.1 Specific target organ toxicity-repeated exposure (STOT RE) [equivalent to section 10.12 of the CLH report template]

For more detailed data on STOT RE effects please refer to RAR Volume 3CA B-6, sections B.6.3, B.6.5, B.6.6 and B.6.8.

Table 26: Summary table of animal studies on repeated dose toxicity (short-term and long-term toxicity) STOT RE (specific target organ toxicity - repeated exposure)

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance (Batch No; purity w/w), route of exposure, dose levels, duration of exposure	Results - NOAEL/LOAEL (mg/kg bw/d) - target tissue/organ - critical effects at the LOAEL	Reference
28 days rat oral, dietary OECD 407 (1995) GLP Wistar rat (HsdCpb:WU) 6/sex/dose Supportive only A range finding study (no weight of prostate, uterus and thyroid; no histopathology of organs)	Cymoxanil 0972, 98.8 % 0, 750, 1500, 3000, 5000 ppm equal to 0, 74.4, 143.5, 260.0, 400.3 mg/kg bw/d (males) 0, 79.8, 154.3, 287.8, 415.9 mg/kg bw/d (females)	NOAEL = 74.4 (♂) LOAEL = 143.5 (♂) based on ↓ body weight gain at week 1 (17.4%); ↓ food consumption throughout weeks 1 and 2 (>10%), ↑ relative liver (12.7%) and kidneys (11.8%) weight NOAEL = 154.3 (♀)n LOAEL = 287.8 (♀) based on ↓ body weight at week 1 (12.1%); ↓ body weight gain at week 1 and 2 (>20%); ↓ food consumption throughout weeks 1 and 2 (>15%)	RAR B.6.3.1.1., 1999a

28 days mice oral, dietary OECD 407 (1995) GLP Swiss albino mice: HsdOla:MF 1 8/sex/dose Supportive only A range finding study (no weight of prostate, epididymis, uterus and thyroid; no histopathology of organs)	Cymoxanil 0972, 98.8 % 0, 750, 1500, 3000, 6000 ppm Equal* to 0, 172.7, 303.4, 624.4 mg/kg bw/d (males) 0, 179.1, 329.9, 679.3 mg/kg bw/d (females) ** For the 6000 ppm feeding group, the test substance intake could not be calculated because all males and 7 out of 8 females died or moribund sacrificed pre-terminally*	NOAEL = 172.7 (♂) LOAEL = 303.4 (♂) based on ↓ body weight gain (41.8%); ↓ food consumption throughout all dosing period (>10%), ↓absolute kidneys (18%) weight NOAEL = 329.9 (♀) LOAEL = 679.3 (♀) based on ↓ body weight (17.9%) ↓ body weight gain (95%); ↓ food consumption throughout all dosing period (>14%); ↓absolute ovaries (27.3%) and adrenals (25%) weight	RAR B.6.3.1.2., 1999a
90 days rat oral, dietary OECD 408 (1981) GLP Sprague-Dawley rat (Crl:CD®BR) 10/sex/dose Acceptable	Cymoxanil DPX-T3217-107, 96.8% 0, 100, 750, 1500, 3000 ppm equal to 0, 6.54, 47.6, 102, 224 mg/kg bw/d (males) 0, 8.00, 59.9, 137, 333 mg/kg bw/d (females)	NOAEL = 6.54 (♂) LOAEL = 47.6 (♂) based on histopathological findings in testes (bilateral elongate spermatid degeneration) and ↑ relative testes weight (10%) NOAEL = 137 (♀) LOAEL = 333 (♀) based on ↓ body weight gain (19.9%), ↓ overall food conversion efficiency (34.9%)	RAR B.6.3.2.1. Study 1, 1993
90 days rat oral, dietary OECD 408 (1981) GLP Wistar rat (HsdCpb:WU) 10/sex/dose Acceptable	Cymoxanil 0972, 98.8% 0, 500, 1000, 2000 ppm equal to 0, 42.6, 85.1, 174.3 mg/kg bw/d (males) 0, 48.1, 97.8, 187.7 mg/kg bw/d (females) *Additional recovery subgroups* (control and high dose), 28 days: equal to 0, 173.5 mg/kg bw/d (males); equal to 0, 185.7 mg/kg bw/d (females)	NOAEL = 85.1 (♂) LOAEL = 174.3 (♂) based on ↓ body weight (11.3%), ↓ body weight gain (14.6%), ↓ food consumption (15.6%), ↑ creatinine (34%), ↑ total bilirubin (85.8%), ↑ relative kidney weight (17.3%), ↑ relative liver weight (15.7%) NOAEL = 97.8 (♀) LOAEL = 187.7 (♀) based on ↓ body weight (>10%) during first two weeks, ↓ body weight gain (18-42%) during first three weeks, ↓ food consumption (10-22%) during seven weeks intermittently	RAR B.6.3.2.1. Study 2, 1999b
90 days mice oral, dietary OECD 408 (1981) GLP Swiss albino mice: Hsd0la:MF1 10/sex/dose Acceptable	Cymoxanil 0972, 98.8% 0, 150, 450, 1350 ppm equal to 0, 28.7, 84.4, 256.6 mg/kg bw/d (males) 0, 32.9, 97.3, 302.5 mg/kg bw/d (females) *Additional recovery subgroups* (control and high dose), 28 days: equal to 0, 266.7 mg/kg bw/d (males); equal to 0, 300.1 mg/kg bw/d (females)	NOAEL = 84.4 (♂) LOAEL = 256.6 (♂) based on ↓ body weight gain (21.4%), ↑ total bilirubin (114.8%), NOAEL = 97.3 (♀) LOAEL = 302.5 (♀) based on ↓ body weight gain (28.6-33.3%) during first five weeks, ↑ total protein (23.7%), ↑ relative liver weight (11.2%)	RAR B.6.3.2.2., 1999b

90 days dog oral, dietary OECD 409 (1981) GLP Beagle dog 4/sex/dose Acceptable	Cymoxanil DPX-T3217-113, 97.8% 0, 100, 200, 250/500 ppm equal to 0, 3.13, 5.13, 10.56 mg/kg bw/d (males) 0, 3, 5.27, 10.51 mg/kg bw/d (females)	NOAEL = 3.13 (♂) LOAEL = 5.13 (♂) based on clinical signs (↓ defecation), alterations of haematological parameters [↓ RBC (15.9%**), ↓ Hb (16.7%**), ↓ Ht (14.9%)] **at 3.13 mg/kg bw/d** (NOAEL): - no effects on clinical signs, body weight/body weight gain, food consumption, haematological parameters **at 5.13 mg/kg bw/d** (LOAEL): - no effects body weight/body weight gain, food consumption, - further findings see above **at 10.56 mg/kg bw/d:** - ↓body weight (35.1%*), loss overall body weight gain (g), ↓food consumption during eleven weeks (34.5 – 68.9%*), **- alterations of haematological parameters [↓ RBC (23.0%**), ↓ Hb (24.4%**), ↓ Ht (23.4%)],** - the MTD have been reached *The effects on sexual function and fertility (effects on testes and epididymis) are summarised in the section 2.6.6.1.* NOAEL = 3.0 (♀) LOAEL = 5.27 (♀) based on clinical signs (↓ defecation, ↑ diarrhoea), loss in overall body weight gain (g) at termination, ↓ food consumption (g/animal/day) during 11 weeks (31.8 – 46.2%**) **at 3 mg/kg bw/d** (NOAEL): - no effects on clinical signs, body weight/body weight gain, haematological parameters **at 5.27 mg/kg bw/d** (LOAEL): - ↓RBC (11.5%), ↓Hb (12.8%), ↓Ht (9.1%) - the MTD have been reached - further findings see above **at 10.51 mg/kg bw/d**: - ↓body weight from fourth week to termination (21.9 - 41.9%), loss of overall body weight gain (g), ↓food consumption throughout the all dosing period (13 weeks, 40.6-74.0%) **- ↓RBC (25.6%*), ↓Hb (22.2%*), ↑MCV (8.7 %*)**	RAR B.6.3.2.3. Study 1, 1993

90 days dog oral, dietary OECD 409 (1981) GLP Beagle dog 4/sex/dose Acceptable	Cymoxanil 498VF973, 98.8% 0, 200, 400, 800 ppm equal to 0, 4.9, 9.7 and 14.2 mg/kg bw/d (males) 0, 5.2, 9.9 and 15.5 mg/kg bw/d (females)	NOAEL = 4.9 (♂) LOAEL = 9.7 (♂) based on clinical signs ('weakness'), loss body weight gain*, ↓ food consumption (g/animal/day) during 5 weeks (23.2 – 46.7%*); **↓ absolute (>55%) and relative (>45%) thymus weight; histological alterations in thymus (2/4 versus 0 in controls) with increasing severity** **at 4.9 mg/kg bw/d** (NOAEL)**:** - no effects on clinical signs, body weight/body weight gain, food consumption, haematological parameters, thymus and eyes **at 9.7 mg/kg bw/d** (LOAEL): - no effects on haematological parameters and eyes - further findings see above **at 14.2 mg/kg bw/d**: - clinical signs ('weakness'),↓body weight (32.4%), loss body weight gain*, ↓food consumption up to 60.0%*, - no effects on haematological parameters and eyes, - ↓absolute (>52%) and relative (>30%) thymus weight; histological alterations in thymus (3/4 versus 0 in controls) - the MTD have been reached *The effects on sexual function and fertility (effects on testes and epididymis) are summarised in the section 2.6.6.1.* NOAEL = 5.2 (♀) LOAEL = 9.9 (♀) based on clinical signs ('weakness'), loss body weight gain*, alterations of haematological parameters [↓ RBC (13.2%*), ↓ Hb (10.1%)]; alterations of clinical chemistry [↑ GGT (89%*), ↑ Total Bil (17%)]; ↑ relative liver weight (28.6%)*; **↓ absolute (>56%*) and relative (>50%*) thymus weight; histological alterations in thymus (2/4 versus 0 in controls)** **at 5.2 mg/kg bw/d** (NOAEL)**:** - no effects on clinical signs, body weight/body weight gain, food consumption, thymus and eyes - ↓ RBC (9.4%*), ↑MCV (4.7%*), **at 9.9 mg/kg bw/d** (LOAEL): - ↓ body weight (11.2%), - no effects on eyes, - further findings see above - the MTD might have been reached **at 15.5 mg/kg bw/d**: - clinical signs ('weakness'), ↓ body weight (30.8%), loss body weight gain*, ↓ food consumption during five weeks (37.8 – 56.1%*) - alterations of haematological parameters [↓ RBC (14.7%*), ↓ Hb (13.9%*), ↓Ht (11.1%*), ↑MCV (3%)] - alterations of clinical chemistry [↑GGT (111%*), ↑ Total Bil (33%*)] - ↓ absolute (>66%*) and relative (>55%*) thymus weight; histological alterations in thymus **(**2/4 versus 0 in controls)	RAR B.6.3.2.3. Study 2, 1999

1 year dog oral, dietary OECD 452 (1981) GLP Beagle dog 5/sex/dose Acceptable	Cymoxanil DPX-T3217-113, 97.8% males: 0, 50, 100, 200 ppm females: 0, 25, 50, 100 ppm equal to 0, 1.8, 3.0, 5.7 mg/kg bw/d (males) 0, 0.7, 1.6, 3.1 mg/kg bw/d (females)	NOAEL = 3.0 (♂) LOAEL = 5.7 (♂) based on alterations of haematological parameters [↑MCV (4.2%**) at termination, ↓ RBC (18.3%*/10.2%*) at week 12/25, ↓ Hb (11.0%*/18.8%**/10.8%*) at week 2/12/25]; alterations of clinical chemistry [↓ potassium (13.7%**)]; **bilateral cataract (slight, grade 1) in one male versus 0 in controls** **at 1.8 mg/kg bw/d:** - no effects on haematological parameters and eyes **at 3.0 mg/kg bw/d** (NOAEL): - no effects on haematological parameters and eyes **at 5.7 mg/kg bw/d** (LOAEL): - body weight gain loss ** during the weeks 1 and 19 - ↓ mean food consumption (g/animal/day) (>33 %*) during the weeks 1 - further findings see above - the MTD have not been reached *The effects on sexual function and fertility (effects on testes and epididymis) are summarised in the section 2.6.6.1.* NOAEL ≥ 3.1 (♀) no substance related effect could be found **at 3.1 mg/kg bw/d** (♀): **- unilateral cataract (slight, grade 1) in one female versus 0 in controls;** - body weight gain loss ** during the weeks 1 - ↓ mean food consumption (g/animal/day) (>33 %*) during the weeks 1 - no effects on haematological parameters	RAR B.6.3.3.1., 1994

1 year dog oral, dietary OECD 452 (1981) GLP Beagle dog 4/sex/dose Acceptable	Cymoxanil 89800028, 98.8% (first 4 weeks) 19800042, 99.2% (for remainder of study) males: 0, 50, 100, 200 ppm females: 0, 25, 50, 100 ppm equal to 0, 1.3, 2.8, 5.6 mg/kg bw/d (males) 0, 0.8, 1.4, 2.9 mg/kg bw/d (females) (0/4; 2/4; 3/4 and 3/4, respectively)	NOAEL = 1.3 (♂) LOAEL = 2.8 (♂) based on histological changes in testes (minimal/slight bilateral atrophy) with an apparent trend in the incidence and severity **at 1.3 mg/kg bw/d** (NOAEL): - ↓ absolute thymus weight (23.2 %) - ↓ relative thymus weight (24.2%) - microscopic thymic lymphoid atrophy (2/4 versus 0 in controls) **at 2.8 mg/kg bw/d** (LOAEL): - ↓absolute thymus weight (38.5 %) - ↓ relative thymus weight (35.4%) - Microscopic thymic lymphoid atrophy (3/4 versus 0 in controls) **at 5.6 mg/kg bw/d:** **- ↓ absolute thymus weight (52.7 %*)** **- ↓ relative thymus weight (44.4%)** **- microscopic thymic lymphoid atrophy (3/4 versus 0 in controls)** **- lenticular degeneration in both eyes (1/4 versus 0 in controls)** - no toxicologically significant effects on haematological parameters - the MTD have not been reached *The effects on sexual function and fertility (effects on testes and epididymis) are summarised in the section 2.6.6.1.* NOAEL ≥ 2.9 (♀) no substance related effect could be found **at 0.8 mg/kg bw/d:** - ↓ absolute thymus weight (16.7 %) - ↓ relative thymus weight (6.7 %) **at 1.4 mg/kg bw/d:** - ↓ absolute thymus weight (23.5 %) - ↓ relative thymus weight (18.8 %) **at 2.9 mg/kg bw/d** (NOAEL): - ↓ absolute thymus weight (29.6 %) - ↓ relative thymus weight (23.8 %) - no toxicologically significant effects on haematological parameters - no effects on eyes - the MTD have not been reached *The study not suitable to establish a proper LOAEL for females*	RAR B.6.3.3.2., 2003
28 days dermal, rat OECD 410 (1981) GLP Sprague-Dawley rat (Crl:CD®BR) 5/sex/dose Acceptable	Cymoxanil T3217-113, 97.8% 0, 50, 500, 1000 mg/kg bw/d 6 h/d, (10% of the total body surface area, on males 44-51 cm^2; on females 36-40 cm^2), covered porous gauze dressing)	NOAEL ≥ 1000 (♂ & ♀) No treatment related adverse local and systemic effects in all dose groups tested	RAR B.6.3.4., 1996

Combined chronic toxicity /carcinogenicity study OECD 453 (1981) GLP Sprague-Dawley rats, Crl:CD Br 72/sex/dose Interim sacrifice: 10/sex/dose Acceptable	Cymoxanil DPX-T3217-113 (Blend of lots 80317145 and 80321154); 97.8% 0, 50, 100, 700, 2000 ppm equal to 0, 1.98, 4.08, 30.3, 90.1 mg/kg bw/day (males) 0, 2.71, 5.36, 38.4, 126 mg/kg bw/day (females) Chronic / Carcinogenicity (23 months, 702-710 days): 62/sex/dose Interim sacrifice (12 months, 357 days): 10/sex/dose 0, 50, 100, 700, 2000 ppm equal to 0, 2.25, 4.58, 32.8, 97.4 mg/kg bw/day (males) 0, 3.07, 6.09, 43.5, 134 mg/kg bw/day (females)	Long-term NOAEL = 4.08 (♂) Long-term LOAEL= 30.3 (♂), based on clinical findings (↑hyperreactivity), ↓ body weight (15.3%), ↓ body weight gain (21.8%), histopathological findings (elongate spermatid degeneration, **retinal atrophy)** Long-term NOAEL = 5.36 (♀) Long-term LOAEL = 38.4 (♀) based on histopathological findings (polyarteritis/ inflammation in lung/liver, **retinal atrophy, sciatic nerve degradation**) Carcinogenic NOAEL ≥ 90.1 (♂) Carcinogenic NOAEL ≥ 126 (♀) Cymoxanil did not reveal any oncogenic potential	RAR B.6.5.1.1., 1994a
Combined chronic toxicity /carcinogenicity study OECD 453 (1981) GLP Wistar rats, HsdCpb:WU strain, in-house bred 50/sex/dose Interim sacrifice (12 months): 10/sex/control; 20/sex/high dose Acceptable	Cymoxanil 0972, 98.8% and 498 VF973, 99.3% 0, 100, 500, 1200 ppm equal to 0, 4.7, 23.5, 58.8 mg/kg bw/day (males) 0, 6.4, 31.6, 75.8 mg/kg bw/day (females) Chronic / Carcinogenicity 24 months Interim sacrifice (12 months) Cymoxanil 0, 1200 ppm equal to 0, 67.6 mg/kg bw/day (males) 0, 85.5 mg/kg bw/day (females)	Long-term NOAEL = 4.7 (♂) Long-term LOAEL= 23.5 (♂), based on histopathological findings (lymphoid hyperplasia in rectum)* * - *Due to deviations of the study the NOAEL (♂) cannot be set properly with respect to findings on male reproductive organs.* Long-term NOAEL = 31.6 (♀) Long-term LOAEL = 75.8 (♀) based on histopathological findings (lymphoid hyperplasia in colon, suppurative bronchopneumonia in lungs) Carcinogenic NOAEL ≥ 58.8 (♂) Carcinogenic NOAEL ≥ 75.8 (♀) Cymoxanil did not reveal any oncogenic potential	RAR B.6.5.1.2., 2003
Carcinogenicity study OECD 451 (1981) GLP Mouse, Crl:CD-1®BR 90/sex/dose Acceptable.	Cymoxanil DPX-T3217-113, 97.8% 0, 30, 300, 1500, 3000 ppm equal to 0, 4.19, 42.0, 216, 446 mg/kg bw/day (males) 0, 5.83, 58.1, 298, 582 mg/kg bw/day (females) 18 months	Long-term NOAEL = 4.19 (♂) Long-term LOAEL= 42.0 (♂), based on histopathological findings in liver (apoptosis, pigment, granuloma, diffuse centrilobular hypertrophy) and epididymis (tubular dilatation, aggregate lymphoid and sperm cysts) Long-term NOAEL = 5.83 (♀) Long-term LOAEL = 58.1 (♀) based on histopathological findings in stomach (hyperplastic gastropathy) and duodenum (cystic enteropathy) Carcinogenic NOAEL ≥ 446 (♂) Carcinogenic NOAEL ≥ 582 (♀) Cymoxanil did not reveal any oncogenic potential	RAR B.6.5.2.1., 1994b

Carcinogenicity study OECD 451 (1981) GLP Mouse, HsdOla: MF1 50/sex/dose Acceptable.	Cymoxanil 498VF973, 98.8% 0, 60,120, 600, 1200 ppm 0, 9.5, 18.7, 91.4, 178.3 mg/kg bw/day (males) 0, 9.5, 18.6, 92.4, 179.8 mg/kg bw/day (females) 18 months	Long-term NOAEL = 91.4 (♂) Long-term LOAEL= 178.3 (♂), based on macroscopic and histopathological findings in mesenteric lymph nodes (dicolouration and haemorrhage) of males found dead and sacrificed moribund Long-term NOAEL = 92.4 (♀) Long-term LOAEL = 179.8 (♀) based on histopathological findings in ovaries (follicular cysts) in females terminal sacrificed and combined fates Carcinogenic NOAEL ≥ 178.3 (♂) Carcinogenic NOAEL ≥ 179.8 (♀) Cymoxanil did not reveal any oncogenic potential	RAR B.6.5.2.2., 2002
Two-generation reproduction toxicity study OECD 416 (1983) GLP Rat, Hsd Cpb: WU 30/sex/group Acceptable	Cymoxanil 0972 and 498VF973, 98.8% 0, 150, 450, 1350 ppm equal to 0, 10.5, 31.6, 94.0 mg/kg bw/day (F0 males) and 0, 14.9, 42.8, 116.3 mg/kg bw/day (F0 females premating, gestation and lactation) 0, 11.6, 35.1, 111.4 mg/kg bw/day (F1 males) and 0, 15.0, 45.1, 132.4 mg/kg bw/day (F1 females premating, gestation and lactation) Oral: diet Approximate number of dose weeks: mating and throughout the mating period: males F0/F1 (14/18 weeks), females F0/F1 (10/14 weeks) females F0/F1: gestation (21 days) and lactation (21 days)	**Parental** NOAEL: 31.6 (♂) – 42.8 (♀) mg/kg bw/day LOAEL: 94.0 (♂) – 116.3 (♀) mg/kg bw/ day based on ↓bw gain premating (F0 males: 17%, F1 males: 14%, F0 female: 10%), gestation (F1 female: 20%), lactation (F0 female: 78%) ↓FC premating (F1 males: 10%, F0/F1 female: 9%), gestation (F0/F1 female: 11%/8%), lactation (F0/F1 females: 33%/26%) **Reproductive** NOAEL: 31.6 (♂) – 42.8 (♀) mg/kg bw/day LOAEL: 94.0 (♂) – 116.3 (♀) mg/kg bw/day based on F1 generation: ↓ mean number of corpora lutea, ↓mean number of implantations, ↑post-implantation loss (%), ↓mean litter size, ↓ live pups born (%), **Offspring** NOAEL: 10.5 (♂) – 14.9 (♀) mg/kg bw/day LOAEL: 31.6 (♂) – 42.8 (♀) mg/kg bw/day based on ↓bw (both sexes combined F1 pups Days 14 and 21: >10%; F2 pups Days 7, 14 and 21: >8%)	RAR B.6.6.1.1., 2001

Two-generation reproduction toxicity study OECD 416 (1983) GLP Rat, Crl:CD®BR 30/sex/group Acceptable	Cymoxanil, DPX-T3217-113, 97.8% 0, 100, 500, 1500 ppm equal to 0, 6.5, 32.1, 97.9 mg/kg bw/day (F0 males) 0, 6.65, 34.7, 103.0 mg/kg bw/day (F0 females gestation) Oral: diet Approximate number of dose weeks: mating and throughout the mating period: males F0/F1 (16/32 weeks), females F0/F1 (10/15 weeks) females F0/F1: gestation (21 days) and lactation (21 days)	**Parental** NOAEL: 32.1 – 34.7 mg/kg bw/day LOAEL: 97.9 – 103 mg/kg bw/day based on clinical signs (end of tail missing, necrotic tip of tail, sore), ↓bw (>10%) premating (F0 males, F0 female), gestation (F0 female) ↓bw gain premating (F0 males: 18%, F1 males: 14%, F0 female: 23%, F1 female: 14%), gestation (F0 female: 12%) ↓FC (>10%) premating (F0/F1 males, F1 female), 1st gestation (F1 female) **Reproductive** NOAEL: > 97.9 – 103.0 mg/kg bw/day LOAEL: Not obtained Did not cause adverse effects at highest dose tested **Offspring** NOAEL: 6.5 – 6.65 mg/kg bw/day LOAEL: 32.1 – 34.7 mg/kg bw/day based on ↓viability index (%) of F1 pups (days 1-4) ↓bw (both sexes combined F2B pups, Days 4, 7, 14 and 21: 13-18%)	RAR B.6.6.1.2., 1993
One generation reproduction toxicity study OECD 415 (1983) GLP Rat, Hsd Cpb: WU 15/sex/group Supportive only A range finding study	Cymoxanil 0972, 98.8% 0, 750, 1500 and 3000 ppm equal to 0, 57.7, 114.3, 226.2 mg/kg bw/day (males premating) and 0, 75.1, 136.1, 240.8 mg/kg bw/day (females premating, gestation and lactation) 0, 68.4, 127.7, 235.2 mg/kg bw/day (combined) Oral: diet Approximate number of dose weeks: mating and throughout the mating period: males (15 weeks), females (10 weeks) females: gestation (21 days) and lactation (21 days)	**Parental** NOAEL: 68.4 (combined) mg/kg bw/day LOAEL: 127.7 (combined) mg/kg bw/day based on ↓bw gain female (gestation 12%) ↓FC female: all premating (9%), all gestation (≥10%), all lactation (≥20%) periods **Reproductive** NOAEL: 127.7 (combined) mg/kg bw/day LOAEL: 235.2 (combined) mg/kg bw/day based on ↓female fertility index (%), ↓ mean number of corpora lutea, (%), ↓mean number of implantations, ↑pre-implantation loss (%), ↑post-implantation loss (%), ↓mean litter size Gross necropsy: bilateral small & flaccid testes of 5 males **Offspring** LOAEL: 68.4 (combined) mg/kg bw/day based on ↓bw (both sexes combined pups Days 14 and 21: >10%)	RAR B.6.6.1.3., 1998

Developmental toxicity (teratogenicity) study OECD 414 (1981) GLP Rat, Crl:CD BR 25 females/dose group Acceptable	Cymoxanil DPX-T3217-113, 97.8% 0, 10, 25, 75, 150 mg/kg bw/day Days 7-16 of gestation, gavage	**Maternal:** NOEL: 10 mg/kg bw/day LOAEL: 25 mg/kg bw/day based on ↓ body weight gain (45%) over 7-9 days of gestation ↓ food consumption (12%) over 7-9 days of gestation **Developmental:** NOAEL: 10 mg/kg bw/day LOAEL: 25 mg/kg bw/day based on ↑incidence of skeletal variations and delayed ossification Foetal toxicity at higher dose levels: ↑ incidence of skeletal malformations (hemi vertebra at ≥75 mg/kg/day; exencephalic head and fused ribs at 150 mg/kg/day) ↑incidence of skeletal variations (partially ossified sternebra, unossified sternebra, wavy ribs and partially ossified pelvis at 150 mg/kg/day)	RAR B.6.6.2.1., 1993
Developmental toxicity (teratogenicity) study OECD 414 (1981) GLP Wistar rats, 27 females/dose group Acceptable	Cymoxanil 0972, 98.8 % 0, 30, 60, 120 mg/kg bw/day Days 6-15 of gestation, gavage	**Maternal:** NOEL: 60 mg/kg bw/day LOAEL: 120 mg/kg bw/day based on ↓ body weight gain (50%/20%) over 6-15/0-20 days of gestation ↓ food consumption (25%/13%) over 6-15/0-20 days of gestation *Information on corrected maternal body weight is not available* **Developmental:** NOAEL cannot be established LOAEL: 30 mg/kg bw/day based on ↑incidence of skeletal minor anomalies (dumb-bell shaped thoracic vertebra 6/13) Foetal toxicity at higher dose level: ↑incidence of skeletal variations: delayed ossification (servical vertebra: 7/7 and supraoccipital) and minor anomalies (dumb-bell shaped thoracic vertebra 6/13, hypoplasia of sternum: sternebra no. 1/2 and rudimentary 14th rib) at ≥60 mg/kg bw/day ↑incidence of skeletal variations: delayed ossification (sternum, vertebra, phalanges and supraoccipital) and minor anomalies (dumb-bell shaped thoracic vertebra 6/13, hypoplasia of sternum: sternebra no. 1/2, rudimentary 14th rib and vertebra) at ≥120 mg/kg bw/day	RAR B.6.6.2.2., 1998

Developmental toxicity (teratogenicity) study OECD 414 (1981) GLP New Zealand White rabbits 15 females/dose group Supportive only (too low number females with implantations and no maternal toxicity demonstrated)	Cymoxanil, 7800-20-C, 94.2% 0, 4, 8, 16 mg/kg bw/day Days 6-18 of gestation, gavage	**Maternal:** NOAEL: ≥ 16 mg/kg bw/day LOAEL cannot be established No effects even at the highest dose tested **Developmental:** NOAEL ≥ 16 mg/kg bw/day LOAEL cannot be established No effects even at the highest dose tested	RAR B.6.6.2.3., 1980
Developmental toxicity (teratogenicity) study OECD 414 (1981) GLP New Zealand White rabbits 15 females/dose group Acceptable	Cymoxanil, 7800-20-C, 94.2% 0, 8, 16, 32 mg/kg bw/day Days 6-18 of gestation, gavage	**Maternal:** NOEL: 8 mg/kg bw/day LOAEL: 16 mg/kg bw/day based on clinical observations (anorexia/ reduced faecal output) *Information on corrected maternal body weight is not available* **Developmental:** NOAEL: 16 mg/kg bw/day LOAEL: 32 mg/kg bw/day based on ↑incidences of skeletal malformations (vertebra and/or rib alterations linked with scoliosis)	RAR B.6.6.2.4., 1981
Developmental toxicity (teratogenicity) study OECD 414 (1981) GLP New Zealand White rabbits 17-20 females/dose group Acceptable	Cymoxanil, INT-3217-90, 95.8% 0, 1, 4, 8, 32 mg/kg bw/day Days 6-18 of gestation, gavage	**Maternal:** NOAEL: ≥ 32 mg/kg bw/day LOAEL cannot be established No effects even at the highest dose tested **Developmental:** NOAEL: 8 mg/kg bw/day LOAEL: 32 mg/kg bw/day based on ↑incidences of visceral malformations (cleft palate and hydrocephaly)	RAR B.6.6.2.5., 1982
Developmental toxicity (teratogenicity) study OECD 414 (1981) GLP New Zealand White rabbits 17 females/dose group Acceptable	Cymoxanil 0972, 98.8 % 0, 5, 15, 25 mg/kg bw/day Days 6-18 of gestation, gavage	**Maternal:** NOEL: 15 mg/kg bw/day LOAEL: 25 mg/kg bw/day based on ↓body weight gain (160%) over 6-18 days of gestation ↓food consumption (17%) over 6-19 days of gestation **Developmental:** NOAEL: 15 mg/kg bw/day LOAEL: 25 mg/kg bw/day based on ↑incidences of visceral malformations (dilation of heart ventricles), visceral variants (slight renal pelvis dilation), skeletal variants (incomplete/poor ossification of fore limb), skeletal minor anomalies (accessory floating rib no. 13)	RAR B.6.6.2.6., 1999

28-Day immunotoxicity feeding study Immunotoxicity US EPA OPPTS 870.7800 (1998) GLP Rat Crl:CD®(SD)IGS BR 10/sex/group Acceptable	Cymoxanil, DPX-T3217-113, 97.8% 0, 200, 400, 800, 1600 ppm Equal to 0, 13.56, 26.97, 53.86, 107.71 mg/kg bw/day (males) Equal to 0, 15.62, 31.32, 58.98, 117.43 mg/kg bw/day (females) Positive control: cyclophosamide monohydrate	Immunotoxicity NOAEL: ≥107.7 mg/kg bw/day Immunotoxicity LOAEL: Not obtained. No effects on immunotoxicity (thymus, spleen weight and the humoral immune response to SRBC) up to highest dose Systemic NOAEL: 31.3 mg/kg bw/day Systemic LOAEL: 58.98 mg/kg bw /day based on ↓body weight gain (♀25.5%), ↓mean food efficiency (♀20.8%)	RAR B.6.8.2.1., 1999a
28-Day immunotoxicity feeding study Immunotoxicity US EPA OPPTS 870.7800 (1998) GLP Mice Crl:CD-1®(ICR)BR 10/sex/group Acceptable	Cymoxanil, DPX-T3217-113, 97.8% Males: 0, 30, 300, 600 or 1200 ppm equal to 0, 5.15, 55.96, 108.33 and 218.39 mg/kg bw/day Females: 0, 30, 300, 1200 or 2400 ppm equal to 0, 7.15, 71.01, 268.51 and 552.44 mg/kg bw/day Positive control: cyclophosamide monohydrate	Immunotoxicity NOAEL: ≥218.4 mg/kg bw/day Immunotoxicity LOAEL: Not obtained. No effects on immunotoxicity (thymus, spleen weight and the humoral immune response to SRBC) up to highest dose Systemic NOAEL: 268.5 mg/kg bw/day Systemic LOAEL: 552.4 mg/kg bw /day based on ↓body weight gain (♀78.6%), ↓mean food efficiency on day 14-21 (♀33.3%)	RAR B.6.8.2.2., 1999b

*Studies which results were relevant for classification as STOT RE only are highlighted in grey. Significance of difference from control: * p≤0.05, ** p≤0.01; for statistical analyses details please see RAR Volume 3CA_B-6*

No human data on STOT RE (specific target organ toxicity-repeated exposure) are available.

Table 27: Summary table of other studies relevant for repeated dose toxicity STOT RE (specific target organ toxicity-repeated exposure)

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
(Q)SAR analysis using Derek Nexus (v.6.01/Nexus v.2.2.1 (Jan 2018)), OECD Toolbox (v.4.1) and VEGA (v.1.1.3).	Cymoxanil technical	The metabolite of cymoxanil was evaluated for mammalian toxicity by *in-silico* modelling tools using QSAR software	An alert for hepatoxicity was triggered in OECD Toolbox under the profiler "Toxicity-repeated doses (HESS)" for cymoxanil. Additionally, Derek Nexus triggered a 'doubted' HERG channel inhibition alert for mammals For more detailed data please refer to RAR Volume 3CA B-6, point 6.8.1.2 Study 2	RAR B.6.8.1.2. Study 2, 2019b

2.6.3.1.1 Short summary and overall relevance of the provided information on specific target organ toxicity – repeated exposure (short-term and long-term toxicity)

Specific target organ toxicity (repeated exposure) is defined in the CLP Regulation (Section 3.9.1.1 of Annex I) as specific, target organ toxicity arising from repeated exposure to a substance. All significant health effects that can impair function, reversible and irreversible, immediate and/or delayed are included in this definition. The adverse health effects relevant for STOT RE classification include consistent and identifiable toxic effects in humans, or, in experimental animals, toxicologically significant changes which have affected the function or morphology of a tissue/organ, or have produced serious changes to the biochemistry or haematology of the organism and these changes are relevant for human health. With respect to animal data, Annex 1, Section 3.9.2.5 of the CLP Regulation notes that the standard animal studies in rats or mice that provide this information are 28-

day, 90-day or lifetime studies (up to 2 years) that include haematological, clinicochemical and detailed macroscopic and microscopic examination to enable the toxic effects on target tissues/organs to be identified. Data from repeat dose studies performed in other species may also be used, if available and other long-term exposure studies such as carcinogenicity, neurotoxicity or reproductive toxicity may also provide evidence of specific target organ toxicity that could be used in the assessment of STOT RE classification.

Classification with STOT- RE is triggered by the occurrence of *significant* (and/or *severe* for Category 1) toxic effects at doses below specified guidance values. For STOT-RE Category 1, the relevant guidance values for oral exposure are 10 mg/kg bw/day (rat 90-day study) and 30 mg/kg bw/day (rat 28-day study). For STOT-RE Category 2, the relevant guidance values for oral exposure are 100 mg/kg bw/day (rat 90-day study) and 300 mg/kg bw/day (rat 28-day study).

The targets of orally administered cymoxanil were testes, epididymis, blood, thymus, eyes and some changes were seen on liver/kidney parameters.

Short-term oral toxicity

The short term toxicity of cymoxanil has been investigated after oral application in rats (28 and 90 days of exposure), mice (28 and 90 days of exposure) and dogs (90 days and 1 year of exposure). In addition, a 28 days dermal study in rats has been conducted. The results of short term toxicity studies are summarised in the Table above.

Testes and epididymis

According to RAC Opinion (*14 September 2012*): "As regards the adverse effects on testes and epididymis reported in the repeated dose toxicity studies, especially in rats, a classification for fertility is considered more appropriate than a classification for repeated dose toxicity/STOT-RE. This is in accordance with the CLP criteria for reproductive toxicity: *"Adverse effects on sexual function and fertility includes alterations to the female and male reproductive system, adverse effects on onset of puberty, gamete production and transport, reproductive cycle normality, sexual behavior, fertility, parturition, -pregnancy outcomes, premature reproductive senescence, or modifications in other functions that are dependent on the integrity of the reproductive system"*. This indicates that a classification for fertility is justified based on the adverse effects on testes and epididymis in the repeated dose toxicity studies in rats, mice and dogs."

Therefore, see further discussion under the section *Summary of reproductive toxicity (point 2.6.6.1.1 Short summary and overall relevance of the provided information on adverse effects on sexual function and fertility*).

Blood (haematology)

Investigations with respect to haematology in a ***90 days study in dogs*** *(RAR B.6.3.2.3. Study 1, 1993)* showed a statistically significant dose dependent reduction (>10%) number of red blood cells (15.9%** and 23.0%**, respectively), haemoglobin (16.7%** and 24.4%**, respectively) and haematocrit (14.9%* and 23.4%**, respectively) in males of mid and high dose groups (5.13 and 10.56 mg/kg bw/day). Males were more severely affected than females in this study and these findings in males at 5.13 mg/kg bw/day were not related to the presence of general toxicity. Number of red blood cells (25.6%*) and haemoglobin (22.2%*) were statistically significant reduced and mean corpuscular volume (MCV) was increased (8.7 %*) in females at high dose (10.51 mg/kg bw/day), meanwhile in mid dose (5.27 mg/kg bw/day) females number of red blood cells (11.5%), haemoglobin (12.8%) and haematocrit (9.1%) were non-statistically significant reduced. According to the *Guidance on the Application of the CLP Criteria* (2017) a reduction in haemoglobin at ≥ 20% is considered a significant adverse effect in haematology. Furthermore, the increase of MCV as the most significant haematological parameter for assessing anaemia in about 5% should be considered as toxicologically significant change in accordance with *WHO Guidance document (WHO, Guidance document for WHO monographers and reviewers; 2015).* One high dose female was euthanized *in extremis* during study week 10. At the time of necropsy, changes were present in haematology parameters including decreased red cell count, haemoglobin and haematocrit values. Macroscopic examination of this female showed dark red contents and reddened mucosa throughout the gastrointestinal tract. In male dogs at scheduled necropsy, no macroscopic or histopathologic changes were reported in the gastrointestinal tract. It is noteworthy that findings reported in male at 10.56 mg/kg bw/day were observed in the presence of general toxicity (clinical signs such as decreased defecation and diarrhoea; reduced body weight (31.5%), loss overall body weight gain (g), loss body weight gain (g) during first four weeks and reduced mean food consumption up to 68.9%). The MTD for males was stated at 10.56 mg/kg bw/day and at 5.27 mg/kg bw/day the MTD seems to be reached for females. The overall NOAEL is proposed at 3.0 mg/kg bw/day based on clinical observations, reduced body weight gain (females), reduced food consumption (females) and alteration of blood parameters (reduced number of red blood cells, haemoglobin and haematocrit more severely in males) at ≥5.13 mg/kg bw/day.

Investigations with respect to haematology in a ***second 90 days study in dogs*** *(RAR B.6.3.2.3. Study 2, 1999)* showed a statistically significant dose dependant reduction (>10%) number of red blood cells (13.2%* and

14.7%*, respectively) in females of mid and high dose groups (equal to 9.9 and 15.5 mg/kg bw/day); however, these values were within the HCD submitted (No of females 105; Mean 1-SD range-L: 5.88 – 7.18 x10^6/µL). On the other hand, it should be noted that the historical control data (HCD) for haematology parameters provided was of limited relevance with respect to the method of collection. Number of red blood cells (10.4%) was non-statistical significantly/dose dependently reduced in males at high dose and the value was within the HCD range submitted (No of males 98; Mean 1-SD range-L: 5.55 – 6.59 x10^6/µL). A dose-related reduction (>10%) in haemoglobin in female was reported (10.1% and 13.9%* at 9.9 and 15.5 mg/kg bw/day, respectively) that reached statistical significance at highest dose (15.5 mg/mg/kg bw/day) only. The latter Hb value (136 g/L) was lower of HCD range (No of females 105; mean 1-SD range-L: 137.54 – 160.66 g/L). The statistically significant increase of MCV in females of the low and mid dose group (4.7%* and 3%*, respectively) could not be shown for the highest dose group (3%). The first value (67.0 fL) was higher and the second value (66.3 fL) was just at the upper edge of HCD range and all three values were above the mean of HCD (No of females 105; mean: 63.64, Mean 1-SD range-L: 60.90 – 66.38 fL). Females were more severely affected than males with respect to haematology in this study. At 9.7 mg/kg bw/day and 9.9 mg/kg bw/day the MTD might have been reached for males and females, respectively. The overall NOAEL was proposed at 4.9 mg/kg bw/day based on clinical observations, loss body weight gain, reduced food consumption, alterations of haematological parameters (reduced RBC and Hb in females), alterations of clinical chemistry (GGT and Total Bil in females), histological findings in the thymus as well as organ weight changes (liver and thymus in females) at ≥ 9.7 mg/kg bw/day for males and 9.9 mg/kg bw/day for females.

Investigations with respect to haematology in the **first 1 year dog dietary study** (*RAR B.6.3.3.1., 1994)* showed a statistically significant, however, not always dose dependant reduction (>10%) number of red blood cells in males of high dose groups (5.7 mg/kg bw/day) at week 12 and 25 (18.3%* and 10.2%*, respectively). In addition, at highest dose a statistically significant, however, not always dose-related reduction (>10%) in haemoglobin in male was reported at week 2, 12 and 25 (11.0%*, 18.8%** and 10.8%*, respectively). Furthermore, at termination males of the high dosage group showed a statistically significant increase (4.2 %**) in MCV (mean corpuscular volume) with concomitant slight reduction (3 %**) of MCHC (mean corpuscular haemoglobin concentration) when compared with control. The increase of this most significant haematological parameter (MCV) for assessing anaemia in about 5% might indicate morphological abnormality of erythrocytes and should be considered as toxicologically significant change in accordance with *WHO Guidance document (WHO, Guidance document for WHO monographers and reviewers; 2015).* Neither toxicologically relevant nor statistically significant changes with respect to haematology (number of RBC, Hb, MCV and MCHC) were shown in female dogs at any of dose levels tested. The NOAEL was proposed at 3.0 mg/kg bw/day based on alterations of haematological parameters (reduced RBC, Hb and increased MCV), alterations of clinical chemistry (reduced potassium) and bilateral cataract in males at 5.7 mg/kg bw/day.
Males in the highest dose group (200 ppm) experienced a loss of mean body weight gain during the weeks 1 and 19 of the test administration, i.e. a mean body weight loss was of approximately 0.5 and 0.3 kilogram, respectively. Females in the highest dose group (100 ppm) experienced the same loss of mean body weight gain during the week 1 too. All these differences from the control group were statistically significant ($p<0.01$). This transient body weight gain effect coincided with commensurately reduced food consumption during the first week: reduced mean food consumption (g/animal/day) (>33 %*) was observed in both sexes at the highest dose. However, for the overall study period (weeks 0 to 52) mean body weight gain in the highest dose group of males was 35% higher than of the control group and of females was nearly identical to that in the control group. Hence, body weights in all treatment groups were shown to be of no statistical significant difference when compared to control; body weight gain as well as food consumption at the end of the study period was not affected by treatment with the test compound, too. The MTD has not been reached at the highest dose level for both sexes. Furthermore, with respect to females, no substance related effect could be found at the highest dose level 3.1 mg/kg bw/day and, therefore, this study was considered not suitable to establish a proper NOAEL/LOAEL for females. Regarding unilateral cataract in one female, please see point 'Eyes' and point 2.6.3.1.2.). For more detailed data please refer to RAR Volume 3, section B.6.3.3.1.
In addition, it is noteworthy that according to Regulation (EU) No. 283/2013 an assessment of the analytical methods and of their validation data should be provided for all toxicity studies with non-radiolabelled test active substances. Some issues could represent sources of uncertainties during the re-evaluation of old studies, e.g. the amount of test substance fed to animals and the extraction efficiency / appropriate extraction method (please see *Technical report on the outcome of the pesticides peer review meeting on general recurring issues in mammalian toxicology. EFSA supporting publication 2016:EN-1074. 24 pp.*). Analytical method for diet analysis submitted for this old study and results of the analytical determination of cymoxanil in diet of 100 ppm group of animals represent sources of uncertainties: the concentrations of cymoxanil in diet of this group were 56.1 – 65.4% of expected values for about one third of the study duration.

There was no evidence for toxicologically significant effects on haematological parameters in both sexes during the **second 1 year dog study** (*RAR B.6.3.3.2., 2003*), however, considering the top dose selection (5.6 mg/kg bw/day for males and 2.9 mg/kg bw/day for females), the amount of compound administered was not high

enough to elicit clear evidence of toxicity. The MTD have not been clear reached for both sexes at the highest dose. The NOAEL was proposed at 1.3 mg/kg bw/day based on histological findings in the testes (minimal/slight bilateral atrophy) at 2.8 mg/kg bw/day. This study was considered not suitable to establish a proper NOAEL/LOAEL for females. No haematological examinations were performed in the two **28 days dietary** range finding studies on **rats** (*RAR B.6.3.1.1., 1999a*) and **mice** (*RAR B.6.3.1.2., 1999a*)**.** Changes among the haematology parameters in rats and mice were not considered to be of toxicological significance or haematological investigations didn't show adverse effects in **90 days dietary studies** (*RAR B.6.3.2.1. Study 1, 1993; RAR B.6.3.2.1. Study 2, 1999b* and *RAR B.6.3.2.2., 1999b*).

Thymus atrophy

With respect to organ weights and histopathological correlation, in one ***90 day dog study*** *(RAR B.6.3.2.3. Study 2, 1999)* there was dose-dependent and statistically significant reduction in absolute (>56%) and relative (>50%) thymus weight at ≥9.9 mg/kg bw/day dose in females with histological evidence of lymphoid atrophy in the thymus. Females affected were 2/4 (minimal and moderate) at 9.9 mg/kg bw/day and 4/4 (2 minimal and 2 moderate) females at 15.5 mg/kg bw/day indicating a dose relationship. A dose dependent increase in lymphoid atrophy of male thymus with increasing severity was reported from 9.7 mg/kg bw/day [2/4 (mild and moderate)] to 14.2 mg/kg bw/day [3/4 (2 moderate and 1 severe)], however, reduction in absolute (>52%) and relative (>30%) thymus weight at ≥9.7 mg/kg bw/day dose in males was non-statistically significant as well as not clear dose-dependent. No atrophy was reported in control animals and low dose animals.

It is noteworthy that the thymus is primary lymphoid organ and is exquisitely sensitive to stress and toxic insult. A decrease in thymus weight and/or thymic atrophy cannot be used as stand-alone criterion of immunosuppression, whereas a dose-response relationship as well as change in other lymphoid tissues might be supportive (*Pearse G, Histopathology of the thymus, Toxicologic Pathology, 34:515-547; 2006; USEPA 2013, A Retrospective Analysis of the Immunotoxicity Study).* In Greaves *("Histopathology of preclinical toxicity studies, interpretation and relevance in drug safety evaluation, Fourth edition, Peter Greaves, 2012*) also it is noted that it can be difficult to distinguish between substance induced thymus weight loss and atrophy, and substances which produce similar changes as a result of generalised high-dose stress response. However, the dose-response relationship may indicate whether the effect is a non-specific stress related effect or is a substance-specific effect. A substance-specific effect is considered to appear in a dose-related manner with decreased thymus weigh and atrophy starting at non-toxic dose-levels. However, non-specific thymus atrophy as a stress response is usually limited to high doses where other toxic effects are reported such as significant weight loss or other severe toxic effects.

A dose-response relationship in thymus weight might be only partly supportive in this case: there was clear dose-dependent and statistically significant reduction in relative thymus weight from 5.2 to 15.5 mg/kg bw/day dose in females (42%*, 51.9%* and 55.6%*, respectively), whereas there was not significant loss of body weight gain as well as lymphoid atrophy in the thymus in females at 5.2 mg/kg bw/day. It is noteworthy that there were changes in other lymphoid tissues (e.g. of bene marrow and lymph nodes) of female at the highest dose (15.5 mg/kg bw/day), whereas these tissues were not examined at other doses. The following histopathological findings of lymphoid tissues were noted in one emaciated female: lymphoid atrophy in thymus (moderate), lymphoid atrophy in mesenteric lymph nodes (mild), atrophy in bone marrow (severe) and in sternum marrow (severe). No such histopathological findings of lymphoid tissues were noted in one emaciated male with lymphoid atrophy in the thymus (severe) at high dose. There were not such histopathological findings of lymphoid tissues in both control groups. At 9.7 mg/kg bw/day and 9.9 mg/kg bw/day the MTD might have been reached for males and females, respectively. The overall NOAEL was proposed at 4.9 mg/kg bw/day based on clinical observations, loss body weight gain, reduced food consumption, alterations of haematological parameters (reduced RBC and Hb in females), alterations of clinical chemistry (GGT and Total Bil in females), histological findings in the thymus as well as organ weight changes (liver and thymus in females) at ≥ 9.7 mg/kg bw/day for males and 9.9 mg/kg bw/day for females.

A statistically significant decrease of absolute thymus weight (52.7 %*) in males of the high dose (5.6 mg/kg bw/day) group was observed in a **1 year dog study** (*RAR B.6.3.3.2., 2003*). Dose-dependent decrease in mean absolute and relative thymus weights of all three male groups treated (1.3, 2.8 and 5.6 mg/kg bw/day) was observed (23.2-57.7% and 24.2-44.4%, respectively), however, the values did not attain statistical significance except the value mentioned above. Macroscopic examination exhibited a reduced in size of thymus in two males of the high dose group and in one male of middle dose (2.8 mg/kg bw/day) group. Microscopically, thymic lymphoid atrophy - involution was observed in three dose groups of treated males (1.3, 2.8 and 5.6 mg/kg bw/day) but not in the control group (0/4; 2/4; 3/4 and 3/4, respectively). The severity of thymus atrophy from minimal to severe was reported: in Group 2 (2, 3), Group 3 (2, 2, 3) and Group 4 (1, 2, 4), where grade 1 - minimal, 2 - slight, 3 - moderate and 4 - severe. These microscopic observations correlated with thymus weights findings in male groups. Females were less affected than males with respect to thymus weight and thymus histopathology. Dose-dependent decrease in absolute and relative thymus weights of all three groups (0.8, 1.4 and

2.9 mg/kg bw/day) of treated females was reported (16.7-29.6% and 6.7-23.8%, respectively) and none of these values attained statistical significance. Microscopically, thymic lymphoid atrophy - involution was observed in all groups of females including control (0, 0.8, 1.4, 2.9 mg/kg bw/day; 4/4; 2/4; 2/4; and 3/4, respectively) and these microscopic findings likely to be attributable to normal age-associated thymic involution. The severity of thymus atrophy from minimal to moderate was reported (Group 1 - 1, 2, 2, 3; Group 2 - 1, 2; Group 3 - 1, 2 and Group 4 - 1, 2, 2). Given that a dose-response relationship in absolute and relative thymus weights of males was observed, these thymus weights findings correlated with the microscopic observations (thymic lymphoid atrophy – involution) and the MTD have not been reached at the highest dose, these findings indicate that changes in male thymus at the high dose (5.6 mg/kg bw/day) group might be a substance-specific effect but not a result of generalised high-dose stress response. The NOAEL is proposed at 1.3 mg/kg bw/day based on histological findings in the testes (minimal/slight bilateral atrophy) at 2.8 mg/kg bw/day with an apparent trend in the incidence and severity.

The macroscopic examination and histological evaluation provided no effects in thymus in **90 days dog study** (*RAR B.6.3.2.3. Study 1, 1993*) and **1 year dog dietary study** (*RAR B.6.3.3.1., 1994),* whereas weight of thymus was not investigated in these studies.

Eyes

In the first ***1 year dog dietary study*** *(RAR B.6.3.3.1., 1994),* bilateral cataract (slight, grade 1) in one male (out of five) and unilateral cataract (slight, grade 1) in one female (out of five) at high dose level (5.7 mg/kg bw/day and 3.1 mg/kg bw/day, respectively) were identified during ophthalmological examination. Since such finding is uncommon in young dogs (< 2 years), a relationship to treatment cannot be excluded. No information whether retina was included in histopathological examination of eyes in this study. The MTD has not been reached at the highest dose level for both sexes. For more detailed data please refer to RAR Volume 3, section B.6.3.3.1.

In the second ***1 year dog study*** *(RAR B.6.3.3.2., 2003),* lenticular degeneration of a slight degree was recorded in both eyes of one (out of four) high dose (equal to 5.6 mg/kg bw/day) male dog. Since this finding may occur in untreated Beagle dogs at a very low incidence, a relationship to treatment cannot be excluded in this case. No information whether retina was included in histopathological examination of eyes in this study.

In the **28 days dietary study in mice** (RAR *B.6.3.1.2., 1999a*), bilateral cataract in single male at 624.4 mg/kg bw/day dose was recorded during gross necropsy. This study was performed as a range finding study and is considered as supportive only. The maximum tolerated dose (MTD) for males was stated at 303.4 mg/kg bw/day. The NOAEL was proposed at 172.7 mg/kg bw/day based on reduced body weight gain, reduction in food consumption throughout all dosing period and decrease in absolute kidneys weight in males at ≥ 303.4 mg/kg bw/day.

Liver and kidney

In the **second 90 days dietary rat study** (*RAR B.6.3.2.1. Study 2, 1999b*), statistically significant and dose related increase (>10%) in creatinine was observed in males at ≥ 1000 ppm (equal to 85.1 mg/kg bw/day). Statistically significant and non-dose-related increase (85.8-115.8%) in total bilirubin was observed in males at ≥1000 ppm (equal to 85.1 mg/kg bw/day). In the male dose groups a statistically significant increase in relative kidney weight was observed at doses of ≥1000 ppm. This increase was dose related and relative kidney weight was higher than 15% of the control at 2000 ppm. No histopathological effects were seen in male kidney at high dose, except slight higher amount of hyaline casts (control 1/10, high dose 3/10). A statistically significant increase (15.7%) in relative liver weight of males was observed at dose 2000 ppm. No histopathological changes were seen in male liver at high dose in this study. It should be noted that some significant clinical biochemistry parameters (good indicators of effects on liver or kidney) were not investigated, e.g. total cholesterol and urea in plasma/serum.

In females, bilirubin statistical significantly increased at 2000 ppm (equal to 187.7 mg/kg bw/day) during the treatment and recovery periods (89.3% and 17.7%, respectively), while creatinine appeared raised (12.5%) at the end of the recovery period only. A statistically significant increases (<10%) in relative liver and kidney weight of females considered to be of no toxicological importance. No histopathological changes were seen in liver and kidney of females at high dose in this study.

In the ***90 days dietary mice study*** *(RAR B.6.3.2.2., 1999b),* the histopathological findings were vacuolar changes of liver cells of males and females (2/10, 2/10, 4/10, 5/10 and 2/10, 2/10, 4/10, 5/10, respectively). Multifocal vacuolar changes (minimal to mild/moderate) of liver cells have been observed in all treated animals groups (28.7, 84.4, 256.6 mg/kg bw/day for males and 32.9, 97.3, 302.5 mg/kg bw/day for females) with highest incidences in the high dose groups. No statistical analysis has been performed with respect to histopathological changes. Additionally, the quite small size of the difference in numbers of affected animals compared to concurrent controls should be noted. The aetiology of this change was uncertain. Furthermore, such dose-dependent mild or slight histopathological or histological findings without statistical significance are difficult to

interpret in terms of whether they are treatment related and adverse or not. Slight increases in vacuoles of hepatocytes were considered as not toxicologically relevant changes according to WHO Guidance document (*WHO, Guidance document for WHO monographers and reviewers; 2015*). However, if any associated changes suggesting toxicity are detected at the same dose as minor findings, they might be considered to be adverse according to this guidance. It should be noted that there were some changes in relative liver weight and clinical chemistry at the highest dose tested. A statistically significant increase (11.2%) in relative liver weight and total protein increase (23.7%) in females at 302.5 mg/kg bw/day were observed. Additionally, a non-statistically significant increase (10.4%) in relative liver weight and total bilirubin statistically significant increases (114.8%) in males at 256.6 mg/kg bw/day were detected. However, these findings observed were at most transient and not permanent based on the results of recovery group: they appeared resolved after four weeks of recovery. It is noteworthy that the macroscopic examination provided no information on damage to liver, there was no effect on liver enzymes in this study, there was the quite small size of the difference in numbers of affected animals compared to concurrent controls and vacuolar changes of liver cells were not reproducible in other toxicity studies on cymoxanil. Therefore, vacuolar changes of liver cells were disregarded in this study and the overall NOAEL was proposed at 84.4 mg/kg bw/day based on decreased body weight gain (males and females), increased total bilirubin (males), increased total protein (females) and increased relative liver weight (females) at 256.6 mg/kg bw/day.

Short-term dermal toxicity

No systemic effects were observed in any group of rats administered up to 1000 mg/kg bw/day cymoxanil in ***28 days dermal toxicity study*** *(RAR B.6.3.4., 1996)*, therefore, the NOAEL was proposed at 1000 mg/kg bw/ day for males and females.

Long term toxicity / carcinogenicity

The long term toxicity and carcinogenicity has been investigated in rats and mice (two studies each): 2-year combined chronic toxicity/carcinogenicity study in rats (*RAR B.6.5.1.1., 1994a* and *RAR B.6.5.1.2., 2003*); carcinogenicity study in mice (*RAR B.6.5.2.1., 1994b* and *RAR B.6.5.2.2., 2002*).

Testes and epididymis

See further discussion under the section *Summary of reproductive toxicity (point 2.6.6.1.1 Short summary and overall relevance of the provided information on adverse effects on sexual function and fertility*).

Blood (haematology)

A ***two years study in rats*** *(RAR B.6.5.1.1., 1994a)* showed a statistically significant increase (approximately 5.8 %*) in MCV (mean corpuscular volume) at 3-month and 6-month time point in males of the high dosage group (at 0, 3.0, 6.07, 42.6 and 123 mg/kg bw/day on test day 0-91). The increase of this most significant haematological parameter (MCV) for assessing anaemia in about 5% might indicate morphological abnormality of erythrocytes and should be considered as toxicologically significant change in accordance with *WHO Guidance document (WHO, Guidance document for WHO monographers and reviewers; 2015*). These alterations were not accompanied by alterations in other relevant haematological parameters. The overall NOAEL for long-term effects was set at 4.08 mg/kg bw/day, based on clinical signs (hyperreactivity), reduced body weight and body weight gain as well as the histological findings (polyarteritis/ inflammation in the lung/liver, elongate spermatid degeneration, retinal atrophy, sciatic nerve degradation) at 30.3 mg/kg bw/day. At 30.3 mg/kg bw/day the MTD was reached for males after 23 months exposure of cymoxanil.

There were no toxicologically significant changes in haematology in the second ***two years study in rats*** *(RAR B.6.5.1.2., 2003).* The overall NOAEL for long-term effects was set at 4.7 mg/kg bw/day, based on histopathological findings (lymphoid hyperplasia in rectum) in males at 23.5 mg/kg bw/day. At 58.8 mg/kg bw/day the MTD was reached for males.

The ***first carcinogenicity study in mice*** *(RAR B.6.5.2.1., 1994b)*, showed a statistically significant increase (6.4 % and 10.6%, respectively) in MCV in males of the two highest dose groups (216 and 446 mg/kg bw/day) after 18-months of treatment. The similar statistically significant increase (6.1 %) in MCV was seen in males of the high dose groups after 3-months of treatment. The increase of this most significant haematological parameter for assessing anaemia in about 5% might indicate morphological abnormality of erythrocytes and should be considered as toxicologically significant change. These alterations in high dose group of males after 18-months of treatment were accompanied also by statistically significant decrease in RBC (22.4%). There were no toxicologically significant changes in haematology parameters of females. The overall NOAEL for long-term effects was set at 4.19 mg/kg bw/day, based on histopathological findings in liver (apoptosis, pigment, granuloma, diffuse centrilobular hypertrophy) and epididymis (tubular dilatation, aggregate lymphoid and sperm cysts) in males at 42.0 mg/kg bw/day.

Eyes

Effects on eyes were reported in a ***two years study in rats*** *(RAR B.6.5.1.1., 1994a)*. Histological evaluation showed statistically significant retinal (photoreceptor cell) atrophy incidence in males from 30.3 mg/kg bw/day (10/45, 18/46, 19/46, 35/46* and 52/54* at 0, 1.98, 4.08, 30.3 and 90.1 mg/kg bw/day) and in females from 38.4 mg/kg bw/day (33/55, 34/54, 28/48, 47/52* and 54/55* at 0, 2.71, 5.36, 38.4 and 126.0 mg/kg bw/day). The incidence and severity of retinal atrophy in both males and females behaved in a dose response manner. The atrophy occurred either bilaterally or unilaterally and ranged in severity from single discrete foci to complete loss of photoreceptor and outer nuclear layers. This lesion is typical of age-related photoreceptor cell atrophy described in albino rats and was observed in all groups of rats at termination. However, it should be recognised that retinal atrophy over the 23-month study was exacerbated either directly or indirectly by the test compound. It is noteworthy that the 1-year interim sacrifice revealed statistically significant increases in the incidence of retinal atrophy in males from the 32.8 mg/kg bw/day (1/9, 1/10, 1/10, 4/10* and 9/10* at 0, 2.25, 4.58, 32.8, 97.4 mg/kg bw/day) and in females from 134 mg/kg bw/day (3/10, 3/10, 5/10, 3/10 and 9/10* at 0, 3.07, 6.09, 43.5, 134 mg/kg bw/day). This lesion was atypical in 1-year control rats and therefore, a treatment related effect of cymoxanil to male retina at two high doses the first year of treatment (32.8 and 97.4 mg/kg bw/day) could not be excluded. Regarding the dose level spacing selected, even seven-fold interval was used, instead of recommended two to four fold intervals for setting the descending dose levels. It should be noted that no information was available whether retina was included in histopathological examination of eyes in two 1-year dog studies.

The ocular examination of eyes after 24 months with cymoxanil (animals terminally sacrificed including animals found dead and sacrificed moribund) in the second ***two years study in rats*** *(RAR B.6.5.1.2., 2003)* revealed increased of retinal atrophy incidence up to 10% in both sexes at high dose (58.8 mg/kg bw/day for males and 75.8 mg/kg bw/day for females), however, this was likely toxicologically non-relevant change.

Ophthalmoscopic examinations at the end of the ***first carcinogenicity study in mice*** *(RAR B.6.5.2.1., 1994b)* revealed no substance related effects up to and including the highest dose level tested (3000 ppm equal to 446 mg/kg bw/day for males and 582 mg/kg bw/day for females). Histological evaluation of eyes at study termination showed that there were no toxicologically relevant differences between eyes lesions incidences (e.g. retinal atrophy, cataract) in control and treated groups of both sexes.

Liver

At ***two years study in rats*** *(RAR B.6.5.1.1., 1994a)* termination, substance related and adverse histopathological changes (inflammation and/or polyarteritis) were reported in the liver of female rats at 38.4 and 126 mg/kg bw/day. At 126 mg/kg bw/day the MTD was reached for females, based on statistically significant reductions in body weight (15.5%) and body weight gain (g) (25.8%) at termination. The female NOAEL for long-term effects was set at 5.36 mg/kg bw/day, based on the histological findings (polyarteritis/ inflammation in the lung/liver, retinal atrophy, sciatic nerve degradation) at 38.4 mg/kg bw/day.

With respect to organ weight changes, in the ***first carcinogenicity study in mice*** *(RAR B.6.5.2.1., 1994b)* absolute and relative liver weight showed a statistically significant increase at the two high dose (298 and 582 mg/kg bw/day) groups (10.3/18.1% and 13.0/26.8%, respectively) of females and this was accompanied by statistically significant treatment-related histological findings in liver (centrilobular apoptotic hepatocytes, macrophages containing a pigment, granuloma, diffuse centrilobular hypertrophy). Such significant treatment-related histological liver findings in males of three dose groups (42.0, 216 and 446 mg/kg bw/day) were not accompanied by liver weight changes. Cymoxanil did not induce alterations in hepatic cellular proliferation as well as in the rate of hepatic peroxisomal β -oxidation or the content of hepatic cytochrome P-450 after approximately one month of feeding (5 mice/group). The overall NOAEL for long-term effects was set at 4.19 mg/kg bw/day, based on histopathological findings in liver (apoptosis, pigment, granuloma, diffuse centrilobular hypertrophy) and epididymis (tubular dilatation, aggregate lymphoid and sperm cysts) in males at 42.0 mg/kg bw/day.

Neuropathy

Effects were also reported on the sciatic nerve in a ***two years study in rats*** *(RAR B.6.5.1.1., 1994a)* as an increase of axon/myelin degeneration of the sciatic nerve without clinical signs in females at 38.4 mg/kg bw/day, indicative of peripheral neuropathy. The MTD was reached at 126 mg/kg bw/day for females.

Reproductive toxicity

With respect to reproductive toxicity, two multigeneration studies in rats have been submitted. An additional reduced-size one-generation study in rats has been submitted for the purpose of renewal. Developmental toxicity of cymoxanil was investigated in two developmental toxicity studies in rats and four developmental toxicity studies in rabbits.

In one two generation study *(RAR B.6.6.1.2., 1993)* no adverse effects on fertility parameters were reported, whereas in other two generation study *(RAR B.6.6.1.1., 2001)* minor effects on fertility parameters were reported in the F1 generation. The effects on reproduction reported in F1 may not have been related to slight maternal

toxicity. The NOAEL for parental toxicity was set at 450 ppm (equal to 31.6 mg/kg bw/day for males and 42.8 mg/kg bw/day for females) based on reduced bodyweight gain in the F0 and F1 parental animals; reduced food consumption in the F1 males; reduced food consumption in the F0 and F1 females at the high dose level. Organs (testes, seminal vesicles with coagulating gland, epididymis, prostate, uterus (with cervix), and ovaries) were not weighed but were subject to gross pathology and microscopic examinations; there were no unusual gross necropsy or microscopic findings in males and females of either parental generation. In other two generation study *(RAR B.6.6.1.2., 1993),* the NOAEL for parental toxicity is proposed at 500 ppm (equal to 32.1 mg/kg bw/day in males and 34.7 mg/kg bw/day in females) based on reduced bodyweight gain in the F0/F1 parental animals; reduced bodyweight in the F0 parental animals and reduced food consumption in the F0/F1 males and F1females at the high dose level. Mean relative testes weight of F0 parental males was statistically significantly greater than controls at 500 ppm and 1500 ppm (10% and 19%, respectively), whereas the absolute weight of the testes was 12% lower than controls in F1 males at 1500 ppm only. See further discussion under the section *Summary of reproductive toxicity (point 2.6.6.1.1 Short summary and overall relevance of the provided information on adverse effects on sexual function and fertility*).

In the one generation study *(RAR B.6.6.1.3., 1998)* minor effects on fertility parameters were reported. The effects reported can be related to maternal toxicity (reduction in body weight gain, food consumption of females). Additionally, at necropsy 5 parent males in the 3000 ppm (equal to 226.2 mg/kg bw/day) group had bilateral small and flaccid testes, rendering them infertile. See further discussion under the section *Summary of reproductive toxicity (point 2.6.6.1.1 Short summary and overall relevance of the provided information on adverse effects on sexual function and fertility*).

Variations and malformations above the historical control values were demonstrated in two developmental toxicity studies in rats (variations from 30 mg/kg bw/day and malformations from 75 mg/kg bw/day) and in three out of four developmental toxicity studies in rabbits (variations and malformations from 25 mg/kg bw/day). These effects were considered not to be related to marked maternal toxicity. There were no test substance-related effects with respect to mortality in all dose groups of these studies.

Neurotoxicity

The neurotoxic potential of cymoxanil has been investigated in a combined neurotoxicity study with the 90-day dietary study (complies with OECD TG 424, 1997) and a developmental neurotoxicity (gavage) study (complies with OECD 426); both were conducted in rats. Overall, there is no evidence that cymoxanil has the potential to affect the nervous system or the developing nervous system in two well-conducted, guideline-compliant rat studies.

In a **90-day neurotoxicity study** (*RAR B.6.7.1.1., 1993*), after 90-days dietary exposure in rats, there was no evidence of a neurotoxic effect in the FOB or motor activity assessments and there were no lesions noted in any examined tissues that would indicate a specific effect on the nervous system. Histology did not show any statistical significant difference between control and the animals of the highest dose group tested. In the absence of specific evidence of neurotoxicity, the NOAEL for subchronic neurotoxicity was ≥3000 ppm for both sexes (equal to 224 mg/kg bw /day for males and 333 mg/kg bw/day for females), the highest tested dose. Based on decreased body weight and/or body weight gain of this sub-study, the NOAEL for systemic toxicity could be set at 1500 ppm (equal to 102 mg/kg bw /day for males and 137 mg/kg bw/day for females). However, it is noteworthy that based on reduced relative testes weight and correlated histopathological findings in testes at 750 ppm (equal to 47.6 mg/kg bw) and above, the overall NOAEL was set at 100 ppm (equal to 6.54 mg/kg bw/day/day) in 90 days rat oral sub-study.

In the **developmental neurotoxicity study** (*RAR B.6.7.1.2., 2001*), there was no evidence of neurotoxicity up to the highest-dose tested in a battery of functional tests; furthermore, examination of the nervous system tissues revealed no unusual findings. Maternal toxicity was characterised by reductions in body weight gain and food consumption at gestation days 6 – 9 at 50 mg/kg bw/day. In pups of the highest dose (100 mg/kg bw/day) group, following treatment-related statistically significant changes were observed: statistically significantly increased number of pups (%) found dead or presumed cannibalized, reduced viability index, reduced lactation indexes, lower number of surviving pups/litter and live litter size. Regarding clinical signs, in the pre-weaning period in top dose (100 mg/kg bw/day) group there were four pups that were cold to the touch, two that were not nursing, two not nesting, one dehydrated and one emaciated pup. A NOAEL for maternal toxicity of 5 mg/kg bw/d, a NOAEL for offspring toxicity of 50 mg/kg bw/d is therefore proposed and a NOAEL for developmental neurotoxicity was ≥100 mg/kg bw/day.

Immunotoxicity

Two studies investigating immunotoxic potential of cymoxanil have been provided. In none of these two studies immunotoxic effects were evident up to high doses (107.7 mg/kg bw/day in rats and 218.4 mg/kg bw/day in mice). The NOAEL for immunotoxicity was be established at > 1600 ppm (equal to 107.7 mg/kg bw/day in male rats) as no effects on immunotoxicity (thymus, spleen weight and the humoral immune response to SRBC) were

observed in rats (*RAR B.6.8.2.1., 1999a*). The overall NOAEL for immunotoxicity was established at > 1200 ppm (equal to 218.39 mg/kg bw/day for males) as no effects on immunotoxicity (thymus, spleen weight and the humoral immune response to SRBC) were observed in mice (*RAR B.6.8.2.2., 1999b*).

Table 28: Extrapolation of equivalent effective dose for toxicity studies of greater or lesser duration than 90 days

Study reference	Effective dose (mg/kg/day) and Effect	Length of exposure	Extrapolated effective dose when extrapolated to 90-day exposure	Classification supported by the study
RAR B.6.3.2.1. Study 2, 1999b	174.3	90 days	174.3	No
RAR B.6.3.2.2., 1999b	256.6	90 days	256.6	No
RAR B.6.3.2.3. Study 1, 1993	10.51 Blood	90 days	10.51	Yes
RAR B.6.3.2.3. Study 2, 1999	9.7 Thymus	90 days	9.7	Yes
RAR B.6.3.3.1., 1994	5.7 Eyes	1 year	22.8	Yes
RAR B.6.3.3.2., 2003	5.6 Thymus/Eyes	1 year	22.4	Yes
RAR B.6.5.1.1., 1994a	32.8 Eye	1 year	131.2	No
	30.3 Eyes	2 year	307.2	No

2.6.3.1.2 Comparison with the CLP criteria regarding STOT RE (specific target organ toxicity-repeated exposure)

Substances are classified in STOT RE Category 1 based on evidence of significant toxicity in humans or where there is evidence from studies in experimental animals that they can be presumed to have the potential to produce significant toxicity in humans following repeated exposure. For classification in Category 1, either reliable good quality human data (evidence from human cases or epidemiological studies) or animal data (observations from appropriate studies in experimental animals in which significant and/or severe toxic effects, of relevance to human health, were observed at generally low exposure concentrations) is required. Annex I, Section 3.9.2.9.6 of the CLP Regulation provides a 'guidance value' of ≤10 mg/kg bw/day from a 90-day rat study to assist in Category 1 classification. For a 28 day study the guidance value of ≤30 mg/kg bw/day to assist in Category 1 classification.

Substances are classified in STOT RE Category 2 based on evidence from studies in experimental animals that they can be presumed to have the potential to be harmful to human health following repeated exposure. For classification in Category 2, animal data (observations from appropriate studies in experimental animals in which significant toxic effects, of relevance to human health, were observed at generally moderate exposure concentrations) is required. Annex I, Section 3.9.2.9.7 of the CLP Regulation provides a 'guidance value' of 10-100 mg/kg bw/day from a 90-day rat study to assist in Category 2 classification. For a 28 day study the guidance value of ≤300 mg/kg bw/day to assist in Category 2 classification.

The targets of orally administered cymoxanil were testes, epididymis, blood, thymus and eyes.

Based on RAC Opinion (*14 September 2012*) a classification for fertility is justified based on the adverse effects on testes and epididymis in the repeated dose toxicity studies in rats, mice and dogs. Therefore, see further discussion under the section *Summary of reproductive toxicity (point 2.6.6.1.1 Short summary and overall relevance of the provided information on adverse effects on sexual function and fertility*).

According to the *Guidance on the Application of the CLP Criteria* (2017) a reduction in the haemoglobin ≥ 20% is considered an adverse effect on haematology and should be considered in the classification for STOT-RE.

Statistically significant reductions in haemoglobin in males (24.4%**) at 10.56 mg/kg bw/day and females (22.2%*) at 10.51 mg/kg bw/day were reported in a *90 days study in dogs (RAR B.6.3.2.3. Study 1, 1993)*. A similar but weaker effect was repeated in a second 90 days study in dogs *(RAR B.6.3.2.3. Study 2, 1999)*: a dose-related and statistically significant reduction (13.9%*) in haemoglobin in female was reported at 15.5 mg/kg bw/day. In addition, at highest dose (5.7 mg/kg bw/day) a statistically significant reduction in haemoglobin in male was reported at week 2, 12 and 25 (11.0%*, 18.8%** and 10.8%*, respectively) in 1 year dog dietary study (*RAR B.6.3.3.1., 1994).*

A dose-dependent increase in male and female thymus atrophy from 9.7 mg/kg bw/day was reported in a 90 day study in dogs (*RAR B.6.3.2.3. Study 2, 1999*). Male thymus atrophy was repeated at 5.6 mg/kg bw/day in a 1 year dog study (*RAR B.6.3.3.2., 2003*). Some findings indicate that changes in thymus might be a substance-specific effect but not a result of generalised high-dose stress response.

Similar effects on eyes were observed in two 1 year dog dietary studies (*RAR B.6.3.3.1., 1994*and *RAR B.6.3.3.2., 2003*): slight bilateral cataract in one male out of five at 5.7 mg/kg bw/day dose and bilateral lenticular degeneration of a slight degree was recorded in a single male out of four at 5.6 mg/kg bw/day dose, respectively. In addition, slight unilateral cataract in one female (out of five) was recorded at 3.1 mg/kg bw/day dose level in one 1 year dog study (*RAR B.6.3.3.1., 1994*). Given small amount of dogs, young age of animals and similar dose level in two studies, it is considered that three incidents of eye damage were not incidental and might represent an effect of substance treatment. Additionally, at from 30.3 mg/kg bw/day and above, both males and females showed statistically significant retinal atrophy in a two years study in rats *(RAR B.6.5.1.1., 1994a)*. It is noteworthy that the 1-year interim sacrifice revealed statistically significant increases in the incidence of retinal atrophy in males from the 32.8 mg/kg bw/day by using seven-fold interval in the dose level spacing. Therefore, the retina degeneration incidence is at the edge of classification.

The effects on haematology and the thymus atrophy reported in dogs were on the border between the guidance values for a classification in STOT-RE 1 and RE 2. These effects were not reported in all short-term dog studies available for evaluation and/or in others repeated exposure studies on other species. Therefore, it is considered that the effects reported are in accordance with a classification of cymoxanil in STOT-RE 2.

The effects on eyes (bilateral cataract/bilateral lenticular degeneration) reported in two 1 year dog studies are in accordance with a classification of cymoxanil in STOT-RE 2 too. These results on eyes are supported by the combined chronic toxicity /carcinogenicity (including the 1-year interim sacrifice) study on rats.

2.6.3.1.3 Conclusion on classification and labelling for STOT RE (specific target organ toxicity-repeated exposure)

Effects reported on blood parameters, the thymus and eyes in dogs following 90 days and 1 year exposure to cymoxanil are relevant for a classification for STOT RE 2 H373 (blood, thymus, eyes).

RAC evaluation of specific target organ toxicity – repeated exposure (STOT RE)

Summary of the Dossier Submitter's proposal

The DS highlighted five studies as relevant for classification (two 90 day studies in dogs, two 1-year studies in dogs, one combined chronic toxicity/ carcinogenicity study in rats). The target tissues of orally administered cymoxanil were testes, epididymis, blood, thymus and eyes.

Adverse effects on the testes and epididymis justify classification of cymoxanil as toxic to male fertility (RAC Opinion, 14 September 2012) based on the findings in the repeated dose toxicity studies in rats, mice and dogs. Therefore, further discussion on these effects can be found in the section on reproductive toxicity.

According to the CLP Guidance (2017) a reduction in haemoglobin ≥ 20% is considered as an adverse effect on haematology and should be considered in the classification for STOT RE. Statistically significant reductions in haemoglobin in males (24.4%) at 10.56 mg/kg bw/d and females (22.2%) at 10.51 mg/kg bw/d were reported in a 90-day study in dogs

(Anonymous, 1993). A similar but weaker effect was repeated in a second 90-day study in dogs (Anonymous, 1999): a dose-related and statistically significant reduction (13.9%) in haemoglobin in females was reported at 15.5 mg/kg bw/d. In addition, at the highest dose (5.7 mg/kg bw/d) a statistically significant reduction in haemoglobin in males was reported at week 2, 12 and 25 (11.0%, 18.8% and 10.8%, respectively) in a 1-year dog dietary study (Anonymous, 1994).

A dose-dependent increase of thymus atrophy from 9.7 mg/kg bw/d was reported in a 90-day study in male and female dogs (Anonymous, 1999). Male thymus atrophy was repeated at 5.6 mg/kg bw/d in a 1-year dog study (Anonymous, 2003). Some findings indicate that changes in thymus might be a substance specific effect rather than a result of generalised high dose stress response.

Similar effects on eyes were observed in two 1-year dog dietary studies (Anonymous, 1994 and Anonymous, 2003): slight bilateral cataract in one male out of five at a dose of 5.7 mg/kg bw/d and slight bilateral lenticular degeneration were recorded in a single male (out of four) at 5.6 mg/kg bw/d, respectively. In addition, slight unilateral cataract in one female (out of five) was recorded at 3.1 mg/kg bw/d in one 1-year dog study (Anonymous, 1994). Given the small number of dogs, the young age of animals, and similar dose levels in two studies, it is considered that three incidents of eye damage were not incidental and might represent a treatment-related effect. Additionally, from 30.3 mg/kg bw/d and above, both males and females showed statistically significant retinal atrophy in a two-year study in rats (Anonymous, 1994a). It is noteworthy that the 1-year interim sacrifice revealed statistically significant increases in the incidence of retinal atrophy in males from the 32.8 mg/kg bw/d dose group.

The effects on haematology and the thymus atrophy reported in dogs were at doses at the boundary between the guidance values for a classification in STOT RE Cat. 1 and Cat. 2. These effects were not reported in all short-term dog studies available for evaluation and/or in other repeated exposure studies in other species. Therefore, it is considered that a classification of cymoxanil for these effects as STOT RE 2 is more appropriate.

The effects on eyes (bilateral cataract/bilateral lenticular degeneration) reported in two 1-year dog studies are also relevant to a classification of cymoxanil in STOT RE 2. These results on eyes are supported by the combined chronic toxicity/carcinogenicity (including at the 1-year interim sacrifice) study on rats.

Conclusion on classification

The DS stated that the effects reported on blood parameters, thymus and eyes in the dogs following 90-day and 1-year exposure to cymoxanil are relevant for classification for **STOT RE 2, H373 (blood, thymus, eyes)**.

Comments received during consultation

One MSCA commented on this endpoint. They considered classification as STOT RE 1, H372 (blood, thymus) appropriate according to the effects observed in 90-day studies in dog below the cut off values (10 mg/kg bw/d).

- In the first 90-day dog study (1993) at 5.13 mg/kg bw/d in males, effects on haematology were observed: mild anaemia with a decrease of red blood cells (RBC, 15.9%), haemoglobin concentration (Hb, 16.7%) and haematocrit (Ht, 14.9%)
- In the second 90-day dog study (1999) thymus toxicity in males was observed at 9.7

mg/kg bw/d: ↓ absolute (> 55%) and relative (> 45%) thymus weight and histological alterations (lymphoid atrophy) with increasing severity

- In addition, in the second 90-day dog study (1999) in females, effects on haematology as well as liver and thymus toxicity were observed at 9.9 mg/kg bw/d:
    - Haematology effects: mild anaemia with a decrease of RBC (13.2%) and haemoglobin concentration (10.1%)
    - Liver toxicity: ↑ relative liver weight (28.6%) with alterations of clinical chemistry [↑ gamma-glutamyl transferase (GGT, 89%), ↑ total bilirubin (17%)]
    - Thymus toxicity: ↓ absolute (> 56%) and relative (> 50%) thymus weight and histological alterations (lymphoid atrophy)

The DS argued that the effects on haematology and thymus reported in dogs were at doses at the boundary between the guidance values for a classification in STOT RE 1 and 2. These effects were not reported in all short-term dog studies available for evaluation and/or in others repeated exposure studies on other species. Therefore, it is considered that the effects reported are in accordance with a classification of cymoxanil in STOT RE 2, H373.

Assessment and comparison with the classification criteria

To evaluate the specific target organ toxicity of cymoxanil after repeated exposure, 26 studies were assessed for relevance for classification. In the table below, the relevant studies for classification and their results are listed. Findings from other studies are not sufficient for classification and are regarded as supportive data.

***Table**: Summary of animal studies on repeated dose toxicity relevant for classification STOT RE (modified from the CLH report table 26). * p < 0.05, ** p < 0.01, effects relevant for classification are in **bold** text*

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance (Batch No; purity w/w), route of exposure, dose levels, duration of exposure**	**Results relevant for STOT RE classification** **Cat. 1: > 10 mg/kg bw/d** **Cat. 2: between 10 and 100 mg/kg bw/d** (based on the 90d oral toxicity study in rats)	**Reference**
90 days dog oral, dietary OECD TG 409 GLP Beagle dog 4/sex/dose	Cymoxanil DPX-T3217-113, 97.8% 0, 100, 200, 250/500 ppm equal to 0, 3.13, 5.13, 10.56 mg/kg bw/d (males) 0, 3, 5.27, 10.51 mg/kg bw/d (females)	<u>Effects in males</u> **At 5.13 mg/kg bw/d:** - alterations of haematological parameters [↓ RBC (15.9%**), ↓ Hb (16.7%**), ↓ Ht (14.9%)] **At 10.56 mg/kg bw/d:** - ↓ body weight (35.1%*), loss overall body weight gain (g), ↓ food consumption during eleven weeks (34.5 – 68.9%*) - **↓ RBC (23.0%**), ↓ Hb (24.4%**), ↓ Ht (23.4%)** <u>Effects in females</u>	Anonymous, 1993

		At 5.27 mg/kg bw/d: -↓ RBC (11.5%), ↓ Hb (12.8%), ↓ Ht (9.1%) -loss in overall body weight gain (g) at termination, ↓ food consumption (g/animal/day) during 11 weeks (31.8 – 46.2%**) **At 10.51 mg/kg bw/d:** -↓ body weight from fourth week to termination (21.9 - 41.9%), loss of overall body weight gain (g), ↓ food consumption throughout all the dosing period (13 weeks, 40.6 - 74.0%) -↓ **RBC (25.6%*),** ↓ **Hb (22.2%*),** ↑ MCV (8.7%*)	
90 days dog oral, dietary OECD TG 409 GLP Beagle dog 4/sex/dose	Cymoxanil 498VF973, 98.8% 0, 200, 400, 800 ppm equal to 0, 4.9, 9.7 and 14.2 mg/kg bw/d (males) 0, 5.2, 9.9 and 15.5 mg/kg bw/d (females)	<u>Effects in males</u> **At 9.7 mg/kg bw/d:** -↓ **absolute (> 55%) and relative (> 45%) thymus weight; histological alterations in thymus (2/4 versus 0 in controls) with increasing severity** **At 14.2 mg/kg bw/d:** -clinical signs ('weakness'), ↓ body weight (32.4%), loss body weight gain*, ↓ food consumption up to 60.0%* -↓ **absolute (> 52%) and relative (> 30%) thymus weight; histological alterations in thymus (3/4 versus 0 in controls)** <u>Effects in females</u> **At 5.2 mg/kg bw/d:** -↓ RBC (9.4%*), ↑ MCV (4.7%*) **At 9.9 mg/kg bw/d:** -↓ body weight (11.2%) -↓ RBC (13.2%*), ↓ Hb (10.1%) -↓ **absolute (> 56%*) and relative (> 50%*) thymus weight; histological alterations in thymus (2/4 versus 0 in controls)** **At 15.5 mg/kg bw/d:** -clinical signs ('weakness'), ↓ body weight (30.8%), loss body weight gain*, ↓ food consumption during five weeks (37.8 – 56.1%*) -↓ RBC (14.7%*), ↓ Hb (13.9%*), ↓ Ht (11.1%*), ↑ MCV (3%) -↑ **GGT (111%*),** ↑ **total bilirubin (33%*)** -↓ **absolute (> 66%*) and relative (>**	Anonymous, 1999

		55%*) thymus weight; histological alterations in thymus (2/4 versus 0 in controls)	
1 year dog oral, dietary OECD TG 452 (1981) GLP Beagle dog 5/sex/dose	Cymoxanil DPX-T3217-113, 97.8% males: 0, 50, 100, 200 ppm females: 0, 25, 50, 100 ppm equal to 0, 1.8, 3.0, 5.7 mg/kg bw/d (males) 0, 0.7, 1.6, 3.1 mg/kg bw/d (females)	Effects in males **At 5.7 mg/kg bw/d** (LOAEL): -body weight gain loss ** during the weeks 1 and 19 -↓ mean food consumption (g/animal/day) (> 33%*) during the weeks 1 -↑ MCV (4.2%**) at termination, ↓ RBC (18.3%*/10.2%*) at week 12/25, ↓ Hb (11.0%*/18.8%**/10.8%*) at week 2/12/25 -**bilateral cataract (slight, grade 1) in one male versus 0 in controls** Effects in females **At 3.1 mg/kg bw/d:** -**unilateral cataract (slight, grade 1) in one female versus 0 in controls** -stat. sign. body weight loss during week 1 -↓ mean food consumption (g/animal/day) (> 33%*) during the week 1	Anonymous, 1994
1 year dog oral, dietary OECD TG 452 (1981) GLP Beagle dog 4/sex/dose	Cymoxanil 89800028, 98.8% (first 4 weeks) 19800042, 99.2% (for remainder of study) males: 0, 50, 100, 200 ppm females: 0, 25, 50, 100 ppm equal to 0, 1.3, 2.8, 5.6 mg/kg bw/d (males) 0, 0.8, 1.4, 2.9 mg/kg bw/d (females)	Effects in males **At 1.3 mg/kg bw/d:** -↓ absolute thymus weight (23.2%) -↓ relative thymus weight (24.2%) -microscopic thymic lymphoid atrophy (2/4 versus 0 in controls) **At 2.8 mg/kg bw/d:** -↓ absolute thymus weight (38.5%) -↓ relative thymus weight (35.4%) -Microscopic thymic lymphoid atrophy (3/4 versus 0 in controls) **At 5.6 mg/kg bw/d:** -↓ **absolute thymus weight (52.7%*)** -↓ **relative thymus weight (44.4%)** -**microscopic thymic lymphoid atrophy (3/4 versus 0 in controls)** -**lenticular degeneration in both eyes (1/4 versus 0 in controls)** Effects in females **At 0.8 mg/kg bw/d:** -↓ absolute thymus weight (16.7%) -↓ relative thymus weight (6.7%) **At 1.4 mg/kg bw/d:** -↓ absolute thymus weight (23.5%)	Anonymous, 2003

		-↓ relative thymus weight (18.8%) **At 2.9 mg/kg bw/d (NOAEL):** -↓ absolute thymus weight (29.6%) -↓ relative thymus weight (23.8%)	

MCV: mean corpuscular volume

According to the existing RAC opinion from 2012, based on the adverse effects on testes and epididymis, reported in repeated dose toxicity studies, a classification for fertility is more appropriate that a classification for STOT RE. This is in line with the CLP criteria for reproductive toxicity, where it is indicated that a classification for fertility is justified based on the adverse effects on testes and epididymis in repeated dose toxicity studies. For further information and discussion on these effects, please refer to the section on reproductive toxicity.

Blood (haematology)

One 90-day study in dogs (Anonymous, 1993) showed a significant dose dependent reduction (> 10%) in the number of red blood cells (15.9% and 23.0%), haemoglobin (16.7% and 24.4%) and haematocrit (14.9% and 23.4%) in mid and high dose males (5.13 and 10.56 mg/kg bw/d). The findings in males at 5.13 mg/kg bw/d were not related to the presence of general toxicity. Findings in males at 10.56 mg/kg bw/d were observed in the presence of general toxicity. The number of red blood cells (25.6%) and haemoglobin (22.2%) were statistically significantly reduced in females of the high dose group (10.51 mg/kg bw/d). In the same dose group, the mean corpuscular volume (MCV) was increased (8.7%). In females of the mid dose group the number of red blood cells (11.5%), haemoglobin (12.8%) and haematocrit (9.1%) were not statistically significantly reduced. During week 10 of the study one high dose female was euthanized in extremis, and at necropsy changes in blood parameters were present. Macroscopic examination revealed dark red contents and reddened mucosa throughout the gastrointestinal tract. In males at scheduled necropsy, no such changes were reported.

In the **second 90-day study in dogs** (Anonymous, 1999) a statistically significant dose dependent reduction (> 10%) in the number of red blood cells (13.2% and 14.7%) was observed in females of the mid and high dose groups (9.9 and 15.5 mg/kg bw/d). These values were within the range of the historical control data (HCD) submitted (No of females 105; mean 1-SD range: 5.88 – 7.18 x 10^6/µL) but it should be noted that the HCD for haematology parameters provided was of limited relevance due to the method of collection. In males of the high dose group the number of red blood cells (10.4%) was not statistically significantly or dose dependently reduced and the value was within the HCD submitted (No of males 98; mean 1-SD range-L: 5.55 – 6.59 x 10^6/µL). A dose related reduction in haemoglobin (> 10%) was reported for females of the mid and high dose groups (10.1% and 13.9% at 9.9 and 15.5 mg/kg bw/d, respectively), which reached statistical significance at the high dose. The latter Hb value (136 g/L) was lower than the HCD range (No of females 105; mean 1-SD range-L: 137.54 – 160.66 g/L). The MCV was statistically significantly increased in females of the low and mid dose groups (4.7% and 3%, respectively) but not in the high dose group (3%). Regarding haematology, females were more severely affected than males in this study.

In a **one-year study in dogs** (Anonymous, 1994) a statistically significant reduction (> 10%) in the number of red blood cells in males of the high dose group (5.7 mg/kg bw/d) was recorded at week 12 and 25 (18.3% and 10.2%, respectively). However, these

finding were not always dose dependent. Additionally, in the high dose group a statistically significantly reduction (> 10%) in haemoglobin was reported in males at week 2, 12 and 25 (11.0%, 18.8% and 10.8%, respectively). This effect was also not dose dependent. At termination MCV was statistically significantly increased (4.2%) in males of the high dose group. This reduction was concomitant with slight reduction (3%) of mean corpuscular haemoglobin concentration when compared to control. No relevant changes in haematology parameters were reported for female dogs in any of the dose groups.

It should be noted that the results of the analytical determination of cymoxanil in the diet of 100 ppm group of animals represent sources of uncertainties, as the concentrations of the substance were 56.1 – 65.4% of expected values for about one third of the study duration.

The **two-year study in rats** (Anonymous, 1994a) revealed a statistically significant increase (5.8%) in MCV at the 3-month and 6-month time points in males of the high dose group (123 mg/kg bw/d). These alterations were not accompanied by changes in other relevant haematological parameters.

In one **carcinogenicity study in mice** (Anonymous, 1994b) a statistically significant increase (6.4% and 10.6%, respectively) in MCV was seen in males of the two highest dose groups (216 and 446 mg/kg bw/d) after 18 months of treatment. A similar statistically significant increase in MCV (6.1%) in males was seen after 3 months of treatment. This increase was accompanied by a statistically significantly decreased number of red blood cells (22.4%). No significant changes in haematology were found in females.

Thymus atrophy

In one **90-day dog study** (Anonymous, 1999) a dose-dependent statistically significantly reduction in absolute (> 56%) and relative (> 50%) thymus weight at ≥ 9.9 mg/kg bw/d dose was observed in females. Additionally, there was histological evidence of lymphoid atrophy in the thymus. In the 9.9 mg/kg bw/d dose group two, out of four females were affected (minimal and moderate), whereas in the 15.5 mg/kg bw/d dose group 4 out of four females showed these effects (two minimal, two moderate). These findings indicate a dose-response relationship. A dose dependent increase in lymphoid atrophy of male thymus with increasing severity was reported for the 9.7 mg/kg bw/d dose group (2/4, minimal and moderate) and for the 14.2 mg/kg bw/d (3/4, 2 moderate and 1 severe). A reduction in absolute and relative thymus weights in males was non-statistically significant and not clearly dose-dependent. No effects on the thymus were reported in the control and low dose groups.

It has to be noted, that the thymus is sensitive to stress and toxic insult. A decrease in thymus weight and/or thymus atrophy cannot be used as a stand-alone parameter for classification. However, a dose-response relationship and changes in other lymphoid tissues might be supportive evidence. It can be difficult to distinguish between substance related effects and changes which are a result of general stress. Substance-related effects are considered to appear in a dose-dependent manner with decreased thymus weight and atrophy starting at non-toxic dose levels. Thymus atrophy as a response to stress is usually limited to high doses.

In this case, there was a clear dose-dependent and statistically significant reduction in relative thymus weight in females (42%, 51.9% and 55.6% for 5.2, 9.9 and 15.5 mg/kg

bw/d). However, there was no significant loss of body weight gain as well as lymphoid atrophy in the thymus in females at 5.2 mg/kg bw/d. Changes were found in other lymphoid tissues in females of the high dose group (e.g. bone marrow, lymph nodes) but these tissues were not examined in females of other dose groups. In one emaciated female the following histopathological findings of lymphoid tissues were noted: lymphoid atrophy in the thymus (moderate), lymphoid atrophy in the mesenteric lymph nodes (mild), atrophy in the bone marrow (severe) and in the sternum marrow (severe). There was no such finding in one emaciated male with lymphoid atrophy in the thymus (severe) at high dose. Neither of the control groups also revealed such histopathological findings in the lymphoid tissues.

In a **one-year dog study** (Anonymous, 2003) a statistically significant decrease in absolute thymus weight was observed in high dose males (5.6 mg/kg bw/d). Additionally, a dose-dependent but not statistically significant decrease in mean absolute and relative thymus weights in males of all three treated groups were recorded (23.2-57.7% and 24.2-44.4% for 1.3, 2.8 and 5.6 mg/kg bw/d). In the macroscopic examination, the thymus size was found to be reduced in two males of the high dose group and in one male of the mid dose group. Microscopic examination revealed thymic lymphoid atrophy – involution in males of all dose groups (1.3, 2.8 and 5.6 mg/kg bw/d) but not in the control group (0/4; 2/4; 3/4; 3/4, respectively). In females the absolute and relative thymus weights of all three treated groups (0.8, 1.4 and 2.9 mg/kg bw/d) were found to be dose-dependently decreased (16.7, 23.5, 29.6% and 6.7, 18.8, 23.8%, respectively). These findings did not reach statistical significance. A microscopic examination revealed that thymic lymphoid atrophy – involution was found in all treated groups of females and also in females of the control group (4/4, 2/4, 2/4 and 3/4 for 0, 0.8, 1.4 and 2.9 mg/kg bw/d, respectively). These microscopic findings are considered to be normal age-associated thymic involution.

The dose-response relationship in absolute and relative thymus weights of males and the microscopic observations indicate that the effects seen in high dose males are not due to stress but might be a substance specific effect.

In a **90-day dog study** (Anonymous, 1993) and a **one-year dog study** (Anonymous, 1994) the thymus weights were not investigated. Macroscopic and histologic examination did not show any effects in thymus.

Eyes

In a **one-year dog study** (Anonymous, 1994), bilateral cataract in one male out of five (slight, grade 1) and unilateral cataract in one female out of five (slight, grade 1) were recorded at high dose level (5.7 mg/kg bw/d and 3.1 mg/kg bw/d, respectively). These findings might be treatment-related, since they are uncommon in young dogs.

Another **one-year dog study** (Anonymous, 2003) showed lenticular degeneration of a slight degree in both eyes of one out of four high dose males (5.6 mg/kg bw/d). This finding might be treatment-related, since this effect occurred in untreated beagle dogs at a very low incidence. No information was included on whether the retina was included in histopathological examinations of the eyes.

In a **two-year rat study** (Anonymous, 1994a) the histological evaluation revealed retinal atrophy incidence in males (10/45, 18/46, 19/46, 35/46 and 52/54 at 0, 1.98, 4.08, 30.3 and 90.1 mg/kg bw/d, respectively) and females (33/55, 34/54, 28/48, 47/52 and 54/55 at 0, 2.71, 5.36, 38.4 and 126.0 mg/kg bw/d, respectively). The incidence and severity of these findings behaved in a dose dependent manner and reached statistical significance

at the two highest dose levels (males: 30.3 and 90.1 mg/kg bw/d; females: 38.4 and 126.0 mg/kg bw/d). Either bilateral or unilateral atrophy ranged in severity from single discrete foci to complete loss of photoceptor and outer nuclear layers. As this lesion is typical of age-related photoceptor cell atrophy described for albino rats, it was observed in all groups at termination. However, it should be noted, that retinal atrophy was exacerbated by the test compound. At the one-year interim sacrifice significant increase in the incidences of retinal atrophy were observed in males of the two highest dose groups (1/9, 1/10, 1/10, 4/10 and 9/10 at 0, 2.25, 4.58, 32.8 and 97.4 mg/kg bw/d, respectively) and in females of the highest dose group (3/10, 3/10, 5/10, 3/10 and 9/10 at 0, 3.07, 6.09, 43.5, 134 mg/kg bw/d, respectively). As this lesion is unusual in one year control rats, a treatment related effect cannot be excluded.

One **28-day mice study** (RAR B.6.3.1.2., 1999a) recorded a bilateral cataract in a single male at 624.4 mg/kg bw/d during gross necropsy. As this study was performed as range finding study it is considered supportive only.

Conclusion on classification

No human data on the specific target organ toxicity after repeated exposure are available.

Several animal studies did not record any toxicologically relevant findings for effects of cymoxanil on the liver or kidney. There is also no evidence for neurotoxicity and immunotoxicity.

Target organs for repeated exposure with cymoxanil were testes, epididymis, blood, thymus and eye. In line with the DS and the RAC opinion from 2012 a classification for fertility is justified based on adverse effects on testes and epididymis in the repeated dose toxicity studies in rats, mice and dogs. For further discussion please refer to the reproductive toxicity section.

***Table**: Extrapolation of equivalent effective dose for relevant toxicity studies (modified from CLH report)*

Study reference	Effective dose (mg/kg bw/d) and affected organ	Duration of exposure	Extrapolated effective dose when extrapolated to 90-day exposure	Guidance values for oral repeated exposure studies (mg/kg bw/d)
90-day dog study (Anonymous, 1993)	10.51 Blood	90 days	10.51	STOT RE 1: C ≤ 10 STOT RE 2: 10 < C ≤ 100
90-day dog study (Anonymous, 1999)	9.7 Thymus	90 days	9.7	
1-year dog study (Anonymous, 1994)	5.7 Eyes	1 year	22.8	
1-year dog study (Anonymous, 2003)	5.6 Thymus/Eyes	1 year	22.4	

The table above summarises the most relevant animal studies for STOT RE classification. Statistically significant reductions in haemoglobin in males (24.4%) at 10.56 mg/kg bw/d and females (22.2%) at 10.51 mg/kg bw/d were recorded in a 90-day dog study (Anonymous, 1993). Similar but weaker effects were reported in another 90-day dog study (Anonymous, 1999). Such effects were also reported to some extent in rats and

mice.

According to the CLP Guidance (2017), a reduction in haemoglobin at ≥ 20% is considered a significant adverse effect in haematology. The increase of the MCV, as the most significant haematological parameter for assessing anaemia, of about 5% might indicate morphological abnormality of erythrocytes and should be considered as a toxicologically significant change, according to WHO Guidance document (WHO, Guidance document for WHO monographers and reviewers, 2015). Thus, haematological changes observed in dogs at or above but not below 10.5 mg/kg bw/d (supported by findings in other species) warrant classification as STOT RE 2.

One study in dogs (2003) showed a dose-dependent increase in male and female thymus atrophy from 9.7 mg/kg bw/d in females. Male thymus atrophy was recorded in a one-year dog study (Anonymous, 2003) at 5.6 mg/kg bw/d. Some findings indicate that changes in the thymus might be a substance related effect and not a high-dose stress response. These effects were borderline between guidance values for classification as STOT RE 1 and STOT RE 2. Moreover, in the 1-year dog study effects of similar severity were observed at considerably higher doses indicating a shallow dose-response curve. Although thymus effects were also observed at doses below the guidance value for category 1, RAC considers these not sufficiently adverse for classification. In the RAC opinion from 2012, it was considered that the effects reported are in accordance with a classification as STOT RE 2, as the effects were not reported in all available studies. This is in line with the evaluation by the DS. Therefore, RAC supports classification as STOT RE 2 for the target organs blood and thymus.

With respect to eyes as target organ, two one-year dog studies (Anonymous, 1994 and Anonymous, 2003) reported slight bilateral cataract in one out of five males at 5.7 mg/kg bw/d and bilateral lenticular degeneration of a slight degree in one male out of four at 5.6 mg/kg bw/d. Additionally, a slight unilateral cataract was reported in one female out of five at 5.6 mg/kg bw/d in the one-year dog study (Anonymous, 1994). These three incidents of eye damage in dogs of young age are not considered to be incidental and might represent a substance-related effect. These effects are supported by a statistically significant retinal atrophy in males and females in a two-year study in rats (Anonymous, 1994a). It is noted that the 1-year interim sacrifice revealed statistically significant increases in the incidence of retinal atrophy in males at 32.8 mg/kg bw/d. Although effects in dogs and rodents are different, classification is based on target organ toxicity and is not restricted to a specific effect. Therefore, RAC considers eye effects in rodents to be supportive of eyes as the target organs. Effects observed in dogs are in line with classification as STOT RE 2.

Conclusively, RAC supports the dossier submitter's proposal to classify cymoxanil as **STOT RE 2 H373 (blood, thymus, eye).**

This recommendation is broadly in line with RAC's previous evaluation (RAC, 2012) of Cymoxanil. However, 'blood' has been amended to 'blood system' in line with the nomenclature used in recent RAC opinions and 'eye' has been added as a target organ.

2.6.4 Summary of genotoxicity / germ cell mutagenicity

Table 29: Summary table of genotoxicity/germ cell mutagenicity tests *in vitro*

Method, guideline, deviations if any	Test substance (Batch No; purity)	Relevant information about the study including rationale for dose selection (as applicable)	Observations /Results	Reference
Bacterial Reverse Mutation Test (Ames test)				
OECD 471 and 472 (1983) GLP Acceptable	Cymoxanil, DPX-T3217-113, 97.8%	Organism/ Strain(s): *Salmonella typhimurium* strains TA 98, TA 100, TA 1535, TA 1537 Concentrations tested : 0, 31.3, 62.5, 125, 250, 500, 1000 and 2000 µg/plate (-/+ S9) *E. coli* WP2 uvrA Concentrations tested: 0, 313, 625, 1250, 2500 and 5000 µg/plate (-/+ S9) Dissolved in DMSO	Negative (+/- S9 mix) Cytotoxicity: TA100, TA98 at 2000 µg/plate (-S9); TA1535, TA1537 at ≥1000 µg/plate (-S9); TA100, TA1535, TA98 at 2000 µg/plate (+S9); TA1537 at 1000 µg/plate (+S9)	RAR B.6.4.1.1. Study 1, 1994
OECD 471 (1983) GLP Not acceptable Lack of sensitivity to detect cross-linking agents & too low dose levels tested	Cymoxanil, 0972, 98.8%	Organism/ Strain(s): *Salmonella typhimurium* strains TA 98, TA 100, TA 1535, TA 1537, TA 1538 Concentrations tested: 0, 50, 85, 140, 235 and 400 µg/plate (-/+S9) Dissolved in DMSO	Negative (+/- S9 mix) Cytotoxicity (preliminary dose range finding): TA100 at ≥800 µg/plate (+/-S9)	RAR B.6.4.1.1. Study 2, 1997
OECD 471 (1997) GLP Acceptable	Cymoxanil, DPX-T3217-266/ LS1207012, 99.7%	Organism/ Strain(s): *Salmonella typhimurium* strains TA 98, TA 100, TA 1535, TA 1537 *E. coli* WP2 uvrA Concentrations tested (range): I and II Experiment: 333 - 5000 µg/plate (-/+S9) II Experiment: TA 98 - 66.7, 100, 333, 667, 1000 µg/plate (-S9) Dissolved in DMSO	Negative (+/- S9 mix) Cytotoxicity (toxicity-mutation test): TA98, TA1535, TA1537 (-/+ S9) TA100 (+ S9) at ≥3333 µg/plate	RAR B.6.4.1.1. Study 3, 2013
OECD 471 (1997) GLP Acceptable	Cymoxanil, 080906, 100.85%	Organism/ Strain(s): *Salmonella typhimurium* strains TA 98, TA 100, TA 102, TA 1535, TA 1537 Concentrations tested (range): 12 - 1250 µg/plate (-/+ S9) Dissolved in DMSO	Negative (+/- S9 mix) Cytotoxicity (preliminary dose range finding): TA100 (-/+S9) at 2500 µg/plate	RAR B.6.4.1.1. Study 4, 2008a
OECD 471 (1997) GLP Acceptable	Cymoxanil, 143/092013, 98.3%	Organism/ Strain(s): *Salmonella typhimurium* strains TA 98, TA 100, TA 1535, TA 1537 *E. coli* WP2 uvrA Concentrations tested (range): 5 - 5000 µg/plate (-/+ S9) Dissolved in DMSO	Negative (+/- S9 mix) Cytotoxicity: all *S.typhimurium* strains Plate Incorporation Method: at 5000 µg/plate (-/+ S9) Pre-Incubation Method: at 1581 and 5000 µg/plate (-/+ S9)	RAR B.6.4.1.1. Study 5, 2015

Method, guideline, deviations if any	Test substance (Batch No; purity)	Relevant information about the study including rationale for dose selection (as applicable)	Observations /Results	Reference
OECD 471 (1997) GLP Acceptable	Cymoxanil, Batch No not available, 99% (CoA not available)	Organism/ Strain(s): *Salmonella typhimurium* strains TA 98, TA 100, TA102, TA 1535, TA 1537 Concentrations tested (range): 5 - 5000 µg/plate (-/+ S9) Dissolved in DMSO	Negative (+/- S9 mix) Cytotoxicity (preliminary toxicity test): TA100 at ≥1500 µg/plate (-/+ S9)	RAR B.6.4.1.1. Study 6, 2006
OECD 471 (1997) GLP Acceptable	Cymoxanil, U028/09-B, 99.09%	Organism/ Strain(s): *Salmonella typhimurium* strains TA 98, TA 100, TA 1535, TA 1537 *E. coli* WP2 uvrA (pKM101) Concentrations tested (range): 5 - 5000 µg/plate (-/+ S9) Dissolved in DMSO	Negative (+/- S9 mix) Cytotoxicity: all *S. Typhimurium* strains at 5000 µg/plate (-/+ S9)	RAR B.6.4.1.1. Study 7, 2009
Mammalian Cell Gene Mutation Test				
OECD 476 (1984) GLP Supportive only The low number of cells plated for mutagenicity leading to a low number of spontaneous mutant frequency	Cymoxanil, DPX-T3217-113 (Blend of lots 80317145 and 80321154), 97.8% (CoA not available)	HPRT-locus in Chinese hamster ovary (CHO K1-BH4) cells 0.005, 0.01, 0.05, 0.1, 0.25, 0.50, 0.75, 1.25 and 1.5 mg/ml Dissolved in DMSO	Negative (+/- S9 mix)	RAR B.6.4.1.2. Study 1, 1993
OECD 476 (1984) GLP Not acceptable The highest exposure concentration chosen was too low	Cymoxanil, 0972, 98.6%	HPRT-locus in Chinese hamster ovary (CHO K1) cells 0, 100, 160, 250 and 400 µg/ml dissolved in DMSO	Negative (+/- S9 mix)	RAR B.6.4.1.2. Study 2, 1998
OECD 476 (1997) GLP Acceptable	Cymoxanil, 2008112201, 98.4%	TK-locus in Mouse lymphoma cells L5178Y 62.5- 1000 µg/ml (-/+S9) 4h exposure; 12.5- 125 µg/ml (-S9) 24 h exposure, 200 – 1000 µg/ml (+S9) 4 h exposure Dissolved in DMSO	Negative (+/- S9 mix)	RAR B.6.4.1.2. Study 3, 2009
Mammalian Chromosome Aberrations Test				
OECD 473 (1983) GLP Supportive only Too low number of total cells analysed per dose and no long term treatment (- S9) available	Cymoxanil, DPX-T3217-113 (Blend of lots 80317145 and 80321154), 97.8% (CoA not available)	Human lymphocytes 0, 0.1, 0.5, 0.75, 0.85, 1.0, 1.25, 1.5 mg/ml Dissolved in DMSO	**Clastogenic** (+/-S9 mix) 3-4 h exposure	RAR B.6.4.1.3. Study 1, 1993

Method, guideline, deviations if any	Test substance (Batch No; purity)	Relevant information about the study including rationale for dose selection (as applicable)	Observations /Results	Reference
US EPA OPPTS 870.5375 (1998), equivalent to OECD 473 (1997) GLP Acceptable	Cymoxanil, 498VF973 98.8%	Chinese hamster ovary (CHO K1) cells 0, 16, 19, 36, 38, 76 and 81 µg/ml Dissolved in DMSO	Negative (+/- S9 mix) 3 and 19-21 hours exposure	RAR B.6.4.1.3. Study 2, 2000
OECD 473 (1997) GLP Acceptable	Cymoxanil, PUS-056200-07/05 > 97.5 % (CoA not available)	Chinese hamster ovary (CHO K1) cells 0, 5 , 12.5, 25 and 50 µg/ml Dissolved in McCoys 5A culture medium	**Clastogenic:** (-S9) 20 hours exposure	RAR B.6.4.1.3. Study 3, 2005
Unscheduled DNA Synthesis Assay				
OECD 482 (1986), now obsolete (has been deleted on 2014) GLP Acceptable	Cymoxanil, DPX-T3217-113 (Blend of lots 80317145 and 80321154), 97.8%	Primary Sprague-Dawley rat hepatocytes 0 (solvent control), 5, 10, 50, 100, 250, 500 µg/ml Dissolved in DMSO	**Positive:** UDS induced (p<0.05) I trial: at 5, 10, 50,100, 250 and 500 µg/ml II trial: at 5, 10, 100 and 250 µg/ml **Cytotoxicity:** at ≥500 µg/ml (II trial) determined by an elevation of LDH activity	RAR B.6.4.1.4., 1993

Table 30: Summary table of genotoxicity/mutagenicity tests in mammalian somatic or germ cells *in vivo*

Method, guideline, deviations if any	Test substance	Relevant information about the study (as applicable)	Observations /Results	Reference
Micronucleus Test				
OECD 474 (1983) GLP Acceptable	Cymoxanil, DPX-T3217-113 (Blend of lots 80317145 and 80321154), 97.8% (CoA not available)	Crl:CD-1 mice 0, 125, 225, 350/450 mg/kg bw suspended in sterile water Single oral exposure Time points of bone marrow samples: 24, 48 or 72 h	Negative **Cytotoxicity:** depression of PCEs/1000 erythrocytes at 48 h at 350 mg/kg (♀); PCE/NCE ratio not affected	RAR B.6.4.2.1. Study 1, 1993
OECD 474 (1997) GLP Acceptable	Cymoxanil, 0972, 98.6%	Albino Swiss mice, HsdOla: MF1 strain 0, 50, 250, 500 mg/kg bw suspended in 0.5 % aqueous carboxymethyl cellulose Twice oral exposure (24 hour intervals) Time point of bone marrow samples: 24 h	Negative **Cytotoxicity:** reduced PCE/RBC ratio at 50, 250, 500 mg/kg bw (except the low dose, ♀)	RAR B.6.4.2.1. Study 2, 1999

OECD 474 (1997) GLP Acceptable	Cymoxanil, 080906, 100.85%	Albino Swiss mice 0, 125, 250, 500 mg/kg bw suspended in corn oil Twice oral exposure (24 hour intervals) Time point of bone marrow samples: 24 h	Negative **Cytotoxicity:** P/E ratio not affected	RAR B.6.4.2.1. Study 3, 2008b
Bone Marrow Cytogenetic Test – Chromosomal Analysis				
Not stated Original guideline OECD 475 only adopted in 1984. The study complies with it. Quality assurance statement Supportive only Too low number of metaphases analysed for each animal and/or group	Cymoxanil, Batch No not available, 98% assumed (CoA not available)	Sprague-Dawley rats (bone marrow cells) 0, 50, 100, 500 mg/kg bw suspended in corn oil Single oral exposure Time points of bone marrow samples: 6, 12, 24 or 48 h after dosing	Negative **No cytotoxicity:** no changes in the mitotic indices	RAR B.6.4.2.2., 1982
Unscheduled DNA synthesis test				
Draft OECD GLP 486 (1997) and reference Butterworth, B.E. et all (1987) GLP Supportive only Dose selection criteria are not fulfilled	Cymoxanil, DPX-T3217-113 (Blend of lots 80317145 and 80321154), 97.8%	Sprague-Dawley rats (hepatocytes and spermatocytes) 0, 500, 1000 mg/kg bw suspended in 0.5 % methyl cellulose Single oral exposure Time points of samples: 2 and 16 h after dosing	Negative **No cytotoxicity:** hepatocytes /spermatocytes viability > 90%	RAR B.6.4.2.3., 1994

No human data relevant for genotoxicity / germ cell mutagenicity are available.

2.6.4.1 Short summary and overall relevance of the provided information on genotoxicity / germ cell mutagenicity

The genotoxicity of cymoxanil was addressed in a number of studies conducted *in vitro* and in the rat and mouse *in vivo* measuring different mutagenic endpoints like gene mutation in bacterial and mammalian cells *in vitro,* and chromosomal mutations and unscheduled DNA synthesis *in vitro* as well as *in vivo*.

Studies previously evaluated in the Cymoxanil DAR (2007) included for *in vitro* studies three Ames tests, two Chinese hamster ovary (CHO) cells gene mutation assays, UDS assay and two chromosome aberration tests and for *in vivo* studies a chromosome aberration test in rat bone marrow, two Micronucleus tests and one UDS assay. The majority of the studies submitted originally were old studies conducted in the 80s and 90s near the time of the first adoption of the OECD guidelines for genotoxicity. The majority of these OECD guidelines have been since revised extensively. Re-evaluation of all studies has been performed by the RMS. The summaries of old studies are found in this section below.

In addition to the above studies, the various applicants involved in the process of renewal submitted a wide range of new OECD compliant *in vitro* and *in vivo* studies: five Ames tests, one Mouse lymphoma gene mutation test, one Chinese hamster ovary cells chromosome aberrations assay and one *in vivo* Micronucleus test. They have been evaluated by the RMS and their summaries are found in this section below too.

In vitro:

Cymoxanil did not show mutagenic potential in bacterial cells: six Ames tests performed on strains of *Salmonella typhimurium* and *Escherichia coli* with and without metabolic activation were negative. The seventh assay (*RAR B.6.4.1.1. Study 2, 1997*) was considered by the RMS as not acceptable due to lack of sensitivity to detect cross-linking agents and too low dose levels tested.

Studies on mammalian cell gene mutation *in vitro* (one HPRT tests on Chinese hamster ovaries and one TK locus assay on Mouse lymphoma cells) did not show any mutagenic potential caused by cymoxanil. However, the first assay (*RAR B.6.4.1.2. Study 1, 1993*) was considered by the RMS as supportive only: too low number of cells was plated for mutagenicity leading to a low number of spontaneous mutant frequencies. Additionally, third assay (*RAR B.6.4.1.2. Study 2, 1998*) was considered by the RMS as not acceptable: the highest exposure concentration chosen was too low. Overall, both the bacterial and mammalian cells *in vitro* tests showed no mutagenic potential.

With respect to chromosomal aberrations, two of three *in vitro* studies showed positive results indicating structural chromosomal damage in human lymphocytes and Chinese hamster ovary cells induced by cymoxanil. However, the first one (*RAR B.6.4.1.3. Study 1, 1993*) was considered by the RMS as supportive only: too low number of total cells was analysed per dose and no long term treatment (- S9) was available. The second study showed a significant increase in chromosome aberrations after 20 hours treatment of CHO-K1 cell lines with cymoxanil without metabolic activation. The results of a third study submitted on chromosomal aberrations on Chinese hamster ovary cells did not find evidence of clastogenic activity of cymoxanil in CHO-K1 cells, neither with or without metabolic activation.

An *in vitro* UDS assay using rat hepatocytes indicated that cymoxanil induced unscheduled DNA synthesis. It should be noted that the OECD TG 482 has been deleted on 2nd April 2014 as this test was not considered sensitive enough and this assay is now non-preferred. The results of an *in vivo* UDS assay (*RAR B.6.4.2.3., 1994*) in rat hepatocytes and spermatocytes did not confirm this finding: no statistically significant increases or increasing dose-related trends in UDS response were observed at any dose of cymoxanil technical at any harvest time. However, this study was considered by the RMS as supportive only.

In vivo:

Four *in vivo* studies provided (three micronucleus tests in mice and one bone marrow chromosomal aberration test in rats) did not show any potential of cymoxanil to produce chromosomal damage. Cymoxanil was evaluated for induction of micronuclei to mouse bone marrow polychromatic erythrocytes (PCEs) in three studies. No statistically significant increases in the frequency of micronucleated PCEs were observed in all treated groups. A reduction in the proportion of immature erythrocytes among total erythrocytes (sufficient evident of bone marrow exposure) was observed in two micronucleus assays (*RAR B.6.4.2.1. Study 1, 1993 and RAR B.6.4.2.1. Study 2, 1999*). Based on *Scientific Opinion on the clarification of some aspects related to genotoxicity assessment (EFSA Journal 2017;15(12):5113, 25 pp.)* additional lines of evidence of bone marrow exposure by cymoxanil were obtained from other signs: systemic toxicity was observed in all bone marrow micronucleus studies at high doses, the radiolabelled cymoxanil was detected in the bone and it was detected systemically in the blood/plasma in toxicokinetic studies. Therefore, cymoxanil was not genotoxic (it was neither clastogen nor aneugen) based on three negative mammalian erythrocyte MN tests outcomes with evidence of bone marrow exposure.

In the bone marrow chromosomal aberration test in rats no increases in the frequency of chromosomal aberrations at any of the cymoxanil dosages were observed (RAR B.6.4.2.2., 1982). The test substance was considered able to reach the bone marrow. However, this study was considered supplementary only: too low number of metaphases was analysed for each animal and/or group.

As already mentioned before, the results of an *in vivo* UDS assay (*RAR B.6.4.2.3., 1994*) in rat hepatocytes and spermatocytes were negative: no statistically significant increases or increasing dose-related trends in UDS response were observed at any dose of cymoxanil technical at any harvest time. Additionally, it was assumed that the target tissues/organs exposure to the substance occurred. However, this study was considered by the RMS as supportive only.

Based on a weight of evidence of all data available is concluded that cymoxanil does not pose genotoxic concern *in vivo*.

2.6.4.2 Comparison with the CLP criteria regarding genotoxicity / germ cell mutagenicity

Annex I Section 3.5.1.1 of the CLP regulation defines mutation as a permanent change in the amount or structure of the genetic material in a cell. The term ‘mutation‘ applies both to heritable genetic changes that may be manifested at the phenotypic level and to the underlying DNA modifications. The term ‘mutagenic’ and ‘mutagen’ are used for agents giving rise to an increased occurrence of mutations in populations of cells and/or organisms. This hazard class is primarily concerned with substances that may cause mutations in the germ cells of humans that can be transmitted to the progeny. However, the results from mutagenicity or genotoxicity tests *in vitro* and in mammalian somatic and germ cells *in vivo* are also considered in classifying substances within this hazard class.

Classification for mutagenicity in Category 1 is appropriate for substances known to induce heritable mutations (Category 1A) or for substances regarded as if they induce heritable mutations in the germ cells of humans (Category 1B).

Classification in Category 1A is based on positive evidence from human epidemiological studies.

No human epidemiological studies are known for cymoxanil. From the results of animal studies there are no indications that cymoxanil could induce heritable mutations in the germ cells of humans.

Classification in Category 1B is based on positive result(s) from in vivo heritable germ cell mutagenicity tests in mammals; or positive result(s) from in vivo somatic cell mutagenicity tests in mammals, in combination with

evidence that the substance has potential to cause mutations to germ cells; or positive results from tests showing mutagenic effects in the germ cells of humans, without demonstration of transmission to progeny.

Data with cymoxanil are not available illustrating the induction of mutagenic effects in germ cells. Results from *in vivo* studies in somatic cell in mammals do not give any indication for potential mutagenicity: cymoxanil was not genotoxic *in vivo* (it was neither clastogen nor aneugen) based on three negative mammalian erythrocyte MN tests outcomes with evidence of bone marrow exposure.

Classification for mutagenicity in Category 2 is appropriate for substances which cause concern for humans owing to the possibility that they may induce heritable mutations in the germ cells of humans. Classification in Category 2 is based on positive evidence obtained from somatic cell mutagenicity tests in mammals and/or in some cases from somatic cell mutagenicity tests in mammals and supporting data from in vitro experiments.

There were no positive *in vivo* micronucleus tests. There were some equivocal results in some of the *in vitro* assays but overall the evidence suggests there is no concern for mutagenicity.

2.6.4.3 Conclusion on classification and labelling for genotoxicity / germ cell mutagenicity

Overall, considering available data on Ames and mammalian gene mutation tests, the compound is unlikely to be of gene mutation concern. Based on negative micronucleus tests *in vivo* where evidence of bone marrow exposure was demonstrated, cymoxanil is unlikely to be genotoxic *in vivo*. The criteria for classification for mutagenicity were not met. On the basis of the available data, no hazard classification of cymoxanil for mutagenicity is warranted according to Regulation (EC) No 1272/2008.

RAC evaluation of germ cell mutagenicity

Summary of the Dossier Submitter's proposal

In vitro

Six Ames tests performed on strains of *Salmonella typhimurium* and *Escherichia coli* with and without metabolic activation were negative. Another assay was considered not acceptable due to lack of sensitivity to detect cross-linking agents, and too low dose levels tested.

Studies on mammalian cell gene mutation *in vitro* (one HPRT tests on Chinese hamster ovaries and one TK locus assay on mouse lymphoma cells) did not show any mutagenic potential caused by cymoxanil. A third assay was considered not acceptable because the highest exposure concentration chosen was too low.

Two of three *in vitro* studies on chromosomal aberrations showed positive results indicating structural chromosomal damage in human lymphocytes and Chinese hamster ovary cells induced by cymoxanil.

One *in vitro* UDS assay using rat hepatocytes indicated that cymoxanil induced unscheduled DNA synthesis. It should be noted that the OECD TG 482 has been deleted on 2nd April 2014 as this test was not considered sensitive enough and this assay is now non-preferred. The results of an *in vivo* UDS assay in rat hepatocytes and spermatocytes did not confirm this finding.

In vivo

Four *in vivo* studies provided (three micronucleus tests in mice and one_bone marrow chromosomal aberration test in rats) did not show any potential of cymoxanil to produce chromosomal damage.

The results of an *in vivo* UDS assay (RAR B.6.4.2.3., 1994) in rat hepatocytes and spermatocytes were negative: no statistically significant increases or increasing dose-

related trends in UDS response were observed at any dose of cymoxanil technical at any harvest time.

Conclusion on classification

The DS summarised that, considering available data from Ames and mammalian gene mutation tests, the compound is unlikely to be of gene mutation concern. Based on negative micronucleus tests *in vivo* where evidence of bone marrow exposure was demonstrated, cymoxanil is unlikely to be genotoxic *in vivo*. The DS concluded that **no classification** of cymoxanil for mutagenicity is warranted.

Comments received during consultation

No comments were received.

Assessment and comparison with the classification criteria

A number of studies conducted *in vitro* and *in vivo* address the genotoxicity of cymoxanil. Twelve Studies have been previously evaluated in the Cymoxanil DAR (2007) including eight *in vitro* studies (three Ames tests, two Chinese hamster ovary (CHO) cells gene mutation assays, one UDS assay and two chromosome aberration tests) and four *in vivo* studies (one chromosome aberration test in rat bone marrow, two micronucleus tests and one UDS assay). In addition to these studies, a wide range of new OECD compliant *in vitro* and *in vivo* studies are available (*in vitro*: five Ames tests, one mouse lymphoma gene mutation test, one Chinese hamster ovary cells chromosome aberrations assay; *in vivo*: one micronucleus test).

In vitro

All available studies on genotoxicity/ germ cell mutagenicity *in* vitro are listed in the table below.

Table*: Summary table of genotoxicity/ germ cell mutagenicity tests in vitro (modified from CLH Report table 29)*

Method, guideline, deviations if any	**Test substance (Batch No; purity)**	**Relevant information about the study including rationale for dose selection (as applicable)**	**Observations /Results**	**Reference**
Bacterial Reverse Mutation Test (Ames test)				

OECD TG 471 and 472 (1983) GLP Acceptable	Cymoxanil DPX-T3217-113 97.8%	Organism/ Strain(s): *S. typhimurium* strains TA98, TA100, TA1535, TA1537 Concentrations tested: 0, 31.3, 62.5, 125, 250, 500, 1000 and 2000 µg/plate (-/+ S9) *E. coli* WP2 uvrA Concentrations tested: 0, 313, 625, 1250, 2500 and 5000 µg/plate (-/+ S9) Dissolved in DMSO	Negative (+/- S9 mix) Cytotoxicity: TA100, TA98 at 2000 µg/plate (-S9); TA1535, TA1537 at ≥ 1000 µg/plate (-S9); TA100, TA1535, TA98 at 2000 µg/plate (+S9); TA1537 at 1000 µg/plate (+S9)	Anonymous, 1994
OECD TG 471 (1983) GLP Not acceptable Lack of sensitivity to detect cross-linking agents & too low dose levels tested	Cymoxanil 0972 98.8%	Organism/ Strain(s): *S. typhimurium* strains TA98, TA100, TA1535, TA1537, TA1538 Concentrations tested: 0, 50, 85, 140, 235 and 400 µg/plate (-/+S9) Dissolved in DMSO	Negative (+/- S9 mix) Cytotoxicity (preliminary dose range finding): TA100 at ≥ 800 µg/plate (+/-S9)	Anonymous, 1997
OECD TG 471 (1997) GLP Acceptable	Cymoxanil DPX-T3217-266/ LS1207012 99.7%	Organism/ Strain(s): *S. typhimurium* strains TA98, TA100, TA1535, TA1537 *E. coli* WP2 uvrA Concentrations tested (range): I and II Experiment: 333 - 5000 µg/plate (-/+S9) II Experiment: TA98 - 66.7, 100, 333, 667, 1000 µg/plate (-S9) Dissolved in DMSO	Negative (+/- S9 mix) Cytotoxicity (toxicity-mutation test): TA98, TA1535, TA1537 (-/+ S9), TA100 (+ S9) at ≥ 3333 µg/plate	Anonymous, 2013
OECD TG 471 (1997) GLP Acceptable	Cymoxanil 080906 100.85%	Organism/ Strain(s): *S. typhimurium* strains TA98, TA100, TA102, TA1535, TA1537 Concentrations tested (range): 12 - 1250 µg/plate (-/+ S9) Dissolved in DMSO	Negative (+/- S9 mix) Cytotoxicity (preliminary dose range finding): TA100 (-/+S9) at 2500 µg/plate	Anonymous, 2008a

OECD TG 471 (1997) GLP Acceptable	Cymoxanil 143/092013 98.3%	Organism/ Strain(s): *S. typhimurium* strains TA98, TA100, TA1535, TA1537 *E. coli* WP2 uvrA Concentrations tested (range): 5 - 5000 µg/plate (-/+ S9) Dissolved in DMSO	Negative (+/- S9 mix) Cytotoxicity: all *S. typhimurium* strains Plate Incorporation Method: at 5000 µg/plate (-/+ S9) Pre-Incubation Method: at 1581 and 5000 µg/plate (-/+ S9)	Anonymous, 2015
OECD TG 471 (1997) GLP Acceptable	Cymoxanil Batch No not available 99% (CoA not available)	Organism/ Strain(s): *S. typhimurium* strains TA98, TA100, TA102, TA1535, TA1537 Concentrations tested (range): 5 - 5000 µg/plate (-/+ S9) Dissolved in DMSO	Negative (+/- S9 mix) Cytotoxicity (preliminary toxicity test): TA100 at ≥ 1500 µg/plate (-/+ S9)	Anonymous, 2006
OECD TG 471 (1997) GLP Acceptable	Cymoxanil U028/09-B 99.09%	Organism/ Strain(s): *S. typhimurium* strains TA98, TA100, TA1535, TA1537 *E. coli* WP2 uvrA (pKM101) Concentrations tested (range): 5 - 5000 µg/plate (-/+ S9) Dissolved in DMSO	Negative (+/- S9 mix) Cytotoxicity: all *S. typhimurium* strains at 5000 µg/plate (-/+ S9)	Anonymous, 2009
Mammalian Cell Gene Mutation Test				
OECD TG 476 (1984) GLP Supportive only The low number of cells plated for mutagenicity leading to a low number of spontaneous mutant frequency	Cymoxanil DPX-T3217-113 (Blend of lots 80317145 and 80321154) 97.8% (CoA not available)	HPRT-locus in Chinese hamster ovary (CHO K1-BH4) cells 0.005, 0.01, 0.05, 0.1, 0.25, 0.50, 0.75, 1.25 and 1.5 mg/mL Dissolved in DMSO	Negative (+/- S9 mix)	Anonymous, 1993

OECD TG 476 (1984) GLP Not acceptable The highest exposure concentration chosen was too low	Cymoxanil 0972 98.6%	HPRT-locus in Chinese hamster ovary (CHO K1) cells 0, 100, 160, 250 and 400 µg/mL dissolved in DMSO	Negative (+/- S9 mix)	Anonymous, 1998
OECD TG 476 (1997) GLP Acceptable	Cymoxanil 2008112201 98.4%	TK-locus in Mouse lymphoma cells L5178Y 62.5- 1000 µg/mL (-/+S9) 4h exposure; 12.5- 125 µg/mL (-S9) 24 h exposure, 200 – 1000 µg/mL (+S9) 4 h exposure Dissolved in DMSO	Negative (+/- S9 mix)	Anonymous, 2009
Mammalian Chromosome Aberrations Test				
OECD TG 473 (1983) GLP Supportive only Too low number of total cells analysed per dose and no long term treatment (-S9) available	Cymoxanil DPX-T3217-113 (Blend of lots 80317145 and 80321154) 97.8% (CoA not available)	Human lymphocytes 0, 0.1, 0.5, 0.75, 0.85, 1.0, 1.25, 1.5 mg/mL Dissolved in DMSO	**Clastogenic** (+/-S9 mix) 3-4 h exposure	Anonymous, 1993
US EPA OPPTS 870.5375 (1998), equivalent to OECD TG 473 (1997) GLP Acceptable	Cymoxanil 498VF973 98.8%	Chinese hamster ovary (CHO K1) cells 0, 16, 19, 36, 38, 76 and 81 µg/mL Dissolved in DMSO	Negative (+/- S9 mix) 3 and 19-21 hours exposure	Anonymous, 2000
OECD TG 473 (1997) GLP Acceptable	Cymoxanil PUS-056200-07/05 > 97.5% (CoA not available)	Chinese hamster ovary (CHO K1) cells 0, 5 , 12.5, 25 and 50 µg/mL Dissolved in McCoys 5A culture medium	**Clastogenic** (-S9) 20 hours exposure	Anonymous, 2005
Unscheduled DNA Synthesis Assay				

OECD TG 482 (1986), now obsolete (has been deleted on 2014) GLP Acceptable	Cymoxanil DPX-T3217-113 (Blend of lots 80317145 and 80321154) 97.8%	Primary Sprague-Dawley rat hepatocytes 0 (solvent control), 5, 10, 50, 100, 250, 500 µg/mL Dissolved in DMSO	**Positive:** UDS induced ($p < 0.05$) I trial: at 5, 10, 50,100, 250 and 500 µg/mL II trial: at 5, 10, 100 and 250 µg/mL Cytotoxicity: at ≥ 500 µg/mL (II trial) determined by an elevation of LDH activity	Anonymous, 1993

Six out of seven Ames tests performed on strains of *S. typhimurium* and *E. coli* with and without metabolic activation were negative. One Ames test (Anonymous, 1997) has a lack of sensitivity to detect cross-linking agents and too low dose levels. Therefore, it is considered not acceptable.

Both studies on mammalian germ cell gene mutation in vitro (HPRT test on Chinese hamster ovaries and TK locus assay on mouse lymphoma cells) did not show any mutagenic potential caused by cymoxanil. The HPRT test on Chinese hamster ovaries (Anonymous, 1993) is considered as supportive only due to the low number of cells plated for mutagenicity. A third study (Anonymous, 1998) is considered to be not acceptable because the highest exposure concentration was chosen too low.

The chromosomal aberration test in human lymphocytes and one chromosomal aberration test in Chinese hamster ovary cells showed positive results indicating structural chromosomal damage induced by cymoxanil. In the chromosome aberration test in human lymphocytes (Anonymous, 1993), the number of total cells analysed per dose was too low and no long-term treatment without metabolic activation was available. This study is regarded as supporting evidence only. One additional chromosome aberration test in Chinese hamster ovary cells (Anonymous, 2000) did not find any evidence of clastogenic activity of cymoxanil.

The *in vitro* UDS assay using rat hepatocytes was positive and indicated that cymoxanil induced unscheduled DNA synthesis. It is noted that the OECD TG 482 was not considered sensitive enough and has been deleted on 2nd April 2014. Therefore, it is now not a preferred test.

In vivo

Three studies evaluated cymoxanil for induction of micronuclei to mouse bone marrow polychromatic erythrocytes (PECs). None of the treated groups showed statistically significant increase in the frequency of micronucleated PCEs. Evidence of bone marrow exposure were obtained from different signs, such as reduction in proportion of immature erythrocytes among total erythrocytes and systemic toxicity observed in all bone marrow studies at high doses. Furthermore, the radiolabelled cymoxanil was detected in the bone and systematically in the blood/plasma in toxicokinetic studies. Cymoxanil showed no genotoxic potential in the mammalian erythrocyte micronucleus tests.

The bone marrow chromosomal aberration test in rats also revealed a negative outcome as no increase in the frequency of chromosomal aberration at any of the cymoxanil

dosages were observed. Due to a too low number of metaphases analysed for each animal/group, this study is considered supplementary only.

One *in vivo* UDS assay in rat hepatocytes and spermatocytes showed negative results.

Study results are summarized in the table below.

***Table**: Summary table of genotoxicity/mutagenicity tests in mammalian somatic or germ cells in vivo (modified from CLH report table 30)*

Method, guideline, deviations if any	Test substance	Relevant information about the study (as applicable)	Observations/Results	Reference
Micronucleus Test				
OECD TG 474 (1983) GLP Acceptable	Cymoxanil DPX-T3217-113 (Blend of lots 80317145 and 80321154) 97.8% (CoA not available)	Crl:CD-1 mice 0, 125, 225, 350/450 mg/kg bw suspended in sterile water Single oral exposure Time points of bone marrow samples: 24, 48 or 72h	Negative Cytotoxicity: depression of PCEs/1000 erythrocytes at 48 h at 350 mg/kg (♀); PCE/NCE ratio not affected	Anonymous, 1993
OECD TG 474 (1997) GLP Acceptable	Cymoxanil 0972 98.6%	Albino Swiss mice, HsdOla: MF1 strain 0, 50, 250, 500 mg/kg bw suspended in 0.5% aqueous carboxymethyl cellulose Twice oral exposure (24 hour intervals) Time point of bone marrow samples: 24h	Negative Cytotoxicity: reduced PCE/RBC ratio at 50, 250, 500 mg/kg bw (except the low dose, ♀)	Anonymous, 1999
OECD TG 474 (1997) GLP Acceptable	Cymoxanil 080906 100.85%	Albino Swiss mice 0, 125, 250, 500 mg/kg bw suspended in corn oil Twice oral exposure (24 hour intervals) Time point of bone marrow samples: 24h	Negative Cytotoxicity: P/E ratio not affected	Anonymous, 2008b
Bone Marrow Cytogenetic Test – Chromosomal Analysis				

Not stated Original guideline OECD TG 475 only adopted in 1984. The study complies with it. Quality assurance statement Supportive only Too low number of metaphases analysed for each animal and/or group	Cymoxanil Batch No not available 98% assumed (CoA not available)	Sprague-Dawley rats (bone marrow cells) 0, 50, 100, 500 mg/kg bw suspended in corn oil Single oral exposure Time points of bone marrow samples: 6, 12, 24 or 48h after dosing	Negative No cytotoxicity: no changes in the mitotic indices	Anonymous, 1982
Unscheduled DNA synthesis test				
Draft OECD GLP 486 (1997) and reference Butterworth *et al*., 1987 GLP Supportive only Dose selection criteria are not fulfilled	Cymoxanil DPX-T3217-113 (Blend of lots 80317145 and 80321154) 97.8%	Sprague-Dawley rats (hepatocytes and spermatocytes) 0, 500, 1000 mg/kg bw suspended in 0.5% methyl cellulose Single oral exposure Time points of samples: 2 and 16h after dosing	Negative No cytotoxicity: hepatocytes /spermatocytes viability > 90%	Anonymous, 1994

Conclusion on classification

There are no human epidemiological data available for cymoxanil. The animal studies did not show any indication that cymoxanil could induce heritable mutations in the germ cells of humans. The criteria for classification for mutagenicity were not met. Therefore, RAC supports the DS proposal that **no classification** for mutagenicity is warranted.

This recommendation is in line with RAC's previous evaluation (RAC, 2012) of Cymoxanil.

2.6.5 Summary of long-term toxicity and carcinogenicity [equivalent to section 10.9 of the CLH report template]

For more detailed data on effects on long-term toxicity and carcinogenicity please refer to RAR Volume 3CA B-6, section B.6.5.

Table 31: Summary table of animal studies on long-term toxicity and carcinogenicity

Method, guideline, deviations[1] if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results** **- NOAEL/LOAEL** **- target tissue/organ** **- critical effects at the LOAEL**	**Reference**

Method, guideline, deviations[1] if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results - NOAEL/LOAEL - target tissue/organ - critical effects at the LOAEL**	**Reference**
Combined chronic toxicity /carcinogenicity study OECD 453 (1981) GLP Sprague-Dawley rats, Crl:CD Br 72/sex/dose Interim sacrifice: 10/sex/dose Acceptable	Cymoxanil DPX-T3217-113 (Blend of lots 80317145 and 80321154); 97.8% 0, 50, 100, 700, 2000 ppm equal to 0, 1.98, 4.08, 30.3, 90.1 mg/kg bw/day (males) 0, 2.71, 5.36, 38.4, 126 mg/kg bw/day (females) Chronic / Carcinogenicity (23 months, 702-710 days): 62/sex/dose Interim sacrifice (12 months, 357 days): 10/sex/dose 0, 50, 100, 700, 2000 ppm equal to 0, 2.25, 4.58, 32.8, 97.4 mg/kg bw/day (males) 0, 3.07, 6.09, 43.5, 134 mg/kg bw/day (females)	Long-term NOAEL = 4.08 (♂) Long-term LOAEL= 30.3 (♂), based on clinical findings (↑hyperreactivity*), ↓ body weight (15.3%*), ↓ body weight gain (21.8%*), **histopathological findings (elongate spermatid degeneration[1] 17/56* versus 7/63 in control; retinal atrophy[2] 35/46* versus 10/45 in control)** **at 1.98 mg/kg bw/day:** - no effects on clinical findings, body weight/body weight gain, - elongate spermatid degeneration[1] (5/62 versus 7/63 in control), - retinal atrophy[2] (18/46 versus 10/45 in control) **at 4.08 mg/kg bw/day (NOAEL):** - clinical findings (↑hyperreactivity*), - no effects on body weight/body weight gain, - elongate spermatid degeneration[1] (4/62 versus 7/63 in control), - retinal atrophy[2] (19/46 versus 10/45 in control) **at 30.3 mg/kg bw/day (LOAEL):** - findings see above, - the MTD has been reached **at 90.1 mg/kg bw/day:** - clinical findings (↑hyperreactivity*, ↑aggressiveness*), - ↓ body weight (23.8%*), ↓ body weight gain (36.2%*), - no effects on food consumption (no statistics) - ↑ haematological parameter MCV (5.8%*) at 3-month and 6-month time points (2000 ppm was equal to 123 mg/kg bw/day on test day 0-91), - ↑relative testes weight (30.7%*), - ↑elongate spermatid degeneration[1] (29/62* versus 7/63 in control), - inflammation in lung* - retinal atrophy[2] 52/54* versus 10/45 in control Long-term NOAEL = 5.36 (♀) Long-term LOAEL = 38.4 (♀) based on histopathological findings (polyarteritis/ inflammation in lung/liver, retinal atrophy*[2], sciatic nerve degradation*[2]) **at 2.71 mg/kg bw/day:** - no effects on clinical findings, body weight/body weight gain **at 5.36 mg/kg bw/day (NOAEL):** - no effects on clinical findings, body weight/body weight gain **at 38.4 mg/kg bw/day (LOAEL):** - ↓ body weight (2.2%), ↓ body weight gain (3.4%), - further findings see above **at 126 mg/kg bw/day:** - no effects on clinical findings, food	RAR B.6.5.1.1., 1994a

Method, guideline, deviations[1] if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results - NOAEL/LOAEL - target tissue/organ - critical effects at the LOAEL**	**Reference**
		consumption (no statistics), - ↓ body weight (15.5%*), ↓ body weight gain (25.8%*), - histopathological findings (retinal atrophy*[2], sciatic nerve degradation*[2]), - polyarteritis/ inflammation in lung/liver/pancreas*, - the MTD has been reached Carcinogenic NOAEL ≥ 90.1 (♂) Carcinogenic NOAEL ≥ 126 (♀) Cymoxanil did not reveal any oncogenic potential	
Combined chronic toxicity /carcinogenicity study OECD 453 (1981) GLP Wistar rats, HsdCpb:WU strain, in-house bred 50/sex/dose Interim sacrifice (12 months): 10/sex/control; 20/sex/high dose Acceptable	Cymoxanil 0972, 98.8% and 498 VF973, 99.3% 0, 100, 500, 1200 ppm equal to 0, 4.7, 23.5, 58.8 mg/kg bw/day (males) 0, 6.4, 31.6, 75.8 mg/kg bw/day (females) Chronic / Carcinogenicity 24 months Interim sacrifice (12 months) Cymoxanil 0, 1200 ppm equal to 0, 67.6 mg/kg bw/day (males) 0, 85.5 mg/kg bw/day (females)	Long-term NOAEL = 4.7 (♂) Long-term LOAEL= 23.5 (♂), based on histopathological findings (lymphoid hyperplasia in rectum*) *Due to deviations of the study the NOAEL (♂) cannot be set properly with respect to findings on male reproductive organs.* **at 4.7 mg/kg bw/day (NOAEL) :** - - no effects on clinical findings, body weight, body weight gain, food consumption **at 23.5 mg/kg bw/day(LOAEL) :** - ↓body weight (6.2%*), ↓ body weight gain (7.3%*), - - seminiferous tubule atrophy in the testis (6/50 versus 4/50 in control), - further findings see above **at 58.8 mg/kg bw/day:** - ↓body weight (12.8%*), ↓ body weight gain (15.3%*), - seminiferous tubule atrophy in the testis (12/50* versus 4/50 in control), epididymal oligospermia with aspermia (11/50 versus 3/50 in control), - histopathological findings (lymphoid hyperplasia in rectum*, suppurative bronchopneumonia in lungs*), - the MTD has been reached Long-term NOAEL = 31.6 (♀) Long-term LOAEL = 75.8 (♀) based on histopathological findings (lymphoid hyperplasia in colon*, suppurative bronchopneumonia in lungs*) **at 6.4 mg/kg bw/day:** - - no effects on clinical findings, body weight, body weight gain, food consumption **at 31.6 mg/kg bw/day (NOAEL):** - no effects on clinical findings, body weight, body weight gain, food consumption **at 75.8 mg/kg bw/day (LOAEL):** - no effects on clinical findings, body weight, body weight gain, food consumption, - histopathological findings (lymphoid hyperplasia in colon*, suppurative bronchopneumonia in lungs*), - further findings see above,	RAR B.6.5.1.2., 2003

Method, guideline, deviations[1] if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results** **- NOAEL/LOAEL** **- target tissue/organ** **- critical effects at the LOAEL**	**Reference**
		- the MTD has not been reached Carcinogenic NOAEL ≥ 58.8 (♂) Carcinogenic NOAEL ≥ 75.8 (♀) Cymoxanil did not reveal any oncogenic potential	
Carcinogenicity study OECD 451 (1981) GLP Mouse, Crl:CD-1®BR 90/sex/dose Acceptable.	Cymoxanil DPX-T3217-113, 97.8% 0, 30, 300, 1500, 3000 ppm equal to 0, 4.19, 42.0, 216, 446 mg/kg bw/day (males) 0, 5.83, 58.1, 298, 582 mg/kg bw/day (females) 18 months	Long-term NOAEL = 4.19 (♂) Long-term LOAEL= 42.0 (♂), based on histopathological findings in liver* (apoptosis, pigment, granuloma, diffuse centrilobular hypertrophy)[2] and **epididymis (tubular dilatation*, aggregate lymphoid* and sperm cysts*)[1]** **at 4.19 mg/kg bw/day (NOAEL):** - no effects on clinical findings, body weight, body weight gain, food consumption **at 42.0 mg/kg bw/day(LOAEL):** - no effects on clinical findings, body weight, body weight gain, food consumption, - further findings see above **at 216 mg/kg bw/day:** - ↓body weight (7.4%*), ↓ body weight gain (34.3%*), - no effects on clinical findings, food consumption - histopathological findings in liver*[2], jejunum* and epididymis*[1] - the MTD has been reached **at 446 mg/kg bw/day:** - clinical findings* (pallor, stained fur) - ↓body weight (11.0%*), ↓ body weight gain (48.0%*), - no effects food consumption - histopathological findings in liver*[2], jejunum* and epididymis*[1] Long-term NOAEL = 5.83 (♀) Long-term LOAEL = 58.1 (♀) based on histopathological findings in stomach (hyperplastic gastropathy*) and duodenum (cystic enteropathy*) **at 5.83 mg/kg bw/day (NOAEL):** - no effects on clinical findings, body weight, body weight gain, food consumption **at 58.1 mg/kg bw/day(LOAEL):** - no effects on clinical findings, body weight, body weight gain, food consumption, - further findings see above **at 298 mg/kg bw/day:** - ↓body weight (5.9%*), ↓ body weight gain (15.0%*), - no effects on clinical findings, food consumption - histopathological findings in liver*[2], stomach*, duodenum* and jejunum* - the MTD has been reached **at 582 mg/kg bw/day:**	RAR B.6.5.2.1., 1994b

Method, guideline, deviations[1] if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results** **- NOAEL/LOAEL** **- target tissue/organ** **- critical effects at the LOAEL**	**Reference**
		- clinical findings* (pallor, weak, hunched over) - ↓body weight (8.8%*), ↓ body weight gain (26.6%*), - no effects food consumption - histopathological findings in liver*[2], stomach*, duodenum*, jejunum*, spleen* and bone marrow* Carcinogenic NOAEL ≥ 446 (♂) Carcinogenic NOAEL ≥ 582 (♀) Cymoxanil did not reveal any oncogenic potential	
Carcinogenicity study OECD 451 (1981) GLP Mouse, HsdOla: MF1 50/sex/dose Acceptable.	Cymoxanil 498VF973, 98.8% 0, 60,120, 600, 1200 ppm 0, 9.5, 18.7, 91.4, 178.3 mg/kg bw/day (males) 0, 9.5, 18.6, 92.4, 179.8 mg/kg bw/day (females) 18 months	Long-term NOAEL = 91.4 (♂) Long-term LOAEL= 178.3 (♂), based on macroscopic and histopathological findings in mesenteric lymph nodes (dicolouration* and haemorrhage*) of males found dead and sacrificed moribund **at 9.5 and 18.7 mg/kg bw/day:** - no effects on clinical findings, body weight, body weight gain, food consumption **at 91.4 mg/kg bw/day (NOAEL):** - no effects on clinical findings, body weight, body weight gain, food consumption, - no effects on mesenteric lymph nodes (dicolouration and haemorrhage) **at 178.3 mg/kg bw/day (LOAEL):** - no effects on clinical findings, body weight, body weight gain, food consumption, - further findings see above, - the MTD has not been reached Long-term NOAEL = 92.4 (♀) Long-term LOAEL = 179.8 (♀) based on histopathological findings in ovaries (follicular cysts*) in females terminal sacrificed and combined fates **at 9.5 and 18.6 mg/kg bw/day:** - no effects on clinical findings, body weight, body weight gain, food consumption **at 92.4 mg/kg bw/day (NOAEL):** - no effects on clinical findings, body weight, body weight gain, food consumption, - no effects ovaries (follicular cysts) **at 179.8 mg/kg bw/day (LOAEL):** - no effects on clinical findings, body weight, body weight gain, food consumption, - further findings see above - the MTD has not been reached Carcinogenic NOAEL ≥ 178.3 (♂) Carcinogenic NOAEL ≥ 179.8 (♀)	RAR B.6.5.2.2., 2002

Method, guideline, deviations[1] if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results - NOAEL/LOAEL - target tissue/organ - critical effects at the LOAEL**	**Reference**
		Cymoxanil did not reveal any oncogenic potential	

*Significance of difference from control: * p≤0.05, ** p≤0.01; for statistical analyses details please see RAR Volume 3CA_B-6*
[1] - The effects on sexual function and fertility (effects on testes and epididymis) are summarised in the section 2.6.6.1.
[2] - The effects on haematological parameters, liver, eyes and sciatic nerve degradation are summarised in the section 2.6.3.

No human data on long-term toxicity and carcinogenicity are available.

Table 32: Summary table of other studies relevant for long-term toxicity and carcinogenicity

Type of study/data	**Test substance**	**Relevant information about the study (as applicable)**	**Observations**	**Reference**
(Q)SAR analysis using Derek Nexus (v.6.01/Nexus v.2.2.1 (Jan 2018)), OECD Toolbox (v.4.1) and VEGA (v.1.1.3).	Cymoxanil technical	The metabolite of cymoxanil was evaluated for mammalian toxicity by *in-silico* modelling tools using QSAR software	With regards to the evaluation of carcinogenicity, tested compound cymoxanil did not trigger any rapid alert in DEREK Nexus "Carcinogenicity" model. Similarly, the OECD Toolbox (Carcinogenicity (genotoxic/ nongenotoxic) alerts by ISS and Oncologic primary classification) and VEGA Carcinogenicity model (CAESAR) did not identify any alert for non-genotoxic carcinogenicity. Cymoxanil can be designated as non-relevant regarding carcinogenicity based on this data. For more detailed data please refer to RAR Volume 3CA B-6, point 6.8.1.2 Study 2	RAR B.6.8.1.2. Study 2, 2019b

2.6.5.1 Short summary and overall relevance of the provided information on long-term toxicity and carcinogenicity

The long term toxicity and carcinogenicity has been investigated in rats and mice (two studies each): 2-year combined chronic toxicity/carcinogenicity study in rats (*RAR B.6.5.1.1., 1994a* and *RAR B.6.5.1.2., 2003*); carcinogenicity study in mice (*RAR B.6.5.2.1., 1994b* and *RAR B.6.5.2.2., 2002*).

Poor survival was observed in all dose-groups (21 - 45%) in ***two years study in rats*** *(RAR B.6.5.1.1., 1994a)*; however, there were no dose related trends in mortality for male and females rats.

Overall survival (%) after 23 months of exposure to cymoxanil

		Dose concentration (ppm in diet)				
	Sex	0	50	100	700	2000
% Survival	Males	26	29	24	45	34
	Females	21	34	34	27	40

With respect to clinical observations, dose related and statistically significantly increased hyperreactivity in males of the 2 highest dose groups (equal to 30.3 and 90.1 mg/kg bw/day) was considered biologically relevant as well as increased aggressiveness at 2000 ppm (equal to 90.1 mg/kg bw/day). The statistically significant and dose related reduced body weight (>15%) and body weight gain (g) (>20%) at termination were observed in males exposure from 700 ppm (equal to 30.3 mg/kg bw/day). These alterations are attributed to test substance-related toxicity and are considered adverse. Over the 1-year interval (test day 0-357), body weight gains were significantly decreased 16.1% and 28.8% in the 700 and 2000 ppm (equal to 32.8 and 97.4 mg/kg bw/day) males groups, respectively. The statistically significant female body weight and body weight gain alterations at 2000 ppm (equal to 126 mg/kg bw/day) are attributed to test substance-related toxicity and are considered adverse too. Statistically significant reductions in body weight (15.5%) and body weight gain (g) (25.8%) at termination were

observed in the 2000 ppm females. Body weight gain decrement was 29.2% for one year interval (test day 0-357) in this group (134 mg/kg bw/day). Although no statistical analysis has been performed with respect to food consumption, for the overall study period, food consumption was comparable in all treated groups when compared to the control. At 700 ppm the MTD was reached for males and at 2000 ppm the MTD was reached for females.

Mean body weights, gains and food consumption after 23 months of treatment

Parameter	Sex	Dose concentration (ppm in diet)				
		0	50	100	700	2000
Body weight (g)	males	870.5	767.6	779.3	737.0[1)]	663.4[1)]
	females	489.8	503.7	557.4	478.9	413.7[1)]
Body weight gain (g)	males	576.2	469.5	486.8	450.7[1)]	367.6[1)]
	females	289.6	306.9	357.1	279.8	215.0[1)]
Food consumption (g/rat/day)[2)]	males	30.4	29.6	29.9	29.8	28.7
	females	22.0	21.9	22.3	22.0	22.4

[1)] *Statistically significant (ANOVA and Dunnett`s test; $p \leq 0.05$)*

Investigations with respect to haematology showed a statistically significant increase (approximately 5.8 %*) in MCV (mean corpuscular volume) at 3-month and 6-month time point in males of the high dosage group (2000 ppm, equal to 123 mg/kg bw/day on test day 0-91). These alterations were not accompanied by alterations in other relevant haematological parameters. The 1-year interim sacrifice revealed statistically significant increases in the incidence of retinal atrophy in males from the 32.8 mg/kg bw/day and in females from 134 mg/kg bw/day. At study termination, the ocular examinations showed that retinal (photoreceptor cell) atrophy incidence was statistical significantly increased in a dose response manner in both sexes at 700 and 2000 ppm. See discussion under the section *Summary of repeated dose toxicity (point 2.6.3.1.1).*

With respect to findings on male reproductive organs weights, a statistically significant increase (30.7%*) of relative testes weight in the high dose (90.1 mg/kg bw/day) group was reported. Histopathological examination of testes revealed statistically significant and test compound-related increase of elongate spermatid degeneration at from 700 (equal to 30.3 mg/kg bw/day). See further discussion under the section *Summary of reproductive toxicity (point 2.6.6.1.1*). For more detailed data on effects on male reproductive organs please refer to RAR Volume 3CA B-6, section B.6.5.1.1.

A statistically significant compound related increase in incidence of axon/myelin degeneration of the sciatic nerve occurred in females at 700 and 2000 ppm, without any other signs of peripheral neuropathy. In the lung, the incidence of polyarteritis was statistical significantly increased in the 700 ppm and 2000 ppm females at the terminal sacrifice. In addition, the 2000 ppm females had significant increases in the incidence of histocytosis, alveolar inflammation, type II cell hyperplasia, alveolar wall squamous metaplasia and fibrosis/inflammation. Electron microscopy suggests test substance-induced phospholipidose. The incidence of the granulomatous inflammation was elevated in the 2000 ppm males and females. At study termination, other substance related and adverse histopathological changes (inflammation and/or polyarteritis) were reported in the liver of female rats at 700 ppm and 2000 ppm. Inflammation and/or polyarteritis were observed in the pancreas/stomach/small intestine/large intestine/urinary bladder and mesenteric lymph node as well as cystic atrophy of the mesenteric lymph node was documented in the 2000 ppm females.

Incidence of relevant histopathological findings after 23 months treatment with cymoxanil (excerpt)

Organ/tissue	Dose concentration (ppm in diet)									
	Males					Females				
	0	50	100	700	2000	0	50	100	700	2000
Lung										
haemorrhage	0/63	1/62	2/62	0/56	**6/61[1)]**	3/62	15/62	19/62	18/62	10/62
histiocytosis	14/63	16/62	19/62	15/56	19/61	15/62	20/62	27/62	24/62	**39/62[1)]**
inflammation (alveolar)	4/63	3/62	3/62	6/56	7/61	7/62	5/62	7/62	4/62	**16/62[1)]**
inflammation (granulomatous)	6/63	3/62	3/62	4/56	**11/61[1)]**	1/62	6/62	9/62	6/62	**15/62[1)]**
fibrosis/inflam-mation	4/63	3/62	1/62	1/56	3/61	0/62	0/62	0/62	1/62	**5/62**[1)]
polyarteritis	-	-	-	-	-	1/62	0/62	0/62	**2/62[1)]**	**7/62[1)]**
metaplasia (alveolar walls)	-	-	-	-	-	0/62	0/62	3/62	0/62	**4/62[1)]**
type II cell hyperplasia	-	-	-	-	-	0/62	2/62	3/62	3/62	**9/62[1)]**
Testes										

	elongate spermatid degeneration	7/63	5/62	4/62	**17/56[1)]**	**29/62[1)]**	-	-	-	-
	multinucleated spermatids	1/63	5/62	1/62	3/56	**8/62[1)]**				
Retina										
	atrophy	10/45	18/46	19/46	**35/46[1)]**	**52/54[1)]**	33/55	34/54	28/48	**47/52[1)]**	**54/55[1)]**
Liver										
	inflammation, (portal)	4/63	0/62	1/62	2/56	4/62	0/62	0/62	1/62	0/61	4/62
	inflammation/ necrosis/fibrosis/ haemorrhage	11/63	7/62	11/62	9/56	14/62	9/62	3/62	7/62	**14/61[1)]**	**15/62[1)]**
Pancreas										
	focal basophilic alteration	2/62	1/51	2/50	0/56	4/62	3/61	4/62	6/62	**13/61[1)]**	**7/62[1)]**
	inflammation	-	-	-	-	-	0/61	0/62	0/62	1/61	**8/62[1)]**
	inflammation/ fibrosis/pigment	10/62	10/51	12/50	8/56	10/62	12/61	7/62	9/62	16/61	**25/62[1)]**
	polyarteritis	7/62	3/51	2/50	2/56	11/62	2/61	0/62	2/62	0/61	**11/62[1)]**
Sciatic nerve										
	axon/myelin degeneration	17/63	7/50	10/48	10/32	20/62	10/61	9/62	14/62	**22/61[1)]**	**28/61[1)]**

[1)] *Statistically significant (Cochran-Armitage trend test or Fisher`s exact test; $p \leq 0.05$)*

There was no significant increase in the incidence of specific neoplasms, nor in the number of rats bearing neoplasms, in either sex. Therefore, Cymoxanil did not reveal any oncogenic potential up to and including the highest dose level tested (2000 ppm).

The overall NOAEL for long-term effects was set at 100 ppm (equal to 4.08 mg/kg bw/day), based on clinical signs (hyperreactivity), reduced body weight and body weight gain as well as the histological findings (polyarteritis/ inflammation in the lung/liver, elongate spermatid degeneration, retinal atrophy, sciatic nerve degradation) at 700 ppm (equal to 30.3 mg/kg bw/day). The NOAEL for carcinogenicity was ≥2000 ppm (90.1 mg/kg bw/day), the highest dose tested.

There were no dose related trends in mortality for male and females rats in ***two years study in rats*** *(RAR B.6.5.1.2., 2003)* and survival to termination was ≥60% in all dose-groups.

Overall survival (%) after 24 months of exposure to cymoxanil

		Dose group levels (ppm)			
	Sex	0	100	500	1200
% survival	males	82	64	70	60
	females	76	86	78	72

The statistically significant and dose related reduced body weight (12.8%) and body weight gain (g) (15.3%) at termination were observed in males exposure to 1200 ppm (equal to 58.8 mg/kg bw/day). At the high dose the mean male body weight and body weight gain was statistical significantly lower (>10%) at termination of the 12 month treatment period too. These alterations are considered adverse. The food consumption was statistical significantly decreased >10% (27.5%) at the termination of the 12 months treatment period at the high dose (equal to 67.6 mg/kg bw/day) in interim sacrifice group only. There were not toxicological relevant changes in the female body weight, body weight gain and the food consumption. Hence, at 1200 ppm the MTD was reached for males and was not reached for females.

Mean body weights, gains and food consumption after 24 months of treatment

		Dose group levels (ppm)			
Parameter	Sex	0	100	500	1200
Body weight [g]	males	533	510	500*	465*
	females	299	304	306	286
Body weight gain [g]	males	451	429	418*	382*
	females	223	229	230	209

Parameter	Sex	Dose group levels (ppm)			
		0	100	500	1200
Food consumption [g/rat/day]	males	20.8	20.8	19.7	20.4
	females	18.3	18.6	17.5	17.2

** statistically significant (Dunnett`s test; level of significance: $p \leq 0.05$)*

There were no toxicologically significant changes in haematology, clinical chemistry and urinalysis parameters in either male or female rats during the course of the study. There were no toxicologically relevant organ weight changes when compared to controls, however, organs weight was determined from 10 animals per sex per group only, instead of all animals. Regarding histopathological examination of animals terminally sacrificed including animals found dead and sacrificed moribund, statistically significant increases in the following non-neoplastic incidences were seen: lymphoid hyperplasia in rectum of males of the mid and high dose group (equal to 23.5 and 58.8 mg/kg bw/day, respectively), lymphoid hyperplasia in colon in females of the highest dose group (equal to 75.8 mg/kg bw/day) and suppurative bronchopneumonia in lungs of males and females of the highest dose group.

Relevant histological findings (number of animals affected) after 24 months with cymoxanil (animals terminally sacrificed including animals found dead and sacrificed moribund) (excerpt)

Tissue	Dose group levels [ppm]							
	Males				Females			
	0	100	500	1200	0	100	500	1200
Colon: lymphoid hyperplasia	4/50	0/50	1/50	7/50	0/50	0/50	2/50	***7/50****
Lungs: suppurative broncho-pneumonia	10/50	6/50	11/50	***22/50****	6/50	5/50	9/50	***15/50****
Testes: mild/moderate atrophy of seminiferous tubules	4/50	6/50	6/50	***12/50***1)***				
Rectum: lymphoid hyperplasia	1/50	2/50	***7/50****	***8/50****	3/50	0/50	0/50	2/50

** statistically significant (Z-test; level of significance: $p \leq 0.05$)*

With respect to findings on male reproductive organs, histopathological examination of testes after 24 months with cymoxanil revealed statistically significantly increased incidence of mild-to-moderate seminiferous tubule atrophy in the testis at 58.8 mg/kg bw/day. See further discussion under the section *Summary of reproductive toxicity (point 2.6.6.1.1)*. For more detailed data on effects on male reproductive organs please refer to RAR Volume 3CA B-6, section B.6.5.1.2.

Concerning the number of rats with benign and/or malignant neoplasms and rats with metastatic/infiltrative neoplasms, the only statistically significant increase was observed for malignant neoplasms in males of the mid dose group found dead or moribund sacrificed; however, this finding was not considered relevant since the incidence in the high dose group males was of no statistical significance and no dose-relationship is evident. Additionally, it should be noted that combined or summarised historical control data are not available (i.e. the range of values, the mean etc). A complete assessment of the relevance of HCD should be provided by the applicant based on the criteria as set out in Commission Regulation (EU) No 283/2013.

The incidences of specific neoplastic findings in liver and uterus of females are presented in the table below.

	Dead and Moribund				Terminal sacrifice				Combined fates			
	C	L	M	H	C	L	M	H	C	L	M	H
No. of rats examined	12	7	11	15	38	43	39	35	50	50	50	50
Liver												
- Adenocarcinoma-metastatic (MM)	1	1	2	5	-	-	-	-	1	1	2	5
- Hepatocellular carcinoma (M)	0	0	0	0	0	0	0	1	0	0	0	1
- Hepatocellular adenoma (B)	-	-	-	-	0	1	0	1	0	1	0	1
No. of rats examined	12	7	11	15	38	17	15	35	50	24	26	50
Uterus												
- Adenocarcinoma (M)	4	2	7	10	6	5	5	2	10	7	12	12
- Adenoma (B)	0	0	0	1	1	6	1	3	1	6	1	4
- Polyp(s) (B)	3	0	0	0	7	4	9	8	10	4	9	8
- Leiomyosarcoma (M)	-	-	-	-	0	0	1	0	0	0	1	0
- Squamous cell carcinoma (M)	-	-	-	-	1	1	1	1	1	1	1	1

B - Benign, M – Malignant and MM - Metastatic

The higher incidence of liver metastatic adenocarcinoma (MM) was observed in the high dose females of dead and moribund animals (D&M), the higher incidence of stomach metastatic adenocarcinoma (MM) was observed in the high dose D&M females as well as a non-statistically significant slight increase was observed for uterus adenocarcinoma (M) in D&M females of the mid and high dose groups. It is noteworthy that these were not primary liver tumours as well as not primary stomach tumours in D&M females. In all these animals the uterine adenocarcinoma was metastatic to several organs including the liver and stomach. The primary liver tumour (hepatocellular carcinoma) was observed only in a single high dose terminally sacrificed female.

For combined subgroup animals (i.e. animals found dead and moribund plus animals sacrificed at study termination), the following incidences of neoplasms were found to be increased with dose but revealed no statistically significance: liver adenocarcinoma (MM) in females, stomach adenocarcinoma (MM) in females, uterus adenocarcinoma (M) and adenoma (B). Though the adenocarcinoma of uterus was metastatic to liver in the five D&M females, the total incidence of primary tumour of uterus (adenocarcinoma) in the high dose females of the combined fate was comparable to the control females. The similar case was with stomach adenocarcinoma (MM) in females (combined fates: 0/50, 1/50, 1/50, 4/50). If a weight of the evidence approach is used, with other factors such as absence of increased liver weight; absence of preneoplastic changes such as hyperplasia, foci, or adenoma; lack of histological evidence of liver cell cytotoxicity; no increases in serum liver enzyme levels indicative of liver cell toxicity; lack of statistical significance and absence of the adenocarcinomas in either males within the study or in a second study conducted in another rat strain, it can be concluded that the very slight increase in female rats is not test substance related.

The NOAEL for long-term effects for females is proposed at 500 ppm (equal to 31.6 mg/kg bw/day) based on histopathological findings (lymphoid hyperplasia in colon and suppurative bronchopneumonia in lungs) at 1200 ppm (equal to 75.8 mg/kg bw/day). The overall NOAEL for long-term effects was set at 100 ppm (equal to 4.7 mg/kg bw/day), based on histopathological findings (lymphoid hyperplasia in rectum) in males at 500 ppm (equal to 23.5 mg/kg bw/day). Due to deviations of the study the NOAEL cannot be set properly with respect to findings on male reproductive organs.

Since no oncogenic effects were observed in study conducted up to and including the highest dose level tested, the NOAEL for carcinogenicity was ≥1200 ppm (equal to 58.8 mg/kg bw/day), the highest dose tested. This available information does not support a classification of cymoxanil for carcinogenicity.

In the ***first carcinogenicity study in mice*** *(RAR B.6.5.2.1., 1994b)*, a statistically significant decrease of survival was found for the high dose (582 mg/kg bw/day) females (survival 57%). Over the entire study interval of 18 months, no treatment related effects on the mortality of male mice were observed.

Overall survival for male and female mice after 18 months of dosing (% survival)

		Dose group levels [ppm]				
	Sex	0	30	300	1500	3000
% survival	males	67	70	78	65	73
	females	69	76	78	74	57[1)]

[1)] *statistically significant (Cochran-Armitage trend test; $p \leq 0.05$)*

With respect to clinical observations, the treatment-related incidences of pallor for both sexes, stained fur for males and weakness with hunched over for females were statistically significantly increased at the highest dose.

The statistically significant and dose related reduced body weight gain (g) at termination were observed in males (>34%) and females (≥15%) exposure from 1500 ppm (equal to 216 mg/kg bw/day and 298 mg/kg bw/day, respectively). The statistically significant female body weight decrease at 3000 ppm (equal to 582 mg/kg bw/day) is attributed to test substance-related toxicity too. Although no statistical analysis has been performed with respect to food consumption, for the overall study period, food consumption of treated mice was comparable in all treated groups when compared to the control. At 1500 ppm the MTD was reached for males and for females.

Mean body weights, body weight gains and food consumption after approx. 18 months of treatment.

Parameter	Sex	Dose group levels [ppm]				
		0	30	300	1500	3000
Body weight [g]	Males	41.7	41.2	40.3	38.6[1)]	37.1[1)]
	females	34.2	34.1	34.7	32.2[1)]	31.2[1)]
Body weight gain [g]	Males	10.2	9.4	8.6	6.7[1)]	5.3[1)]
	females	11.3	11.2	11.4	9.6[1)]	8.3[1)]
Food consumption [g][2)]	Males	5.6	5.6	5.5	5.4	5.3
	Females	5.8	5.9	5.9	5.8	5.6

[1)] *statistically significant (ANOVA and Dunnett`s test: $p \leq 0.05$)*

[2)] *no statistical analysis performed*

Investigations with respect to haematology showed a statistically significant increase (6.4 % and 10.6%, respectively) in MCV in males of the two highest dose groups (216 and 446 mg/kg bw/day) after 18-months of treatment. The similar statistically significant increase (6.1 %) in MCV was seen in males of the high dose groups after 3-months of treatment. These alterations in high dose group of males after 18-months of treatment were accompanied also by statistically significant decrease in RBC (22.4%). There were no toxicologically significant changes in haematology parameters of females as well as no toxicologically significant changes in clinical chemistry parameters in males and females.

Absolute and relative liver weight showed a statistically significant increase at the two high dose groups (10.3/18.1% and 13.0/26.8%, respectively) of females and this was accompanied by statistically significant treatment-related histological findings in liver (centrilobular apoptotic hepatocytes, macrophages containing a pigment, granuloma, diffuse centrilobular hypertrophy). Such significant treatment-related histological liver findings in males of three dose groups (42.0, 216 and 446 mg/kg bw/day) were not accompanied by liver weight changes. Cymoxanil did not induce alterations in hepatic cellular proliferation as well as in the rate of hepatic peroxisomal β-oxidation or the content of hepatic cytochrome P-450 after approximately one month of feeding (5 mice/group).

Histological evaluation exhibited statistically significant treatment-related findings also in other organs: increased incidence of hyperplastic gastropathy in female stomach and cystic enteropathy in female duodenum from 300 ppm; cystic enteropathy in jejunum of males and females from 1500 ppm; diffuse atrophy in spleen, congestion in bone marrow and thymus atrophy in females at 3000 ppm. These changes are considered test substance related and adverse. Additionally, based on pathological evaluation of 5 females which were sacrificed on or before test day 35, pancreatic acinar cell necrosis in females was considered an adverse effect at 3000 ppm.

Chronic dietary dose study in mice: relevant histological findings (number of animals affected) after 18 months of treatment (excerpt)

	Dose group levels [ppm]									
	Males					Females				
Finding	0	30	300	1500	3000	0	30	300	1500	3000
Liver:										
apoptosis/ pigment/ granuloma	1/80	1/80	***8/80[1)]***	***32/80[1)]***	***38/81[1)]***	18/80	15/80	24/80	***42/81[1)]***	***43/88[1)]***
centilobular hypertrophy	29/80	31/80	***45/80[1)]***	***63/80[1)]***	***68/81[1)]***	1/80	0/80	3/80	***12/81[1)]***	***20/88[1)]***
Stomach hyperplastic gastropathy	10/80	8/79	18/80	13/80	15/81	11/79	11/79	***23/79[1)]***	***30/81[1)]***	***36/88[1)]***
Duodenum: cystic enteropathy	1/80	0/80	0/80	0/79	5/80	0/78	0/77	***2/79[1)]***	***3/81[1)]***	***36/88[1)]***
Jejunum cystic enteropathy	0/80	0/80	0/80	***2/80[1)]***	***11/80[1)]***	0/79	0/79	0/78	***9/81[1)]***	***25/68[1)]***
Pancreas acinar cell necrosis	0/80	0/25	0/19	1/29	1/80	1/79	0/79	0/79	0/81	5/86
Spleen diffuse atrophy	1/80	0/33	0/29	1/38	6/81	1/79	1/27	1/23	3/22	***8/88[2)]***
Bone marrow congestion	2/80	1/25	0/17	1/29	6/81	0/79	0/18	0/17	0/24	***9/88[2)]***
Thymus atrophy	0/79	0/23	0/19	1/28	4/77	0/78	0/24	1/22	1/24	***10/84[2)]***
Testes tubular atrophy	18/80	27/80	24/80	30/80	***40/81[1)]***					
Epididymides										
tubular dilatation	0/80	1/80	***5/80[1)]***	***8/79[1)]***	***14/81[1)]***					
lymphoid aggregate	1/80	2/80	***6/80[1)]***	***8/79[1)]***	***10/81[1)]***					
oligospermia bilateral	6/80	3/80	9/80	***14/79[1)]***	***24/81[1)]***					
oligospermia unilateral	4/80	6/80	6/80	***8/79***	***19/81[1)]***					
sperm cyst	0/80	1/80	***5/80[1)]***	***9/79[1)]***	***21/81[1)]***					
sperm granuloma	0/80	1/80	0/80	***7/79[1)]***	***10/81[1)]***					

1) *statistically significant (Cochran-Armitage trend test: $p \leq 0.05$)*

2) *statistically significant (Fisher`s exact test: $p < 0.05$)*

With respect to findings on male reproductive organs, from 300 ppm (equal to 42.0 mg/kg bw/day) tubular dilatation, aggregate lymphoid and sperm cysts/cystic dilation of epididymis were statistically significantly increased in a dose-dependent manner. See further discussion under the section *Summary of reproductive toxicity (point 2.6.6.1.1*). For more detailed data on effects on male reproductive organs please refer to RAR Volume 3CA B-6, section B.6.5.2.1.

Concerning carcinogenicity, there was no significant increase in the incidence of the total number of mice bearing neoplasms, or the total number of specific neoplasms over the 18-month study period in either sex.

The NOAEL for long-term effects for females is proposed at 30 ppm (equal to 5.83 mg/kg bw/day) based on histopathological findings in stomach (hyperplastic gastropathy) and duodenum (cystic enteropathy) at 300 ppm (equal to 58.1 mg/kg bw/day). The overall NOAEL for long-term effects was set at 30 ppm (equal to 4.19 mg/kg bw/day), based on histopathological findings in liver (apoptosis, pigment, granuloma, diffuse centrilobular hypertrophy) and epididymis (tubular dilatation, aggregate lymphoid and sperm cysts) in males at 300 ppm (equal to 42.0 mg/kg bw/day).

Since no oncogenic effects were observed in study conducted up to and including the highest dose level tested, the NOAEL for carcinogenicity was ≥3000 ppm (equal to 446 mg/kg bw/day), the highest dose tested for males. This available information does not support a classification of cymoxanil for carcinogenicity.

In the ***second carcinogenicity study in mice*** *(RAR B.6.5.2.2., 2002)*, survival to termination exceeded 50% in all dose-groups. There were no dose related trends in mortality for male and females mice.

Overall % survival for male and female mice after 18 months of dosing

		Dose group levels [ppm]				
	Sex	0	60	120	600	1200
% survival	Males	64	56	64	76	52
	Females	74	64	76	60	70

Includes mice found dead or found moribund and sacrificed

For more detailed data on effects on long-term toxicity and carcinogenicity please refer to RAR Volume 3CA B-6, section B.6.5.2.2.

No treatment-related clinical signs were evident. No adverse findings were reported in eyes in the ophthalmological examinations.

The MTD was not reached for both sexes. The statistically significant reduced body weight (>10%) on weeks 1&2 only and body weight gain (g) (48.7%) on week 1 only were observed in males exposure at 1200 ppm (equal to 178.3 mg/kg bw/day). The statistically significant reduced body weight gain (g) (>10%) on week 25 only was observed in females exposure from 600 ppm (equal to 92.4 mg/kg bw/day). There were no toxicologically significant changes in haematology as well as effects on absolute or relative organ weights in both sexes. It should be noted that weights of thyroids (with parathyroids) and testes were not investigated.

Historical control data may be valuable in the interpretation of the results of the study. Although historical control data (non-neoplastic and neoplastic findings) from different studies are included in the original study report (Historical data 40), summarised HCD are not available (i.e. with the range of values, the mean etc), no obligatory information (e.g. name of laboratory, age and strain of animals, year of the studies) is available for these studies.

There were no significant increases in any of the gross changes observed except for a significant increase in the incidence of discolouration of the mesenteric lymph nodes in the highest dose males found dead and sacrificed moribund. This dicolouration of mesenteric lymph nodes was a red discolouration resulting from microscopically identified haemorrhage (3/18 [17%], 7/22 [32%], 1/18 [6%], 4/12 [33%], 11/24* [46%]). It should be noted that this gross finding as well as histopathological finding (haemorrhage) were not replicated in the animals reaching terminal sacrifice. The total incidence of haemorrhage in mesenteric lymph nodes over the course of the study was seen in all treatment male groups, however, values did not attain statistical significance.

Regarding other histopathological findings in males, a statistically significant increase in the incidence of heart myocardial degeneration was seen in all treatment male groups found dead and sacrificed moribund (0/18 [0%], 5/22* [22.7%], 4/18* [22.2%], 8/12* [66.7%], 8/24* [33.3%]). This statistically significant increase was not dose related and it was not replicated in the males reaching terminal sacrifice. The total incidence of heart myocardial degeneration over the course of the study was also seen in all treatment male groups, however, values did not attain statistical significance.

Carcinogenic study in mice: relevant macroscopic findings (number of animals affected) of animals terminally sacrificed and animals found dead and sacrificed moribund

Parameter	Dose group levels [ppm]									
	Males					Females				
	0	60	120	600	1200	0	60	120	600	1200
Mesenteric lymph nodes: discolouration										
Animals found dead or sacrificed moribund	0/18 0%	3/22 14%	0/18 0%	0/12 0%	**5/24*** 21%	1/13 8%	2/19 11%	1/12 8%	0/20 0%	2/15 13%
Animals terminally sacrificed	3/32 9%	4/28 14%	1/32 3%	3/38 8%	2/26 8%	1/37 3%	3/31 10%	3/38 8%	3/30 10%	0/35 0%
Total	3/50 6%	7/50 14%	1/50 2%	3/50 6%	7/50 14%	2/50 4%	5/50 10%	4/50 8%	3/50 6%	2/50 4%

** statistically significant (Z-test; level of significance: $p \leq 0.05$)*

The main histopathological finding was a statistically significant increase in ovaries follicular cysts in the highest dose group observed in females terminal sacrificed and combined fates (4 incidences from 50 females, 8%*).

Carcinogenic study in mice: relevant histological findings (number of animals affected) after 18 months of treatment (animals terminally sacrificed including animals found dead and sacrificed moribund) (excerpt)

	Dose group levels [ppm]									
	Males					Females				
Parameter	0	60	120	600	1200	0	60	120	600	1200
Ovary: follicular cysts	-	-	-	-	-	0/50 0%	0/50 0%	0/50 0%	0/50 0%	***4/50**** ***8%***
Mesenteric lymph nodes Haemorrhage	8/50 16%	13/32 41%	9/29 31%	9/26 35%	17/50 34%	10/50 20%	9/30 30%	8/26 31%	5/33 15%	13/50 26%
Heart myocardial degeneration (all animals examined)	3/50 6%	5/23 22%	4/18 22%	8/12 67%	8/50 16%	2/50 4%	0/19 0%	0/12 0%	1/21 5%	1/50 2%
dead or moribund	3	5	4	8	8	0	0	0	1	0
terminal sacrifice	3	0	-	-	0	2	-	-	-	1

** statistically significant increased (Z-test; level of significance: $p \leq 0.05$)*

Concerning the number of mice with benign/malignant neoplasms or mice with metastatic/infiltrative neoplasms no significant increase could be identified when compared with the control groups. The number and types of neoplasms noted in mice of all dose groups were considered to be similar in both treated and control animals and were within historical background. However, HCD provided did not meet the criteria as set out in Commission Regulation (EU) No 283/2013.

Carcinogenic study in mice: neoplastic findings (number of animals affected) (excerpt)

Parameter	Dose group levels [ppm]									
	Males					Females				
	0	60	120	600	1200	0	60	120	600	1200
Malignant lymphoma										
Animals found dead or sacrificed moribund	4/18 22%	3/22 14%	5/18 28%	0/12 0%	6/64 25%	2/13 15%	9/19 47%	6/12 50%	6/20 30%	3/15 20%
Animals terminally sacrificed	3/32 9%	9/28 32%	4/32 13%	8/38 21%	4/26 15%	7/37 19%	8/31 26%	11/38 29%	6/30 20%	9/35 26%
Total	7/50 14%	12/50 24%	9/50 18%	8/50 16%	10/50 20%	9/50 18%	17/50 34%	17/50 34%	12/50 24%	12/50 24%
Total mice with any tumour found dead or sacrificed moribund										
Total mice with benign tumours	2/18	1/22	3/18	2/12	3/24	2/13	5/19	3/12	4/20	4/15
Total mice with malignant tumours	4/18	3/22	6/18	0/12	7/24	7/13	10/19	7/12	7/20	6/15
Total benign and malignant tumours	6/18	3/22	8/18	2/12	9/24	8/13	11/19	8/12	11/20	10/15
Total mice with any tumour animals terminally sacrificed										
Total mice with benign tumours	5/32	3/28	2/32	3/38	3/26	9/37	3/31	6/38	3/30	6/35
Total mice with malignant tumours	4/32	9/28	8/32	10/38	4/26	9/37	9/31	11/38	9/30	9/35
Total benign and malignant tumours	9/32	10/28	10/32	12/38	6/26	13/37	10/31	15/38	11/30	13/35
Total mice with any tumour all animals (50 animals per group)										
Total mice with benign tumours	7	4	5	5	6	11	8	9	7	10
Total mice with malignant tumours	8	12	14	10	11	16	19	18	16	15
Total benign and malignant tumours	15	13	18	14	15	21	21	23	22	23

Considering the top dose selection, the amount of compound administered was not high enough to elicit clear evidence of toxicity. The MTD was not reached for both sexes. With respect to males, no clear substance related effect could be found at the highest dose level (1200 ppm corresponding to 178.3 mg/kg bw/ day) and no robust NOAEL could be established. The overall NOAEL for long-term effects is proposed at 600 ppm (equal to 91.4 mg/kg bw/day) based on macroscopic and histopathological findings in mesenteric lymph nodes (dicolouration and haemorrhage) of males found dead and moribund as well as histopathological findings in the ovaries (follicular cysts) at 1200 ppm (equal to 178.3 mg/kg bw/day).

Since no oncogenic effects were observed in study conducted up to and including the highest dose level tested, the NOAEL for carcinogenicity was ≥1200 ppm (equal to 178.3 mg/kg bw/day), the highest dose tested for males. This available information does not support a classification of cymoxanil for carcinogenicity.

2.6.5.2 Comparison with the CLP criteria regarding carcinogenicity

Annex I Section 3.6.1.1 of the CLP Regulation defines a carcinogen as a substance which induces cancer or increase its incidence. Substances which have induced benign and malignant tumours in well performed experimental studies on animals are considered also to be presumed or suspected human carcinogens unless there is strong evidence that the mechanism of tumour formation is not relevant for humans. Carcinogenic substances are allocated to Category 1 (known or presumed human carcinogens) or Category 2 (suspected human carcinogens).

A substance is classified in Category 1 for carcinogenicity on the basis of epidemiological and/or animal data. Substances known to have carcinogenic potential for humans (based largely on human evidence) are classified in Category 1A. Substances presumed to have carcinogenic potential for humans (based largely on animal evidence) are classified in Category 1B. In addition, on a case-by-case basis, scientific judgement may warrant a decision of presumed human carcinogenicity derived from studies showing limited evidence of carcinogenicity in humans together with limited evidence of carcinogenicity in experimental animals.

The placing of a substance in Category 2 is done on the basis of evidence obtained from human and/or animal studies, but which is not sufficiently convincing to place the substance in Category 1A or 1B, based on strength of evidence together with additional considerations. Such evidence may be derived either from limited evidence of carcinogenicity in human studies or from limited evidence of carcinogenicity in animal studies.

Studies performed with cymoxanil in the rat and mouse do not provide sufficient evidence of carcinogenicity based on an overall weight and strength of evidence approach and in consideration of the important factors in Annex I section 3.6.2.2.6 of the CLP Regulation. However, it should be noted that the MTD was not reached for both sexes in the second carcinogenicity study in mice and for females in second combined chronic toxicity\carcinogenicity studies in rats. The MTD dose is needed for the identification of carcinogenicity and if the highest dose tested had been adequate in the second mouse study, it would give confidence in this negative result, however it is not the case.

The conclusion of the RAC Opinion for cymoxanil (2012) was that "*no oncogenic effects were observed in studies conducted with cymoxanil, either in rat or in mouse carcinogenicity studies*". No new information has been submitted for the renewal of approval of cymoxanil regarding carcinogenicity and no change of the harmonised classification of cymoxanil is proposed.

Table 33: Compilation of factors to be taken into consideration in the hazard assessment

Species and strain	Tumour type and background incidence	Multi-site responses	Progression of lesions to malignancy	Reduced tumour latency	Responses in single or both sexes	Confounding effect by excessive toxicity?	Route of exposure	MoA and relevance to humans
-	-	-	-	-	-	-	-	-

2.6.5.3 Conclusion on classification and labelling for carcinogenicity

Based on the available data, cymoxanil does not require classification for carcinogenicity according to Regulation (EC) No 1272/2008.

RAC evaluation of carcinogenicity

Summary of the Dossier Submitter's proposal

The carcinogenicity of cymoxanil has been investigated in rats and mice (two studies each).

Cymoxanil did not reveal any oncogenic potential in any of the four studies, up to and including the highest dose levels tested. The DS proposed **no classification** of cymoxanil for carcinogenic effects.

Comments received during consultation

No comments were received.

Assessment and comparison with the classification criteria

The conclusion of the RAC Opinion for cymoxanil (2012) was that "*no oncogenic effects were observed in studies conducted with cymoxanil, either in rat or in mouse carcinogenicity studies*". No new information has been submitted for the renewal of approval of cymoxanil regarding carcinogenicity since then with the exception of a QSAR evaluation of the metabolite of cymoxanil, which has yielded no structural alerts for carcinogenicity. Since the 2012 opinion was very condensed, the studies are presented here in a somewhat more comprehensive form.

The long-term toxicity and carcinogenicity have been investigated in rats and mice (two studies each): 2-year combined chronic toxicity/carcinogenicity study in rats (RAR B.6.5.1.1., 1994a and RAR B.6.5.1.2., 2003); carcinogenicity study in mice (RAR B.6.5.2.1., 1994b and RAR B.6.5.2.2., 2002).

In the **first two year study in rats** (RAR B.6.5.1.1., 1994a) the survival rate was decreased in all groups, including the control group without any dose dependency (table below). Therefore, this effect is considered not treatment related.

***Table**: Overall survival (%) after 23 months of exposure to cymoxanil*

	Sex	Concentration in diet (ppm)				
		0	50	100	700	2000
% Survival	Males	26	29	24	45	34
	Females	21	34	34	27	40

Relevant histopathological findings of this study are summarized in the table below.

***Table**: Incidence of relevant histopathological findings after 23 months treatment with cymoxanil. * p < 0.05 Cochran-Armitage trend test or Fisher's exact test*

Organ/tissue	Concentration in diet (ppm)									
	Males					Females				
	0	50	100	700	2000	0	50	100	700	2000
Lung										
haemorrhage	0/63	1/62	2/62	0/56	**6/61***	3/62	15/62	19/62	18/62	10/62
histiocytosis	14/63	16/62	19/62	15/56	19/61	15/62	20/62	27/62	4/62	**16/62***
inflammation (alveolar)	4/63	3/62	3/62	4/56	**11/61***	7/62	5/62	7/62	6/62	**15/62***
inflammation (granulomatous)	6/63	3/62	3/62	1/56	3/61	1/62	6/62	9/62	1/62	**5/62***
fibrosis/inflammation	4/63	3/62	1/62	-	-	0/62	0/62	0/62	**2/62***	**7/62***
polyarteritis	-	-	-	-	-	1/62	0/62	0/62	0/62	**4/62***
metaplasia (alveolar walls)	-	-	-	-	-	0/62	0/62	0/62	3/62	**9/62***
type II cell hyperplasia	-	-	-	-	-	0/62	2/62	3/62		
Testes										
elongate spermatid degeneration	7/63	5/62	4/62	**17/56***	**29/62***	-	-	-	-	-
multinucleated spermatids	1/63	5/62	1/62	3/56	**8/62***					
Retina										
atrophy	10/45	18/46	19/46	**35/46***	**52/54***	33/55	34/54	28/48	**47/52***	**54/55***
Liver										
inflammation, (portal)	4/63	0/62	1/62	2/56	4/62	0/62	0/62	1/62	0/61	4/62
inflammation/ necrosis/fibrosis/	11/63	7/62	11/62	9/56	14/62	9/62	3/62	7/62	**14/61***	**15/62***

haemorrhage										
Pancreas focal basophilic alteration inflammation inflammation/ fibrosis/pigment polyarteritis	2/62 - 10/62 7/62	1/51 - 10/51 3/51	2/50 - 12/50 2/50	0/56 - 8/56 2/56	4/62 - 10/62 11/62	3/61 0/61 12/61 2/61	4/62 0/62 7/62 0/62	6/62 0/62 9/62 2/62	**13/61*** 1/61 16/61 0/61	**7/62*** **8/62*** **25/62*** **11/62***
Sciatic nerve axon/myelin degeneration	17/63	7/50	10/48	10/32	20/62	10/61	9/62	14/62	**22/61***	**28/61***

There was no significant increase in the incidence in specific neoplasms, nor in the number of rats bearing neoplasms, in either sex. Therefore, the NOAEL for carcinogenicity was ≥ 2000 ppm (90.1 and 126 mg/kg bw/d for males and females, respectively), the highest dose tested.

In the **second two year study in rats** (RAR B.6.5.1.2., 2003) no dose related trends in mortality for male or female rats were observed. The survival rate was above 60% in all dose-groups (table below).

***Table**: Overall survival (%) after 24 months of exposure to cymoxanil*

	Sex	**Concentration in diet (ppm)**			
		0	**100**	**500**	**12000**
% Survival	Males	82	64	70	60
	Females	76	86	78	72

Concerning the number of rats with benign and/or malignant neoplasms and rats with metastatic/infiltrative neoplasms, the only statistically significant increase was observed for malignant neoplasms in males of the mid dose group found dead or moribund sacrificed. However, this finding was not considered relevant since the incidence in the high dose group males was of no statistical significance and no dose-relationship is evident. No HCD were available.

The incidences of specific neoplastic findings in liver and uterus of females are presented in the table below.

***Table**: Incidences of neoplastic findings in liver and uterus of female rats in the 2003 rat carcinogenicity study. B – Benign, M – Malignant, MM - Metastatic*

	Dead and moribund				**Terminal sacrifice**				**Combined fates**			
	C	**L**	**M**	**H**	**C**	**L**	**M**	**H**	**C**	**L**	**M**	**H**
No. of rats examined	12	7	11	15	38	43	39	35	50	50	50	50
Liver												
Adenocarcinoma-metastatic (MM)	1	1	2	5	-	-	-	-	1	1	2	5

Hepatocellular carcinoma (M)	0	0	0	0	0	0	0	1	0	0	0	1
Hepatocellular adenoma	-	-	-	-	0	1	0	1	0	1	0	1
No. of rats examined	12	7	11	15	38	17	15	35	50	24	26	50
Uterus												
Adenocarcinoma (M)	4	2	7	10	6	5	5	2	10	7	12	12
Adenoma (B)	0	0	0	1	1	6	1	3	1	6	1	4
Polyp(s) (B)	3	0	0	0	7	4	9	8	10	4	9	8
Leiomyosarcoma (M)	-	-	-	-	0	0	1	0	0	0	1	0
Squamous cell carcinoma (M)	-	-	-	-	1	1	1	1	1	1	1	1

In high dose dead and moribund females, a higher incidence of metastatic adenocarcinoma of liver and stomach was observed. It is noteworthy, that these were neither primary liver tumours nor primary stomach tumours. In dead and moribund females of the mid and high dose, a non-statistically significant increase in the incidence of adenocarcinoma of the uterus was observed. In these animals, the uterine adenocarcinomas were metastatic to several organs including the liver and stomach. A primary liver tumour (hepatocellular carcinoma) was observed in one high dose female.

For combined subgroups (i.e. animals found dead and moribund plus animals sacrificed at study termination), the following incidences of neoplasms appeared to be dose related: liver adenocarcinoma in females, stomach adenocarcinoma in females, uterus adenocarcinoma, and adenoma. However, the difference compared to the control group was not statistically significant for any dose group.

In a weight of evidence approach considering factors such as absence of increased liver weight, absence of preneoplastic changes, lack of histological evidence of liver cell cytotoxicity, no increases in serum liver enzyme levels indicative of liver cell toxicity, lack of statistical significance, and absence of adenocarcinomas in either males within the study or in a second study conducted in another rat strain, it can be concluded that the slight increase of neoplasms in female rats is not test substance related.

Since no oncogenic effects were observed in this study the NOAEL for carcinogenicity was ≥ 1200 ppm (equal to 58.8 and 75.8 mg/kg bw/d for males and females, respectively), the highest dose tested.

The available data from two rat studies do not support a classification of cymoxanil for carcinogenicity.

In the **first carcinogenicity study in mice** (RAR B.6.5.2.1., 1994b), the survival rate was statistically significantly decreased in high dose (582 mg/kg bw/d) females (survival 57%) at the end of the study after 18 months. Male rats, however, did not show increased mortality in any dose group (table below).

***Table**: Overall survival for male and female mice after 18 months of dosing (% survival) * p < 0.051 Cochran-Armitage trend test*

	Sex	**Concentration in diet (ppm)**				
		0	**30**	**300**	**1500**	**3000**
% survival	Males	67	70	78	65	73

	Females	69	76	78	74	57*

There was no significant increase in the incidence of the total number of mice bearing neoplasms, or the total number of specific neoplasms over the whole study period in either sex.

Since no oncogenic effects were observed in the study including the highest dose group, the NOAEL for carcinogenicity was ≥ 3000 ppm (equal to 446 mg/kg bw/d), the highest dose tested for males.

In the **second carcinogenicity study in mice** (RAR B.6.5.2.2., 2002) no dose related trends in mortality in males or females were observed (table below).

***Table**: Overall survival for male and female mice after 18 months of dosing (% survival)*

	Sex	**Concentration in diet (ppm)**				
		0	**60**	**120**	**600**	**1200**
% survival	Males	64	56	64	76	52
	Females	74	64	76	60	70

Includes mice found dead or found moribund and sacrificed

There was no significant increase in the incidence of the total number of mice bearing neoplasms, or the total number of specific neoplasms over the whole study period in either sex.

Since no oncogenic effects were observed in the study including the highest dose group, the NOAEL for carcinogenicity was ≥ 3000 ppm (equal to 446 and 582 mg/kg bw/d for males and females, respectively), the highest dose tested.

In the **second carcinogenicity study in mice** (RAR B.6.5.2.2., 2002) no dose related trends in mortality in males and females were observed (table below).

***Table**: Overall survival for male and female mice after 18 months of dosing (% survival)*

	Sex	**Concentration in diet (ppm)**				
		0	**60**	**120**	**600**	**1200**
% survival	Males	64	56	64	76	52
	Females	74	64	76	60	70

No significant increase in neoplasms could be identified compared to control groups. The number and types of neoplasms noted in mice of all dose groups were similar in both treated and control animals and were within the range of historical controls (see the table below). However, HCD provided did not meet the criteria as set out in Commission Regulation (EU) No 283/2013.

***Table**: Carcinogenic study in mice, neoplastic findings (number of animals affected).*

Parameter	**Concentration in diet (ppm)**	
	Males	**Females**

	0	60	120	600	1200	0	60	120	600	1200
Malignant lymphoma										
Animals found dead or sacrificed moribund	4/18 22%	3/22 14%	5/18 28%	0/12 0%	6/64 25%	2/13 15%	9/19 47%	6/12 50%	6/20 30%	3/15 20%
Animals terminally sacrificed	3/32 9%	9/28 32%	4/32 13%	8/38 21%	4/26 15%	7/37 19%	8/31 26%	11/38 29%	6/30 20%	9/35 26%
Total	7/50 14%	12/50 24%	9/50 18%	8/50 16%	10/50 20%	9/50 18%	17/50 34%	17/50 34%	12/50 24%	12/50 24%
Total mice with any tumour all animals (50 animals per group)										
Total mice with benign tumours	7	4	5	5	6	11	8	9	7	10
Total mice with malignant tumours	8	12	14	10	11	16	19	18	16	15
Total benign and malignant tumours	15	13	18	14	15	21	21	23	22	23

No oncogenic effects were observed in this study. The NOAEL for carcinogenicity was ≥ 1200 ppm (equal to 178.3 and 179.8 mg/kg bw/d for males and females, respectively), the highest dose tested.

Conclusion on classification

Studies performed with cymoxanil in rats and mice did not provide sufficient evidence of carcinogenicity based on overall weight and strength of evidence. However, it should be noted that the maximum tolerated dose was not reached for either sex in the second carcinogenicity study in mice and for females in the second combined chronic toxicity/carcinogenicity study in rats.

No new information has been submitted for the renewal of approval of cymoxanil regarding carcinogenicity. After a second thorough evaluation of the data no change of the harmonised classification of cymoxanil is proposed.

RAC concurs with the DS that **no classification** for cymoxanil for carcinogenicity is warranted.

This recommendation is in line with RAC's previous evaluation (RAC, 2012) of Cymoxanil.

2.6.6 Summary of reproductive toxicity [equivalent to section 10.10 of the CLH report template]

2.6.6.1 Adverse effects on sexual function and fertility – generational studies [equivalent to section 10.10.1 of the CLH report template]

For more detailed data on effects on sexual function and fertility please refer to RAR Volume 3CA B-6, section B.6.3., B.6.5., B.6.6. and section B.6.7.1.2.

Table 34: Summary table of animal studies on adverse effects on sexual function and fertility – generational studies

Method, guideline, deviations[1] if any, species, strain, sex, no/group	**Test substance (Batch No; purity w/w), dose levels duration of exposure**	**Results** **- NOAEL/LOAEL (for sexual function and fertility, parents)** **- target tissue/organ** **- critical effects at the LOAEL**	**Reference**
Two-generation reproduction toxicity study OECD 416 (1983) GLP Rat, Hsd Cpb: WU 30/sex/group Acceptable	Cymoxanil 0972 and 498VF973, 98.8% 0, 150, 450, 1350 ppm equal to 0, 10.5, 31.6, 94.0 mg/kg bw/day (F0 males) and 0, 14.9, 42.8, 116.3 mg/kg bw/day (F0 females premating, gestation and lactation) Oral: diet Approximate number of dose weeks: mating and throughout the mating period: males F0/F1 (14/18 weeks), females F0/F1 (10/14 weeks) females F0/F1: gestation (21 days) and lactation (21 days)	**Parental** NOAEL: 31.6 (♂) – 42.8 (♀) mg/kg bw/day LOAEL: 94.0 (♂) – 116.3 (♀) mg/kg bw/ day based on ↓bw gain premating (F0 males: 17%, F1 males: 14%, F0 female: 10%), gestation (F1 female: 20%), lactation (F0 female: 78%) ↓FC premating (F1 males: 10%, F0/F1 female: 9%), gestation (F0/F1 female: 11%/8%), lactation (F0/F1 females: 33%/26%) **Reproductive** NOAEL: 31.6 (♂) – 42.8 (♀) mg/kg bw/day LOAEL: 94.0 (♂) – 116.3 (♀) mg/kg bw/day based on F1 generation: ↓ mean number of corpora lutea, ↓mean number of implantations, ↑post-implantation loss (%), ↓mean litter size, ↓ live pups born (%) **Offspring** NOAEL: 10.5 (♂) – 14.9 (♀) mg/kg bw/day LOAEL: 31.6 (♂) – 42.8 (♀) mg/kg bw/day based on ↓bw (both sexes combined F1 pups Days 14 and 21: >10%; F2 pups Days 7, 14 and 21: >8%)	RAR B.6.6.1.1., 2001
Two-generation reproduction toxicity study OECD 416 (1983) GLP Rat, Crl:CD®BR 30/sex/group Acceptable	Cymoxanil, DPX-T3217-113, 97.8% 0, 100, 500, 1500 ppm equal to 0, 6.5, 32.1, 97.9 mg/kg bw/day (F0 males) 0, 6.65, 34.7, 103.0 mg/kg bw/day (F0 females gestation) Oral: diet Approximate number of dose weeks: mating and throughout the mating period: males F0/F1 (16/32 weeks), females F0/F1 (10/15 weeks) females F0/F1: gestation (21 days) and lactation (21 days)	**Parental** NOAEL: 32.1 – 34.7 mg/kg bw/day LOAEL: 97.9 – 103 mg/kg bw/day based on clinical signs (end of tail missing, necrotic tip of tail, sore), ↓bw (>10%) premating (F0 males, F0 female), gestation (F0 female) ↓bw gain premating (F0 males: 18%, F1 males: 14%, F0 female: 23%, F1 female: 14%), gestation (F0 female: 12%) ↓FC (>10%) premating (F0/F1 males, F1 female), 1st gestation (F1 female) **Reproductive** NOAEL: > 97.9 – 103.0 mg/kg bw/day LOAEL: Not obtained Did not cause adverse effects at highest dose tested **Offspring** NOAEL: 6.5 – 6.65 mg/kg bw/day LOAEL: 32.1 – 34.7 mg/kg bw/day based on ↓viability index (%) of F1 pups (days 1-4) ↓bw (both sexes combined F2B pups, Days 4, 7, 14 and 21: 13-18%)	RAR B.6.6.1.2., 1993
One generation reproduction toxicity study OECD 415	Cymoxanil 0972, 98.8%	**Parental** NOAEL: 68.4 (combined) mg/kg bw/day LOAEL: 127.7 (combined) mg/kg bw/day based on	RAR B.6.6.1.3., 1998

Method, guideline, deviations[1] if any, species, strain, sex, no/group	**Test substance (Batch No; purity w/w), dose levels duration of exposure**	**Results** **- NOAEL/LOAEL (for sexual function and fertility, parents)** **- target tissue/organ** **- critical effects at the LOAEL**	**Reference**
(1983) GLP Rat, Hsd Cpb: WU 15/sex/group Supportive only A range finding study	0, 750, 1500 and 3000 ppm equal to 0, 57.7, 114.3, 226.2 mg/kg bw/day (males premating) and 0, 75.1, 136.1, 240.8 mg/kg bw/day (females premating, gestation and lactation) 0, 68.4, 127.7, 235.2 mg/kg bw/day (combined) Oral: diet Approximate number of dose weeks: mating and throughout the mating period: males (15 weeks), females (10 weeks) females: gestation (21 days) and lactation (21 days)	↓bw gain female (gestation 12%) ↓FC female: all premating (9%), all gestation (≥10%), all lactation (≥20%) periods **Reproductive** NOAEL: 127.7 (combined) mg/kg bw/day LOAEL: 235.2 (combined) mg/kg bw/day based on ↓female fertility index (%), ↓ mean number of corpora lutea, (%), ↓mean number of implantations, ↑pre-implantation loss (%), ↑post-implantation loss (%), ↓mean litter size Gross necropsy: bilateral small & flaccid **testes** of 5 males **Offspring** LOAEL: 68.4 (combined) mg/kg bw/day based on ↓bw (both sexes combined pups Days 14 and 21: >10%)	
Developmental neurotoxicity study in rats US EPA OPPTS 870.6300 [complies generally with OECD 426 (2007)] GLP Rat CD®(SD)IGS VA/Plus® 25 females/dose Acceptable	Cymoxanil DPX-T3217-113; 97.8 % 0, 5, 50, 100 mg/kg bw/day administered from GD 6 to lactation day 21, gavage	Neurodevelopmental NOAEL: ≥ 100 mg/kg bw/day Neurodevelopmental LOAEL: Not obtained. No developmental neurotoxic effects up to the highest dose level tested Maternal NOAEL: 5 mg/kg bw/day Systemic LOAEL: 50 mg/kg bw /day based on ↓body weight gain at GD 6 – 9 (49.1%) ↓FC (13.2%) Developmental NOAEL = 50 mg/kg bw/day Developmental LOAEL = 100 mg/kg bw/day based on ↑pup mortality Days 2-5/9-12 post-partum ↓viability index on day 5, ↓lactation indexes on day 12/22, ↓average number of live pups/litter, ↓ live litter size, clinical observations in pups (cold to the touch, not nursing and not nesting),	RAR B.6.7.1.2., 2001
90 days rat oral, dietary OECD 408 (1981) GLP Sprague-Dawley rat (Crl:CD®BR) 10/sex/dose Acceptable	Cymoxanil DPX-T3217-107, 96.8% 0, 100, 750, 1500, 3000 ppm equal to 0, 6.54, 47.6, 102, 224 mg/kg bw /day (males) 0, 8.00, 59.9, 137, 333 mg/kg bw /day (females)	NOAEL = 6.54 (♂) LOAEL = 47.6 (♂) based on histopathological findings in **testes** (bilateral elongate spermatid degeneration) and ↑ relative testes weight (10%) **Epididymis:** cell debris and hypospermia at 224 mg/kg bw /day MTD (♂) at 224 mg/kg bw /day based on ↓ body weight gain (14.6%), ↓ body weight gain (21.9%), ↓ overall food conversion efficiency (g wt gain/g food consumption) (18.7%) NOAEL = 137 (♀)	RAR B.6.3.2.1. Study 1, 1993

Method, guideline, deviations[1] if any, species, strain, sex, no/group	**Test substance (Batch No; purity w/w), dose levels duration of exposure**	**Results** **- NOAEL/LOAEL (for sexual function and fertility, parents)** **- target tissue/organ** **- critical effects at the LOAEL**	**Reference**
		LOAEL = 333 (♀) based on ↓ body weight gain (19.9%), ↓ overall food conversion efficiency (34.9%) No toxicologically relevant macroscopic/histopathological changes in ovaries, uterus, cervix and vagina were at the highest dose tested (equal to 333 mg/kg bw/day). Weight of these organs was not investigated. Histopathological examination of mammary glands was not performed. Estrous cyclicity was not performed.	
90 days rat oral, dietary OECD 408 (1981) GLP Wistar rat (HsdCpb:WU) 10/sex/dose Acceptable	Cymoxanil 0972, 98.8% 0, 500, 1000, 2000 ppm equal to 0, 42.6, 85.1, 174.3 mg/kg bw /day (males) *Additional recovery subgroups* (control and high dose), 28 days: equal to 0, 173.5 mg/kg bw /day(males)	NOAEL = 85.1 (♂) LOAEL = 174.3 (♂) based on ↓ body weight (11.3%), ↓ body weight gain (14.6%), ↓ food consumption (15.6%), ↑ creatinine (34%), ↑ total bilirubin (85.8%), ↑ relative kidney weight (17.3%), ↑ relative liver weight (15.7%) MTD at 174.3 mg/kg bw /day Recovery: **Testes:** atrophy of seminiferous tubules (severe and diffuse)/calcification (mild and multifocal) in one male out of 10 at 173.5 mg/kg bw/ day (testes tissues of other 9 males were not examined) **Epididymis:** no histopathological examination	RAR B.6.3.2.1. Study 2, 1999b
90 days mice oral, dietary OECD 408 (1981) GLP Swiss albino mice: Hsd0la:MF1 10/sex/dose Acceptable	Cymoxanil 0972, 98.8% 0, 150, 450, 1350 ppm equal to 0, 28.7, 84.4, 256.6 mg/kg bw / day (males) *Additional recovery subgroups* (control and high dose), 28 days: equal to 0, 266.7 mg/kg bw (males)	NOAEL = 84.4 (♂) LOAEL = 256.6 (♂) based on ↓ body weight gain (21.4%), ↑ total bilirubin (114.8%) MTD at 256.6 mg/kg bw /day Recovery: **Testes:** decreased spermatogenesis in one male from ten at 266.7 mg/kg bw/ day **Epididymis:** no histopathological examination	RAR B.6.3.2.2., 1999b
90 days dog oral, dietary OECD 409 (1981) GLP Beagle dog 4/sex/dose Acceptable	Cymoxanil DPX-T3217-113, 97.8% 0, 100, 200, 250/500 ppm equal to 0, 3.13, 5.13, 10.56 mg/kg bw /day (males) 0, 3.0, 5.27, 10.51 mg/kg bw /day (females)	NOAEL = 3.13 (♂) LOAEL = 5.13 (♂) based on clinical signs (↓ defecation), alterations of haematological parameters [↓ RBC (15.9%**), ↓ Hb (16.7%**), ↓ Ht (14.9%)] MTD at 10.56 mg/kg bw /day based on ↓ body weight (31.5%), loss overall body weight gain (g), loss body weight gain (g) during first four weeks, ↓ FC during eleven weeks (34.5 – 68.9%) **Testes:** "small testes" of one male and aspermatogenesis in the testes (2 animals) at 10.56 mg/kg bw/ day **Epididymis:** decrease of relative (24.1%) and absolute (48.4%) weight at 10.56 mg/kg bw/ day, however, no histopathological findings	*RAR B.6.3.2.3. Study 1, 1993*

Method, guideline, deviations[1] if any, species, strain, sex, no/group	**Test substance (Batch No; purity w/w), dose levels duration of exposure**	**Results** **- NOAEL/LOAEL (for sexual function and fertility, parents)** **- target tissue/organ** **- critical effects at the LOAEL**	**Reference**
		were reported in all groups tested. NOAEL = 3.0 (♀) LOAEL = 5.27 (♀) based on clinical signs (↓ defecation, ↑ diarrhoea), loss in overall body weight gain (g) at termination, ↓ food consumption (g/animal/day) during 11 weeks (31.8 – 46.2%) [MTD] No adverse effects on organ weight (absolute and relative) and histopathological findings in ovaries and uterus.	
90 days dog oral, dietary OECD 409 (1981) GLP Beagle dog 4/sex/dose Acceptable	Cymoxanil 498VF973, 98.8% 0, 200, 400, 800 ppm equal to 0, 4.9, 9.7 and 14.2 mg/kg bw /day (males)	NOAEL = 4.9 (♂) LOAEL = 9.7 (♂) based on clinical signs ('weakness'), loss body weight gain, ↓ food consumption (g/animal/day) during 5 weeks (23.2 – 46.7%*); ↓ absolute (>55%) and relative (>45%) thymus weight; histological alterations in thymus (lymphoid atrophy) with increasing severity MTD might have been reached at 9.7 mg/kg bw/day **Testes:** 30.3% - 38.1% decrease of absolute testes weight from 9.7 mg/kg bw/day; however, no histopathological changes were observed in these groups. **Epididymis:** No histopathological findings were reported in the control and the highest dose groups tested **Prostate:** prostate hypoplasia (1/4, 1/4 and 2/4) was reported at 4.9 mg/kg bw/ day, 9.7 mg/kg bw /day and 14.2 mg/kg bw /day, respectively	RAR B.6.3.2.3. Study 2, 1999
1 year dog oral, dietary OECD 452 (1981) GLP Beagle dog 5/sex/dose Acceptable	Cymoxanil DPX-T3217-113, 97.8% males: 0, 50, 100, 200 ppm equal to 0, 1.8, 3.0, 5.7 mg/kg bw /day (males)	NOAEL = 3.0 (♂) LOAEL = 5.7 (♂) based on alterations of haematological parameters [↑MCV (4.2%**) at termination, ↓ RBC (18.3%*/10.2%*) at week 12/25, ↓ Hb (11.0%*/18.8%**/10.8%*) at week 2/12/25]; alterations of clinical chemistry [↓potassium (13.7%**); bilateral cataract MTD has not been reached up to 5.7 mg/kg bw/day **Testes:** non-statistically significant and non-dose-dependent decrease of absolute and relative testes weight at 5.7 mg/kg bw/day; no histopathological changes in testes **Epididymis:** mild periarteritis (1 animal) at 5.7 mg/kg bw/day **Prostate:** benign adenoma (1 animal) at 5.7 mg/kg bw/day *Not high enough dose levels were selected*	RAR B.6.3.3.1., 1994
1 year dog oral, dietary OECD 452	Cymoxanil 89800028, 98.8% (first 4 weeks) 19800042, 99.2% (for remainder	NOAEL = 1.3 (♂) LOAEL = 2.8 (♂) based on histological changes in **testes** (minimal/slight bilateral atrophy) with	RAR B.6.3.3.2., 2003

Method, guideline, deviations[1] if any, species, strain, sex, no/group	**Test substance (Batch No; purity w/w), dose levels duration of exposure**	**Results** **- NOAEL/LOAEL (for sexual function and fertility, parents)** **- target tissue/organ** **- critical effects at the LOAEL**	**Reference**
(1981) GLP Beagle dog 4/sex/dose Acceptable	of study) males: 0, 50, 100, 200 ppm equal to 0, 1.3, 2.8, 5.6 mg/kg bw /day (males) females: 0, 25, 50, 100 ppm 0, 0.8, 1.4, 2.9 mg/kg bw /day (females)	an apparent trend in the incidence and severity MTD has not been clear reached at 5.6 mg/kg bw /day **Epididymis:** bilateral seminiferous cell debris (minimal) and unilateral atrophy (moderate) with aspermia at 5.6 mg/kg bw/day *Not high enough dose level was selected to elicit clear evidence of general toxicity (MTD)* *Histological examination of epididymis was not specified* NOAEL ≥ 2.9 (♀) no substance related effect could be found *The study not suitable to establish a proper LOAEL for females*	
Combined chronic toxicity /carcinogenicity study OECD 453 (1981) GLP Sprague-Dawley rats, Crl:CD Br 72/sex/dose Interim sacrifice: 10/sex/dose Acceptable	Cymoxanil DPX-T3217-113 (Blend of lots 80317145 and 80321154); 97.8% 0, 50, 100, 700, 2000 ppm equal to 0, 1.98, 4.08, 30.3, 90.1 mg/kg bw/day (males) 0, 2.71, 5.36, 38.4, 126 mg/kg bw/day (females) Chronic / Carcinogenicity (23 months, 702-710 days): 62/sex/dose	Long-term NOAEL = 4.08 (♂) Long-term LOAEL= 30.3 (♂), based on clinical findings (↑hyperreactivity), ↓ body weight (15.3%), ↓ body weight gain (21.8%), histopathological findings (elongate spermatid degeneration, retinal atrophy) Carcinogenic NOAEL ≥ 90.1 (♂) Carcinogenic NOAEL ≥ 126 (♀) Cymoxanil did not reveal any oncogenic potential MTD (♂) at 30.3 mg/kg bw/day based on ↓ body weight (15.3%), ↓ body weight gain (21.8%) **Testes:** elongate spermatid degeneration in testes at 30.3 mg/kg bw/day (7/63, 5/62, 4/62, 17/56*, 29/62* at 0, 1.98, 4.08, 30.3, 90.1 mg/kg bw/day, respectively); increase in multinucleate spermatids at 90.1 mg/kg bw/day **Epididymis:** non-statistically significant increase in bilateral oligospermia at 90.1 mg/kg bw/day (10/63, 8/62, 11/62, 8/56 and 23/62 at 0, 1.98, 4.08, 30.3, 90.1 mg/kg bw/day, respectively) Long-term NOAEL = 5.36 (♀) Long-term LOAEL = 38.4 (♀) based on histopathological findings (polyarteritis/ inflammation in lung/liver, retinal atrophy, sciatic nerve degradation) MTD (♀) at 126 mg/kg bw/day based on ↓ body weight (15.5%), ↓ body weight gain (25.8%) No adverse effects on histopathological findings in ovaries, uterus, vagina and mammary glands as well as on ovaries weight.	RAR B.6.5.1.1., 1994a
	Interim sacrifice (12 months, 357 days): 10/sex/dose 0, 50, 100, 700, 2000 ppm	MTD (♂) at 32.8 mg/kg bw/day base on ↓ body weight gain (16.1%) **Testes:** elongate spermatid degeneration at 32.8	

Method, guideline, deviations[1] if any, species, strain, sex, no/group	**Test substance (Batch No; purity w/w), dose levels duration of exposure**	**Results** **- NOAEL/LOAEL (for sexual function and fertility, parents)** **- target tissue/organ** **- critical effects at the LOAEL**	**Reference**
	equal to 0, 2.25, 4.58, 32.8, 97.4 mg/kg bw/day (males) 0, 3.07, 6.09, 43.5, 134 mg/kg bw/day (females)	mg/kg bw/day (1/9, 0/10, 0/10, 4/10*, 5/10* at 0, 2.25, 4.58, 32.8, 97.4 mg/kg bw/day, respectively); **Epididymis:** increase in multinucleate spermatids at 97.4 mg/kg bw/day (0/9, 0/10, 0/10, 0/10 and 3/10* at 0, 2.25, 4.58, 32.8, 97.4 mg/kg bw/day, respectively) MTD (♀) at 134 mg/kg bw/day based on ↓ body weight gain (29.2%) No adverse effects on histopathological findings in ovaries, uterus, vagina and mammary glands as well as on ovaries weight.	
Combined chronic toxicity /carcinogenicity study OECD 453 (1981) GLP Wistar rats, HsdCpb:WU strain, in-house bred 50/sex/dose Interim sacrifice (12 months): 10/sex/control; 20/sex/high dose Acceptable	Cymoxanil 0972, 98.8% and 498 VF973, 99.3% 0, 100, 500, 1200 ppm equal to 0, 4.7, 23.5, 58.8 mg/kg bw/day (males) Chronic / Carcinogenicity 24 months Interim sacrifice (12 months) Cymoxanil 0, 1200 ppm equal to 0, 67.6 mg/kg bw/day (males)	Long-term NOAEL = 4.7 (♂) Long-term LOAEL= 23.5 (♂), based on histopathological findings (lymphoid hyperplasia in rectum)* ** - Due to deviations of the study the NOAEL (♂) cannot be set properly with respect to findings on male reproductive organs.* Carcinogenic NOAEL ≥ 58.8 (♂) Cymoxanil did not reveal any oncogenic potential MTD at 58.8 mg/kg bw/day **Testes:** seminiferous tubule atrophy at 58.8 mg/kg bw/day **Epididymis:** increased combined incidence of epididymal oligospermia with aspermia at top dose (control 3/50 and 11/50 at 58.8 mg/kg bw/day); however, no statistical analysis has been performed	*RAR B.6.5.1.2.*, 2003
Carcinogenicity study OECD 451 (1981) GLP Mouse, Crl:CD-1®BR 90/sex/dose Acceptable.	Cymoxanil DPX-T3217-113, 97.8% 0, 30, 300, 1500, 3000 ppm equal to 0, 4.19, 42.0, 216, 446 mg/kg bw/day (males) 0, 5.83, 58.1, 298, 582 mg/kg bw/day (females) 18 months	Long-term NOAEL = 4.19 (♂) Long-term LOAEL= 42.0 (♂), based on histopathological findings in liver (apoptosis, pigment, granuloma, diffuse centrilobular hypertrophy) and epididymis (tubular dilatation, aggregate lymphoid and sperm cysts) Carcinogenic NOAEL ≥ 446 (♂) Cymoxanil did not reveal any oncogenic potential MTD (♂) at 216 mg/kg bw/day based on ↓ body weight gain (>34%) **Testes:** tubular atrophy at 446 mg/kg bw/day **Epididymis:** tubular dilatation, aggregate lymphoid and sperm cysts at 42.0 mg/kg bw/day; oligospermia and sperm granuloma in epididymis from 216 mg/kg bw/day	RAR B.6.5.2.1., 1994b

Method, guideline, deviations[1] if any, species, strain, sex, no/group	**Test substance (Batch No; purity w/w), dose levels duration of exposure**	**Results** **- NOAEL/LOAEL (for sexual function and fertility, parents)** **- target tissue/organ** **- critical effects at the LOAEL**	**Reference**
		Long-term NOAEL = 5.83 (♀) Long-term LOAEL = 58.1 (♀) based on histopathological findings in stomach (hyperplastic gastropathy) and duodenum (cystic enteropathy) MTD (♀) at 298 mg/kg bw/day based on ↓ body weight gain (≥15%) No adverse effects on histopathological findings in ovaries, uterus, vagina and mammary glands.	

*Studies which results were relevant for classification as Repr. 2, H361f only are highlighted in grey. Significance of difference from control: * p≤0.05, ** p≤0.01; for statistical analyses details please see RAR Volume 3CA_B-6*

No human data on adverse effects on sexual function and fertility are available. No other studies relevant for toxicity on sexual function and fertility are available.

2.6.6.1.1 Short summary and overall relevance of the provided information on adverse effects on sexual function and fertility – generational studies

With respect to reproductive toxicity, two multigeneration studies in rats have been submitted. An additional reduced-size one generation study in rats has been submitted for the purpose of renewal and was considered supportive only. These data except a new reduced-size one-generation study were evaluated during the first Annex I review of cymoxanil and were presented in the monograph (Vol.3, Annex B, Section B6, Point B.6.6, June 2007). All these studies have been re-evaluated by the RMS under the renewal programme. The results of reproductive toxicity studies are summarised in the Table above. Additionally, studies presented under other hazard classes STOT-RE contain relevant information about the effects on sexual function and fertility (effects on testes and epididymis) and these results are also summarised in this table.

It is noteworthy that while two multigeneration GLP studies were compliant to OECD 416 (1983), some sensitive EAS-mediated parameters as regard endocrine disruption requested in the updated guideline OECD 416 (2001) (e.g. sperm parameters, oestrus cyclicity, assessment of sexual maturation, anogenital-distance, primordial follicle count F1 females and weight of some sexual organs) were not investigated. Furthermore, no EAS-mediated parameters measurements were taken in the one generation study.

In the **first two-generation study** (*RAR B.6.6.1.1.*, 2001), the NOAEL concerning systemic toxicity for parental animals in the 2-generation study is proposed at 450 ppm (equal to 31.6 mg/kg bw/day for males and 42.8 mg/kg bw/day for females) based on reduced bodyweight gain in the F0 and F1 males and in the F0 females (during premating and lactation) and F1 females (during gestation); reduced food consumption in the F1 generation males and in the F0 and F1 females (during all phases) at the high dose of 1350 ppm (equal to 94.0 mg/kg bw/day for males and 116.3 mg/kg bw/day for females). It was considered inappropriate to establish the LOAEL for parental toxicity at 450 ppm (equal to 31.6 mg/kg bw/day for males and 42.8 mg/kg bw/day for females) based on reduced initial body weight in the F1 generation (11% for males and 9% for females) in the absence of changes in body weight gain after administration of cymoxanil, slight reduced food consumption (7% - 9%) in F0 females (during premating and gestation) and reduced body weight gain (23.7%) in the F1 females during the first five days of gestation only. These findings without any other associated adverse effects were considered insufficiently relevant for setting the LOAEL at 450 ppm. It is noteworthy that in the other rat studies, the reduced body weight, body weight gain and average food consumption were observed at higher dose levels, e.g. approximately from 188 mg/kg bw/day (*90 days rat study, Wistar rat (HsdCpb:WU, RAR B.6.3.2.1. Study 2, 1999b*) and from 224 mg/kg bw/day (*90 days rat study, Sprague-Dawley rat, RAR B.6.3.2.1. Study 1, 1993).*

For more detailed data on effects please refer to RAR Volume 3CA B-6, section B.6.6.1.1.

Organs (testes, seminal vesicles with coagulating gland, epididymis, prostate, uterus (with cervix), and ovaries) were not weighed but were subject to gross pathology and microscopic examinations; there were no unusual gross necropsy or microscopic findings in males and females of either parental generation.

With respect to reproductive parameters, the F0 treatment groups did not show any statistically significant differences attributed to treatment of cymoxanil up to 1350 ppm (94.0 mg/kg bw/day for males and 116.3 mg/kg bw/day for females). For the F1 generation parents, there were a statistically significant decrease in mean litter

size, the percentage of live pups born, together with a reduced mean number of corpora lutea and mean number of implantations as well as an increased percentage of post-implantation loss in the high dose only. The HCD provided did not meet requirements as set out in Commission Regulation (EU) No 283/2013 (section 5; 5.6. Reproductive toxicity), e.g. no identification of species, strain, name of the supplier, name of the laboratory, the dates when the study was performed as well as no information on the mean, median and standard deviation is available. Although some of these findings were outside the range of the HCD declared, due to limited information provided by the Applicant the assessment of their relevance was impossible.

Reproduction parameters of females treated with cymoxanil (excerpt)

Parental generation	F_0				F_1				Historical control data (range): 4 studies
Dose level (ppm)	0	150	450	1350	0	150	450	1350	
No. animals/group	30	30	30	30	30	30	30	30	
No. females paired with males	30	30	30	30	30	30	30	30	
No. females impregnated[1)]	30	30	30	30	30	30	30	30	
Mean No. of corpora lutea	12.2	12.7	13.3	13	14.3	14.1	13.8	**12.2[6)]**	**12.6 – 13.3**
Mean number of implantations	10.7	11.8	11.6	10.7	11.7	12	12	**10.1[6)]**	**11.3 – 12.1**
% post implantation loss[4)]	12.2	11.6	12.6	15.1	9.9	9.0	11.2	**19.0[6)]**	**8.2 – 13.5**

[4)] No. implantations – No. live pups/No. implantations x 100,
[6)] statistically significant (Student`s t-test; level of significance: $p \leq 0.05$)

It should be noted that at the high dose female parents of the F1 some maternal effects (reduced initial body weight, reduced body weight gain during gestation and reduced food consumption during all phases) were observed.

Body-weight development and food consumption in F_1 parental females administered cymoxanil (excerpt)

Parameter	Time of investigation	Dose level [ppm]			
		0	150	450	1350
Premating					
Body weight [g] (% difference from controls)	0 weeks	59	56	54[1)] (-9%)	41[1)] (-31%)
	2 weeks	124	122	115[1)] (-7%)	96[1)] (-23%)
	4 weeks	161	164	156 (-3%)	138[1)] (-14%)
	8 weeks	206	211	200 (-3%)	184[1)] (-11%)
	12 weeks	225	231	219 (-3%)	203[1)] (-10%)
	14 weeks	229	239	223 (-3%)	210[1)] (-8%)
Body weight gain [g]	0 – 14 weeks	170	182	170	168
Food consumption [g/rat/day] (% difference from controls)	0 – 14 weeks	14.6	15.1	14.4	13.3[1)] (-9%)
Gestation					
Body weight [g] (% difference from controls)	0 days	231	239	230	212[1)] (-8%)
	5 days	249	258	243	225[1)]
	10 days	259	269	257	236[1)]
	15 days	277	289	275	253[1)]
	20 days	337	346	332	296[1)] (-12%)
Body weight gain [g]	0 – 20 days	106	107	102	84[1)] (-20%)

Parameter	Time of investigation	Dose level [ppm]			
		0	150	450	1350
Food consumption [g/rat/day] (% difference from controls)	0 – 20 days	20.7	20.8	20.4	19.1[1)] (-8%)

[1)] statistically significant (Dunnett`s test; level of significance: p ≤ 0.05)

The NOAEL for parental toxicity is proposed at 450 ppm (equal to 31.6 mg/kg bw/day for males and 42.8 mg/kg bw/day for females) based on reduced bodyweight gain in the F0 and F1 parental animals; reduced food consumption in the F1 males; reduced food consumption in the F0 and F1 females at the high dose level.

The NOAEL for reproductive toxicity was set 450 ppm (equal to 31.6 mg/kg bw/day for males and 42.8 mg/kg bw/day for females) based on a decrease in mean litter size, the percentage of live pups born, reduced mean number of corpora lutea, increased percentage of post-implantation loss and reduced mean number of implantations in the high dose F1 generation parents.

With respect to offspring toxicity, statistically significant reductions have been observed for number of the F1 litter pups (both sexes combined) alive on day 14 and 21 and the respective survival indices of the high dose (1350 ppm) group animals. These findings are associated with the statistically significantly increased number of pups dead/cannibalised on days 8-14 and days 15-21. At the highest dose (1350 ppm) group of the F2 litters, the statistically significantly lower mean litter size including the mean viable litter size was reported and was likely a consequence of the lower number of corpora lutea, an increased percentage of post-implantation loss and a reduced mean number of implantations of the F1 dams. The number of F2 pups (both sexes combined) alive (pre-cull) on day 4 and number of pups alive (post-cull) on day 4 were found to be statistically significantly decreased for the high dose (1350 ppm) group animals. There was again a reduced number of F2 pups alive on day 21 (including the day 21 survival index) related to the increased pups found dead/cannibalised on day 15 – 21 for the high dose group animals. Although some of above mentioned findings were within the range of the HCD declared, due to limited information provided by the Applicant the assessment of their relevance was impossible.

Pup survival and sex ratio (excerpt)

Parental generation	F_0 (F_1 pups)				F_1 (F_2 pups)			
Dose levels [ppm]	0	150	450	1350	0	150	450	1350
Mean litter size / Mean litter size index	9.5	10.7	10.2	9.5	10.8	11	11	**8.6[2)]**
Mean viable litter size	9.4	10.4	10.1	9.1	10.5	10.9	10.7	**8.5[2)]**
No. pups dead/cannibalised (day 1-4)	6	13	2	10	13	7	12	2[1)]
No. pups dead/cannibalised (day 8-14)	0	1	1	**10[1)]**	2	1	2	5
No. of pups alive on day 14	191	203	178	**174[1)]**	199	213	198	189
No. pups dead/cannibalised (day 15-21)	0	0	1	**4[1)]**	0	1	1	**5[1)]**
No. of pups alive on day 4 / No pups. PND 4 (pre-cull)	242	276	243	236	267	291	274	**229[1)]**
Pups PND 4 (post-cull)	191	204	179	184	201	214	203	**194[1)]**
Pups dead cannibalised PND (5-21)	0	1	2	**14[1)]**	2	2	6	**10[1)]**
Pups PND 21	191	203	177	**170[1)]**	199	212	197	**184[1)]**
Day 21 Survival index (%)	100.0	99.5	98.9	**92.4[1)]**	99.0	99.1	97.0	**94.8[1)]**
Survival index PND 21 (lactation index %)	100	99.5	98.9	**92.4[1)]**	99	99.1	97.5	**97.4[1)]**

[1)] statistically significant (Z test; level of significance: p≤0.05),
[2)] statistically significant (Student`s t-test; level of significance: p ≤ 0.05)

Concerning pup development, statistically significantly decreased (10 - 46%) body weights (both sexes combined) for F1 pups were reported from day 4 until day 21 at the high dose level of 1350 ppm. Pup body weights were also statistically significantly reduced (>10%) in the mid-dose group on days 14 and 21. Amongst

the F2 pups statistically significantly decreased (19.5 - 38%) body weights were reported from day 7 until day 21 at the high dose level of 1350 ppm. Pup body weights were also statistically significantly slight reduced (8.4-9.1%) in the mid dose group on days 7, 14 and 21.

Body weight development of F_1 pups administered cymoxanil (excerpt)

Generation	**Time of investigation [days]**		**Dose level [ppm]**			
			0	**150**	**450**	**1350**
F1 generation, both sexes	1	Body weight [g] (% difference from controls)	6.5	6.6	6.7	6.4
	4		10.0	9.9	9.8	9.0[1)] (10%)
	7		15.3	15.0	14.5	11.7[1)] (24%)
	14		30.8	28.9	27.7[1)] (10.1%)	18.9[1)] (39%)
	21		47.6	46.1	42.0[1)] (11.8%)	25.6[1)] (46%)

[1)] statistically significant (Dunnett`s test; level of significance: $p \leq 0.05$)

Body weight development of F_2 pups administered cymoxanil (excerpt)

Generation	Time of investigation [days]		Dose level [ppm]			
			0	150	450	1350
F2 generation (both sexes)	1	Body weight [g] % difference from controls	6.7	6.6	6.5	6.5
	4		10.1	10.0	9.5	9.5
	7		15.4	15.3	14.1[1)] (8.4%)	12.4[1)] (19.5%)
	14		29.6	29.3	26.9[1)] (9.1%)	20.5[1)] (30.7%)
	21		46.4	46.0	42.4[1)] (8.6%)	28.6[1)] (38.4%)

[1)] statistically significant (Dunnett`s test; level of significance: $p \leq 0.05$)

The NOAEL for offspring toxicity was set at 150 ppm (equal to 10.5 mg/kg bw/day for males and 14.9 mg/kg bw/day for females) based on reduced body weight of F1 pups (both sexes combined) on days 14 and 21 as well as reduced body weight of F2 pups (both sexes combined) on days 7, 14 and 21.

In the **second two-generation study** (*RAR B.6.6.1.2.,* 1993), the F1 parental animals at high dose of 1500 ppm (equal to 97.9 mg/kg bw/day for males and 103 mg/kg bw/day for females) showed a statistically significant increase of clinical observations: males showed a statistically significant increase of “end of tail missing”, “necrotic tip of tail” as well as “sore”; females of this group showed statistically significant increase of “sore” (premating until lactation), “end of tail missing”, “necrotic tip of tail”, “stained fur” and “masses”. Body weight gain was statistically significantly impaired (>10%) at 1500 ppm in parental males of the F0/F1 generations as well as in females of the F0 generation (premating and gestation) and F1 generation (premating). The body weight of F0 males and females (premating and gestation) at the top dose groups was statistically significantly reduced (10 - 13%) throughout a long observation period. Meanwhile the initial mean body weights of F1 males and females at high dose were substantially and statistically significantly below the concurrent control values (~30%), that persisted for the remainder of the study (>10% reduction). Food consumption was statistically significantly reduced (>10%) at 1500 ppm in parental males of the F0/F1 generations as well as in females of the F1 generation (premating and 1st gestation). There were no adverse effects (i.e. decrease ≥10% was considered as adverse) on body weight, body weight gain and food consumption of F0/F1 generations of both sexes at the mid dose level and lower.

For more detailed data on effects please refer to RAR Volume 3CA B-6, section B.6.6.1.2.

Selected body weight and food consumption measurements in F_0 females administered cymoxanil during pre-mating and gestation (excerpt)

Parameter	**Time of investigation [days]**	**Dose level [ppm]**			
		0	**100**	**500**	**1500**
Premating					
Body weight [g]	0	162.3	162.6	160.0	159.6
	7	187.6	188.6	185.2	176.6[1)] (6%)
	14	211.1	214.8	208.8	197.3[1)] (7%)

Parameter	Time of investigation [days]	Dose level [ppm]			
		0	100	500	1500
	21	233.1	236.8	225.5	212.5[1)] (9%)
	28	248.4	253.4	242.9	223.6[1)] (10%)
	35	259.7	263.1	253.2	235.0[1)] (10%)
	42	271.0	276.4	260.8	244.9[1)] (10%)
	49	280.3	287.6	272.3	248.4[1)] (11%)
	56	288.1	299.1	280.2	254.6[1)] (12%)
	63	291.4	304.0	283.4	260.3[1)] (11%)
	70	302.0	313.1	290.7	267.6[1)] (11%)
Body weight gain [g]	0 – 70	139.7	150.5	130.7	108.0[1)] (23%)
Food consumption [g/rat]	0 – 70	20.4	20.3	19.9	19.7
Gestation					
Body weight [g]	0	296.1	315.8[1)]	290.0	270.4[1)] (9%)
	7	331.0	345.4	324.6	298.7[1)] (10%)
	14	356.5	372.1	350.2	323.7[1)] (9%)
	21	443.0	449.7	439.2	399.9[1)] (10%)
Body weight gain [g]	0 – 21	146.8	135.3	149.1	129.5[1)] (12%)
Food consumption [g/rat]	0 – 14	23.5	23.7	23.5	21.3

[1)]statistically significant (Dunnett`s test; level of significance: $p \leq 0.05$)

Body-weight development and food consumption of F_1 parental females administered cymoxanil during pre-mating, gestation and lactation (excerpt)

Parameter	Time of investigation [days]	Dose level [ppm]			
		0	100	500	1500
Premating					
Body weight [g]	0	57.6	56.5	53.1[1)]	39.2[1)] (32%)
	7	97.1	94.6	90.6[1)]	67.4[1)] (40%)
	14	142.9	138.3	136.0	106.2[1)] (26%)
	21	179.9	173.1	171.0	140.0[1)] (22%)
	28	207.6	200.5	200.7	167.2[1)] (19%)
	35	234.7	225.8	227.0	190.7[1)] (19%)
	42	255.1	244.9	246.2	208.3[1)] (18%)
	49	268.0	257.8	260.5	222.9[1)] (17%)
	56	280.8	272.6	274.2	231.9[1)] (17%)
	63	292.4	282.9	285.0	241.6[1)] (17%)
	70	299.6	294.2	291.5	249.3[1)] (17%)
	77	306.8	303.7	296.7	256.5[1)] (16%)
	84	315.4	308.1	304.9	258.8[1)] (18%)
	91	318.7	314.2	307.1	263.3[1)] (17%)
	98	324.6	320.7	316.6	270.7[1)] (17%)
	105	330.2	326.0	322.7	272.5[1)] (17%)
Body weight gain [g]	0 – 105	272.6	269.5	269.6	233.4[1)] (14%)
Food consumption [g/rat]	0 – 105	20.8	20.7	20.7	19.3[1)] (7%)
1st Gestation					
Body weight [g]	0	324.1	331.2	312.5	280.7[1] (13%)
	7	358.3	361.3	343.1	306.9[1)] (14%)
	14	387.5	385.2	371.7	331.6[1)] (14%)
	21	465.8	463.1	458.8	407.8[1)] (12%)
Body weight gain [g]	0 – 21	141.8	131.9	146.2	127.1
Food consumption [g/rat]	0 – 14	26.6	25.0	25.4	23.9[1)] (10%)
2nd Gestation					
Body weight [g]	0	379.1	350.0	348.4[1)]	317.3[1)] (16%)
	7	411.0	384.2	380.6[1)]	338.4[1)] (18%)
	14	438.1	408.4	407.4	366.0[1)] (16%)
	21	526.1	492.4	498.5	446.7[1)] (15%)
Body weight gain [g]	0 – 21	146.6	141.3	150.1	129.5
Food consumption [g/rat]	0 – 14	27.0	27.1	27.4	24.5

[1)] statistically significant (Dunnett`s test; level of significance: $p \leq 0.05$)

Mean relative testes weight of F0 parental males was statistically significantly greater than controls at 500 ppm and 1500 ppm (10% and 19%, respectively), whereas the absolute weight of the testes was 12% lower than controls in F1 males at 1500 ppm only. Since there were no histopathological correlates evident that would account for the increase/decrease in the F0/F1 testes weight, these effects on testes were considered not adverse. Organs (seminal vesicles with coagulating gland, epididymis, prostate, uterus (with cervix), and ovaries) were not weighed but were subject to gross pathology and microscopic examinations; there were no unusual gross necropsy or microscopic findings in males and females of either parental generation.

Based on the results obtained, the reproductive parameters investigated did not indicate a possible reproductive influence caused by the test substance. Based on these findings, the NOAEL for parental toxicity is proposed at 500 ppm (equal to 32.1 mg/kg bw/day in males and 34.7 mg/kg bw/day in females). The NOAEL for reproductive toxicity was set at ≥1500 ppm (equal to 97.9 mg/kg bw/day in males and 103 mg/kg bw/day in females).

With respect to offspring toxicity, the viability index of F1 pups during early lactation (days 1-4) was statistically significantly reduced at 500 ppm and 1500 ppm and this finding was not evident at both F2 generations. The litter survival (%) (Number of litters weaned/ Number of viable litters delivered x 100) for F_1 pups at 1500 ppm was also statistically significantly lower than the respective control. There was a statistically significant increase in the total number of affected litters with respect to clinical observations in pups of the highest dose of 1500 ppm group in the F1, F2A and F2B generations: relevant clinical observations comprise “gasping”, “no milkspot”, “subcutaneous haemorrhage” and “weak”. Concerning pup weight (both sexes combined), statistically significant reductions (11-40%) were evident at 1500 ppm (all generations) and also at the mid dose level of 500 ppm for the F2B generation (reduction 13-18%).

Body weight development of male and female F_1, F_{2A} and F_{2B} pups administered cymoxanil

Generation	Time of investigation [days]	Dose level [ppm]			
		0	100	500	1500
F_1 generation	0	6.7	6.7	6.5	6.4
	4 (pre-culling)	10.8	11.6	10.4	9.6[1)] (11%)
	4 (post-culling)	10.9	11.6	10.4	9.7[1)] (11%)
	7	17.7	18.3	16.9	13.9[1)] (21%)
	14	36.5	37.3	34.9	25.4[1)] (30%)
	21	58.6	59.1	55.2	39.6[1)] (32%)
F_{2A} generation	0	6.5	6.5	6.5	6.2
	4 (pre-culling)	10.4	10.8	9.9	9.1
	4 (post-culling)	10.4	10.8	9.9	9.1[1)] (13%)
	7	16.8	17.2	15.6	13.1[1)] (22%)
	14	34.6	35.4	31.2	22.5[1)] (35%)
	21	56.3	56.9	50.7	34.0[1)] (40%)
F_{2B} generation	0	6.8	6.7	6.3[1)] (7%)	6.4[1)] (6%)
	4 (pre-culling)	11.5	11.1	9.5[1)] (17%)	9.3[1)] (19%)
	4 (post-culling)	11.5	11.1	9.4[1)] (18%)	9.3[1)] (19%)
	7	18.3	17.7	15.5[1)] (15%)	13.2[1)] (28%)
	14	37.4	35.3	32.4[1)] (13%)	24.4[1)] (35%)
	21	61.8	58.2	53.1[1)] (14%)	38.7[1)] (37%)

[1)] statistically significant (Kruskal-Wallis test; level of significance: $p<0.05$)

The NOAEL for offspring toxicity was set at 100 ppm (equal to 6.5 mg/kg bw/day in males and 6.65 mg/kg bw/day in females).

The additional **reduced-size one generation study in rats** (*RAR B.6.6.1.3., 1998*) is considered acceptable as a range finding study and supportive only. There were no clinical signs observed, no pre-terminal mortality, and no treatment related ophthalmological findings. The body weight of males at the top dose of 3000 ppm (equal 226.2 mg/kg bw/day for males) was statistically significantly reduced (10 - 13%) throughout the observation period; overall body weight gain (0 – 15 weeks) was statistically significantly decreased by 18%. Food consumption was statistically significantly reduced (10 – 27.6%) at 3000 ppm in parental males throughout the first 6 weeks based on the originally study report. For more detailed data on effects please refer to RAR Volume 3CA B-6, section B.6.6.1.3.

The body weight of females was reduced at the top dose of 3000 ppm marginally, whereas the body weight gain was statistically significantly reduced by 12% and 43% (respectively) during gestation (0 – 20 days) at the mid and high doses levels (equal 136.1 mg/kg bw/day and 240.8 mg/kg bw/day for females). Based on the originally study report, food consumption was statistically significantly reduced (12.9 – 28.4%) at 3000 ppm in parental females throughout all premating period, whereas food consumption was statistically significantly reduced (>10%) during all gestation and all lactation period from the mid dose of 1500 ppm (equal 136.1 mg/kg bw/day).

It is noteworthy that in the other rat studies, the reduced body weight, body weight gain and average food consumption were observed at quite similar dose levels, e.g. approximately from 188 mg/kg bw/day (*90 days rat study, Wistar rat (RAR B.6.3.2.1., 1999b*) and from 224 mg/kg bw/day (*RAR B.6.3.2.1, 1993).*

Mean body weight (g), females

Parameter	Time of investigation [week]	Dose level [ppm]			
		0	**750**	**1500**	**3000**
Body weight [g]	0	108	108	107	105
	1	132	128	126	118* (-11%)
	2	152	145	146	140*
	3	168	163	161	155*[1] (-8%)
	4	178	172	174	166
	5	189	183	182	176
	6	197	190	191	187
	7	204	197	198	191
	8	210	203	205	201
	9	215	207	208	204
	10	220	212	213	209 (-5%)
Body weight gain [g]	0 – 10	111	104	106	104 (-6%)
	GD 0 – 20	114	106	100* (-12%)	65*[1] (-43%)
	LD 1 – 21	30	33	18*	20

** Statistically significant (Dunnett`s test: $p \leq 0.05$) [1] Significant dose correlation*

With respect to reproductive parameters, at necropsy 5 parent males in the 3000 ppm group had bilateral small, flaccid testes, rendering them infertile. The statistically significantly reduced the female fertility index (%), reduced numbers of corpora lutea, increased pre-implantation and post-implantation loss, reduced mean number of implantations as well as reduced mean litter size were observed at 3000 ppm (equal to 226.2 mg/kg bw/day for males, 240.8 mg/kg bw/day for females and combined 235.2 mg/kg bw/day). The statistically significantly lower mean litter size was likely a consequence of the lower number of corpora lutea, an increased percentage of pre and post-implantation losses and a reduced mean number of implantations of the dams.

Based on these findings, the NOAEL for parental toxicity is proposed at 750 ppm (equal to 68.4 mg/kg bw/day combined). The NOAEL for reproductive toxicity was set at 1500 ppm (equal to 127.7 mg/kg bw/day combined).

Group reproductive and litter data

Parameter	Dose level [ppm]			
	0	750	1500	3000
No. of males impregnating females	14	15	13	12
Male fertility index (%)	93.3	100	86.7	80.0
No. of females impregnated	15	15	15	7
No. pregnant	15	15	15	7
Female fertility index (%)	100	100	100	46.7*
Mean corpora lutea	12.0	11.7	11.0	7.9*
Mean implantations	10.9	10.6	10.1	6.1*
Pre-implantation loss (%)	8.9	9.1	7.9	21.8*a
Post-implantation loss (%)	6.7	3.1	9.9	37.2*a
Mean litter size	10.4	10.4	9.3	5.5*
No. of live litters	15	15	15	6
No. of pups dead at birth	3	2	2	6*
No. of live pups on LD 1	153	154	137	27*
No. of pups dead/cannibalised up to day 4	10	6	12	11*
No. of live pups on LD 4	146	150	127	22*
Mean live litter size	10.2	10.3	9.1	4.5*
Live birth index (%)	98.1	98.7	98.6	81.8*
LD 4 survival index (%)	95.4	97.4	92.7	81.5*
LD 7 survival index (%)	99.1	99.2	95.1	100.0
LD 14 survival index (%)	97.4	99.2	94.2	90.9
LD 4 – 21 survival index (lactation index), %	97.4	98.3	94.2	90.9
Mean pup weight, combined sexes (g): LD 1	6.1	6.1	6.1	5.9
LD 4	8.6	8.5	7.8*	7.3*
LD 7	13.3	12.3	10.7*	9.0*[1]
LD 14	27.0	23.7*	20.2*	16.4*[1]
LD 21	40.9	36.3*	27.9*	21.8*[1]

** Statistically significant ($p \leq 0.05$); [1] - Significant dose correlation;*
a - Proportions relative to other groups inflated by the reduced ovulation, implantation and litter size

With respect to offspring toxicity, statistically significant reductions have been observed for number of pups (both sexes combined) alive on LD 1 and LD 4 and the respective LD 4 survival index (%) of the high dose (3000 ppm) group animals. These findings are associated with the statistically significantly increased number of pups dead at birth/on day 1 and dead/cannibalised up to day 4. Additionally, the statistically significantly lower mean live litter size including lower live birth index (%) was reported the high dose. Concerning pup development, statistically significantly decreased (>11%) body weight (both sexes combined) for pups were reported from second week of lactation at 750 ppm, statistically significant reduction (19.6 – 31.2%) of body weight was observed from LD 7 at 1500 ppm and decreased (15.1 – 46.7%) body weight for pups were reported from LD 4 at 3000 ppm. The LOAEL for offspring toxicity was set at 750 ppm (equal to 68.4 mg/kg bw/day combined).

Developmental neurotoxicity

In the **developmental neurotoxicity rat study** (*RAR B.6.7.1.2., 2001*), there was no evidence of neurotoxicity up to the highest-dose tested in a battery of functional tests; furthermore, examination of the nervous system tissues revealed no unusual findings. Maternal toxicity was characterised by reductions in body weight gain and food consumption at gestation days 6 – 9 at 50 mg/kg bw/day.

For more detailed data on effects please refer to RAR Volume 3CA B-6, section B.6.7.1.2.

Developmental neurotoxicity study on rats: body weight, body weight gain and food consumption during gestation of dams

Parameter	Time of investigation [day of gestation]	Dose level [mg/kg bw]			
		0	5	50	100
Body weight [g]	0	241.1	240.6	241.2	241.0
	6	268.4	266.8	270.6	266.6
	7	271.2	269.5	268.3	261.3[1)]
	8	275.9	273.8	270.8	262.6[1)]
	9	279.8	278.8	276.4	266.2[1)]
	10	285.2	282.6	280.6	271.2[1)]
	11	291.4	289.6	287.8	278.5[1)]
	12	296.0	295.0	291.5	281.8[1)]
	13	300.0	299.0	296.1	286.2[1)]
	14	305.8	304.4	300.8	291.3[1)]
	15	313.6	313.4	310.0	299.4[1)]
	16	324.7	324.2	320.7	309.8[1)]
	17	338.6	336.9	334.1	322.6[1)]
	18	351.3	350.0	347.7	336.6[1)]
	19	363.7	362.2	360.4	346.2[1)]
	20	378.8	377.6	376.0	358.2[1)]
	21	390.3	389.2	388.5	368.2[1)]
Body weight gain [g]	0 – 6	27.3	26.2	29.4	25.6[1)]
	6 – 9	11.4	13.0	5.8[1)]	-0.4[1)]
	9 – 12	16.2	16.2	15.1	15.6
	12 – 15	17.6	18.4	18.5	17.6
	15 – 18	37.6	36.7	37.6	37.2
	18 – 21	37.3	36.7	40.8	31.6
	6 – 12	122.2	121.7	117.9	101.7[1)]
	0 – 21	149.7	148.1	147.3	127.3[1)]
Food consumption [g/rat/day]	0 – 6	22.1	21.9	22.4	22.6
	6 – 9	23.5	23.5	20.4[1)]	17.5[1)]
	9 – 12	24.5	24.1	22.9[2)]	21.1[1)]
	12 – 15	25.0	25.5	24.7	22.6[1)]
	15 – 18	25.8	25.6	25.6	25.2
	18 – 21	23.1	21.7	22.2	19.6[1)]
	6 – 21	24.4	24.1	23.2	21.2[1)]
	0 – 21	23.7	23.5	23.0	21.6[1)]

[1)] statistically significant (Dunnett`s test; level of significance: $p \leq 0.01$)
[2)] statistically significant (Dunnett`s test; level of significance: $p \leq 0.05$)

In pups of the highest dose (100 mg/kg bw/day) group, following treatment-related statistically significant

changes were observed: statistically significantly increased number of pups (%) found dead or presumed cannibalized ((13.2% and 2.3% on Days 2-5/9-12, respectively), reduced viability index (number of live pups on day 5 post-partum/number of live born pups on day 1 post-partum), reduced lactation indexes (number of live pups on day 12 post-partum/number of live pups on day 5 post-partum; number of live pups on day 22 post-partum (weaning)/number of live pups in subsets 2 – 4 on day 12 post-partum), lower number of surviving pups/litter (from Day 5 post-partum to Day 22 post-partum) and live litter size (Days 12/18/22 post-partum). The average number of pups delivered, live born and stillborn pups as well as sex ratio remained unaffected. Regarding clinical signs, in the pre-weaning period in top dose (100 mg/kg bw/day) group there were four pups that were cold to the touch, two that were not nursing, two not nesting, one dehydrated and one emaciated pup. In the gross pathology examination on the pups that died prior to weaning the only finding noted was a dose-related increase in pups with no milk in their stomachs (see the table below). 33% of dead pups had no milk in their stomachs at high dose of 100 mg/kg bw/day; however, in percent values it was not dose-related. Body weight, body weight gain and food consumption were not significantly affected by treatment in any dose group. Cymoxanil had no effect on the sex ratio of pups or the timing of sexual maturation.

Developmental neurotoxicity study in rats: relevant litter data/reproductive parameter

Litter data/ reproductive parameter	Dose group levels [mg/kg bw/day]			
	0	5	50	100
Pregnancy and live litters				
Number of females pregnant (out of 25 mated)	25	25	22	24
Number of litters delivered	25	25	22	23
Dams with all pups dying (days 1-4 post-partum)	0	0	0	2 [8.3%]
Dams with all pups dying (days 2-22 post-partum)	0	0	0	0
Pups found dead or presumed cannibalized [number/%]				
Days 2 – 5 post-partum	3/384 [0.8 %]	4/379 [10.0 %]	9/335 [2.7 %]	46/349 [13.2 %][1)]
Days 9 – 12 post-partum	0/249 [0.0 %]	0/248 [0.0 %]	0/220 [0.0 %]	5/215 [2.3 %][1)]
Days 13 – 14 post-partum	0/160 [0.0 %]	0/160 [0.0 %]	0/160 [0.0 %]	2/171 [1.2 %]
Days 15 – 18 post-partum	0/160 [0.0 %]	0/160 [0.0 %]	1/160 [0.6 %]	2/169 [1.2 %]
Days 19 – 22 post-partum	0/160 [0.0 %]	0/160 [0.0 %]	0/159 [0.0 %]	2/167 [1.2 %][1]
Viability index* [%]	98.7	98.4	97.0	85.6[1)]
Lactation index** [%]	99.2	99.2	100.0	95.9[1)]
Lactation index*** [%]	100.0	100.0	99.4	95.4[1)]
Average number of live pups/litter				
Day 1 post-partum	15.4	15.2	15.3	14.8
Day 5 post-partum (preculling)	15.2	15.0	14.8	12.6[2)]
Day 12 post-partum	8.4	8.3	8.2	7.1[1)]
Day 14 post-partum	8.0	8.0	8.0	7.0[1)]
Day 18 post-partum	8.0	8.0	8.0	6.9[1)]
Day 22 post-partum	8.0	8.0	8.0	6.8[1)]
Live litter size				
Day 12 post-partum	10.0	9.9	10.0	9.5[2)]
Day 18 post-partum	8.0	8.0	8.0	7.5[1)]
Day 22 post-partum	8.0	8.0	8.0	7.5[1)]

* *number of live pups on day 5 post-partum/number of live born pups on day one post-partum*

** *number of live pups on day 12 post-partum/number of live pups on day 5 post-partum*

*** *number of live pups on day 22 post-partum (weaning)/number of live pups in subsets 2 – 4 on day 12 post-partum*

[1)] *statistically significant (Dunnett`s test; level of significance: $p \leq 0.01$)*

[2)] *statistically significant (Dunnett`s test; level of significance: $p \leq 0.05$)*

A NOAEL for maternal toxicity of 5 mg/kg bw/d, a NOAEL for offspring toxicity of 50 mg/kg bw/d is therefore proposed and a NOAEL for developmental neurotoxicity was ≥100 mg/kg bw/day.

Repeated exposure toxicity

The results regarding effects on testes and epididymis in rats, mice and dogs in short-term toxicity and combined chronic toxicity / carcinogenicity studies are presented below. For more detailed data on these effects please refer to RAR Volume 3CA B-6, section B.6.3. and section B.6.5. Effects on reproductive organs of females are briefly presented for studies only which results were relevant for classification as Repr. 2, H361f.

Rats:

The **28 days dietary study in rats** (*RAR B.6.3.1.1., 1999a*) was performed as a range finding study and is considered as supportive only: relevant endpoints were not investigated (e.g. weight of prostate, seminal vesicles and histopathology of all organs). In animals of the two highest dose levels (260 mg/kg bw/day and 400.3 mg/kg bw/day) changes in testes and epididymis weight were observed: the absolute weights of testes were statistical significantly and dose related lower (15.2% and 30%, respectively) while the relative weights of epididymis were higher (27.4% and 42.8%, respectively). No histology has been performed in this study. These alterations most likely were linked to the reduction in body weight (28% and 41%, respectively) and body weight gain (45% and 66%, respectively) that occurred at the two highest dose groups. The maximum tolerated dose (MTD) for males was stated at 143.5 mg/kg bw/ day. The NOAEL was proposed at 74.4 mg/kg bw/day, based on reduced body weight gain, reductions in food consumption, increases in relative liver and kidneys weight at ≥ 143.5 mg/kg bw/day in males.

A **90 days dietary rat study** (*RAR B.6.3.2.1. Study 1, 1993*) does not fulfil the requirements of the OECD TG 408 (2018) and some relevant endpoints were not investigated (e.g. weight of epididymis, prostate, seminal vesicles and sperm morphology). Statistical significantly reduced body weight (14.6%), body weight gain (21.9%) and overall food conversion efficiency (g wt gain/g food consumption) (18.7%) in male rats exposed to 224 mg/kg bw/day were considered adverse effects and, therefore, at this dose MTD was reached for males. There were dose-related increases in mean relative testis weights at 47.6, 102, 224 mg/kg bw/day (9.9%, 17.7%* and 15.6%*, respectively); however, the increase at low-intermediate dose was non-statistically significant. These changes in testis weight were correlated with histopathological findings: increased bilateral elongate spermatid degeneration occurred at ≥ 47.6 mg/kg bw/day, the incidence and severity increasing with dosage. Increased elongate spermatid degeneration was observed in three animals of the 47.6 mg/kg bw/day dose group, five of the 102 mg/kg bw/day dose group and seven animals of the 224 mg/kg bw/day dose group: the increased incidence showed a clear dose-relationship and was statistically significant at the highest dose level. Furthermore, one male rat each from the 102 mg/kg bw/day and 224 mg/kg bw/day dose group had multinucleated spermatids: despite of no statistical significance the finding supports a compound related effect to male reproductive organ. The following histopathological changes have been observed with respect to epididymis: cell debris (1 animal and 6 animals of the two highest dose groups, respectively), bilateral hypospermia (4 animals of the highest dose group) and multinucleated spermatids (one animal each of the two highest dose groups tested); statistical significance was shown for cell debris and hypospermia of the highest dose group (224 mg/kg bw/day). No weight of epididymis was assessed in the study. Therefore, despite the lack of statistical significance at 47.6 and 102 mg/kg bw/day for the testicular findings, there was sufficient evidence of dose related trend for the NOAEL to be considered as 6.54 mg/kg/day for males.

It should be noted that no toxicologically relevant macroscopic/histopathological changes in ovaries, uterus, cervix and vagina were observed in this study at the highest dose tested (equal to 333 mg/kg bw/day). Weight of these organs was not investigated. Histopathological examination of mammary glands as well as estrous cyclicity was not performed.

A **second 90 days dietary rat study** (*RAR B.6.3.2.1. Study 2, 1999b*) does not fulfil the requirements of the OECD TG 408 (2018). It should be noted that neither weight of epididymis/prostate/seminal vesicles with coagulating glands was recorded nor histopathological examination of these organs/tissues was performed. Neither changes in weight of testes nor macroscopic/histopathological damage/changes in testes were observed in this study at the highest dose tested (174.3 mg/kg bw/day). However, it is noteworthy that in additional recovery group (173.5 mg/kg bw/day) small sized (-1.5 cm) and flabby bilateral testes were observed in one male (ten animals were investigated) during gross necropsy and later histopathological examination showed atrophy of seminiferous tubules (severe and diffuse)/calcification (mild and multifocal) in this male testes (testes tissues of other males were not examined). At the 174.3 mg/kg bw/day dose the MTD was reached for males. Based on decreased body weight, body weight gain, food consumption and increased creatinine, total bilirubin values as well as increased relative kidney and liver weight at 174.3 mg/kg bw/day in males, the overall NOAEL was set at 85.1 mg/kg bw/day.

In a **first 2 years dietary rat study** (*RAR B.6.5.1.1., 1994a*), a statistically significant increase (30.7%*) of relative testes weight in the high dose group (90.1 mg/kg bw/day) was reported; however, weight of epididymis was not investigated. Histological findings with respect to testes (statistically significant elongate spermatid degeneration) were observed from 30.3 mg/kg bw/day (7/63, 5/62, 4/62, 17/56* and 29/62* at 0, 1.98, 4.08, 30.3 and 90.1 mg/kg bw/day) at termination (all animals excluding interim sacrifice). Although this lesion could be found in control animals, the incidence and severity increased with increasing dietary concentration of the test compound, e.g. grades of lesions were 14 minimal, 3 mild at 30.3 mg/kg bw/day and 20 minimal, 4 mild and 5 moderate at 90.1 mg/kg bw/day. In addition to the elongate spermatid lesion there was a statistically significant increase in multinucleate spermatids in the 90.1 mg/kg bw/day male testes which was secondary to the elongate spermatid abnormality. Furthermore, these histopathological findings in testes were evident also in animals at the one year interim sacrifice: statistically significant increase of elongate spermatid degeneration at 32.8 mg/kg bw/day and 97.4 mg/kg bw/day [1/9, 0/10, 0/10, 4/10* and 5/10*] as well as multinucleated spermatids in testes in one male at 97.4 mg/kg bw/day. Additionally, at the one year interim sacrifice, multinucleated spermatids in epididymis had a statistically significantly increased occurrence in males at 97.4 mg/kg bw/day (0/9, 0/10, 0/10, 0/10 and 3/10* at 0, 2.25, 4.58, 32.8, 97.4 mg/kg bw/day, respectively); however, at study termination increased bilateral oligospermia in epididymis at high dose (10/63, 8/62, 11/62, 8/56 and 23/62 at 0, 1.98, 4.08, 30.3, 90.1 mg/kg bw/day, respectively) did not attain statistical significance. Therefore, these results confirmed the similar microscopic findings in testes in a previous 90-day rat study (see RAR Volume 3CA B-6, point B.6.3.2.1 Study 1, *1993*). Based on the statistically significant and dose related reduced body weight (>15%) and body weight gain (g) (>20%) at termination at 700 ppm (equal to 30.3 mg/kg bw/day) the MTD was reached for males. Over the 1-year interval (test day 0-357), body weight gains were significantly decreased 16.1% and 28.8% in the 700 and 2000 ppm (equal to 32.8 and 97.4 mg/kg bw/day) males groups, respectively. Therefore, the MTD was reached for interim sacrifice males at 32.8 mg/kg bw/day.

It should be noted that no adverse effects on histopathological findings in ovaries, uterus, vagina and mammary glands as well as on ovaries weight were observed in this study at termination and interim sacrifice up to the highest doses tested (134 and 126 mg/kg bw/day, respectively).

In a **second 2 years dietary rat study** (*RAR B.6.5.1.2., 2003*), histopathological examination of testes after 24 months with cymoxanil (animals terminally sacrificed including animals found dead and sacrificed moribund) revealed statistically significantly increased incidence of mild-to-moderate seminiferous tubule atrophy in the testis at 58.8 mg/kg bw/day. It was seen in both testes (bilaterally) in 3 of the 12 cases at 1200 ppm. It should be noted that tissues of male reproductive organs of all rats from control and high dose groups, all the dead / moribund sacrificed rats and all gross lesions were examines for histopathological changes. However, based on the original study report, microscopic histopathological changes in testes not always were identified in individual gross pathological observation. Additionally, the correlation between incidences of gross observation in testes (e.g. 'small' and / or 'soft') and the incidences of microscopic observation in testes (e.g. seminiferous tubule atrophy) was not always observed in previous 2 year rat study (*RAR B.6.5.1.1., 1994a*). Since male reproductive organs (testes and epididymis) are target tissues of cymoxanil and these tissues showed treatment-related changes in the high dose group, histopathological examinations had to be performed from all animals in all dose groups. It is noteworthy that there was a clear increased combined incidence of epididymal oligospermia with aspermia (a low sperm count and the complete lack of semen) at the highest dose level (58.8 mg/kg bw/day); however, no statistical analysis has been performed. In all cases aspermia was exceptionally identified in terminal sacrificed males: this finding was not identified in animals found dead and sacrificed moribund. Due to methodological limitation (no histopathological examination was performed from all animals in all dose groups) the NOAEL cannot be set properly with respect to findings on male reproductive organs. The overall NOAEL for long-term effects was set at 100 ppm (equal to 4.7 mg/kg bw/day), based on histopathological findings (lymphoid hyperplasia in rectum) in males at 500 ppm (equal to 23.5 mg/kg bw/day).

Mice:

The **28 days dietary study in mice** (*RAR B.6.3.1.2., 1999a*) was performed as a range finding study and is considered as supportive only: most of endpoints recommended for the detection of endocrine disrupters were not investigated (e.g. weight of prostate, seminal vesicles, epididymis, uterus; histopathology of all organs and clinical biochemistry determinations). No effects on testes caused by cymoxanil technical were evident in this study. No histology has been performed in this study. The maximum tolerated dose (MTD) for males was stated at 303.4 mg/kg bw/day. The NOAEL was proposed at 172.7 mg/kg bw/day based on reduced body weight gain, reduction in food consumption throughout all dosing period and decrease in absolute kidneys weight in males at ≥ 303.4 mg/kg bw/day.

The **90 days dietary mice study** (*RAR B.6.3.2.2., 1999b*) does not fulfil the requirements of the OECD TG 408 (2018). It is noteworthy that neither weight of epididymis/prostate/seminal vesicles with coagulating glands was recorded nor histopathological examination of these organs/tissues was performed. At 256.6 mg/kg bw/day the MTD seems to be reached for males. Neither changes in weight of testes nor macroscopic/histopathological damage/changes in testes were observed in this study at the highest dose tested (256.6 mg/kg bw/day). It is noteworthy that in additional recovery group (equal to 266.7 mg/kg bw/day) histopathological examination showed decreased spermatogenesis in testes in one male (ten animals were investigated). The overall NOAEL is proposed at 84.4 mg/kg bw/day based on decreased body weight gain, increased total bilirubin, increased total protein and increased relative liver weight at 256.6 mg/kg bw/day.

In the **first 18 months dietary mice study** (*RAR B.6.5.2.1., 1994b*), absolute testes weight in highest dose (446 mg/kg bw/day) group was statistically significant reduced (19.8%) and macroscopic examination showed increased incidences of small and "soft" testes at this dose. Additionally, histopathological examination of testes revealed statistical significantly increased incidence of tubular atrophy of testes at the top dose (446 mg/kg bw/day). Furthermore, from 300 ppm (equal to 42.0 mg/kg bw/day) tubular dilatation, aggregate lymphoid and sperm cysts/cystic dilation of epididymis were statistically significantly increased in a dose-dependent manner. From 1500 ppm (equal to 216 mg/kg bw/day) statistically significantly increased unilateral and bilateral oligospermia and sperm granuloma in epididymis were observed. All these changes are considered test substance related and adverse. At 1500 ppm (equal to 216 mg/kg bw/day) the MTD was reached for males based on the statistically significant and dose related reduced body weight gain (g) at termination (>34%). The overall NOAEL for long-term effects was set at 30 ppm (equal to 4.19 mg/kg bw/day), based on histopathological findings in liver (apoptosis, pigment, granuloma, diffuse centrilobular hypertrophy) and epididymis (tubular dilatation, aggregate lymphoid and sperm cysts) in males at 300 ppm (equal to 42.0 mg/kg bw/day).

It should be noted that no adverse effects on histopathological findings in ovaries, uterus, vagina and mammary glands were observed in this study up to the highest doses tested (582 mg/kg bw/day).

In the **second 18 months dietary mice study** (*RAR B.6.5.2.2., 2002*), no effects on testes/epididymis caused by cymoxanil were evident up to the highest dose tested (178.3 mg/kg bw/day). It should be noted that weights of

testes were not investigated. Considering the top dose selection, the amount of compound administered was not high enough to elicit clear evidence of toxicity. The MTD was not reached for males and no robust NOAEL could be established. The overall NOAEL for long-term effects was proposed at 600 ppm (equal to 91.4 mg/kg bw/day) based on macroscopic and histopathological findings in mesenteric lymph nodes (dicolouration and haemorrhage) of males found dead and moribund as well as histopathological findings in the ovaries (follicular cysts) at 1200 ppm (equal to 178.3 mg/kg bw/day).

Dogs:

With respect to adverse effects on male reproductive organs weights, a statistically significant decrease of relative (24.1%) and absolute (48.4%) epididymis weight of animals in the high dose (10.56 mg/kg bw/day) group were noted in the **first 90 days dog study** (*RAR B.6.3.2.3. Study 1, 1993*), however, no histopathological findings were reported in all groups tested. Non-statistically significant decrease of relative (9.8%) and absolute (35.7%) testes weight of animals in the high dose group was also observed and at the macroscopic examination "small testes" of one high dose male was reported. Histopathology showed aspermatogenesis in the testes of 2 animals of the high dose group. For Male 1 moderate (grade 3) aspermatogenesis bilateral, no evidence of development beyond primary spermatocyte were reported; for Male 2 mild (grade 2) aspermatogenesis, minimal spermatid formation were reported (at the scheduled necropsy "small testes" were described too). It is noteworthy that findings reported in male reproductive organs at 10.56 mg/kg bw/day were observed in the presence of general toxicity (clinical signs such as decreased defecation and diarrhoea; reduced body weight (31.5%), loss overall body weight gain (g), loss body weight gain (g) during first four weeks and reduced mean food consumption up to 68.9%). The MTD for males was stated at 10.56 mg/kg bw/day based on statistical significantly reduced body weight at termination (31.5%), loss overall body weight gain (g), loss body weight gain (g) during first four weeks of the dosing period and reduced mean food consumption (g/animal/day) during eleven weeks (34.5 – 68.9%) at 500 ppm (equal to 10.56 mg/kg bw/ day).. The overall NOAEL is proposed at 3.0 mg/kg bw/day based on clinical observations (diarrhoea and decreased defecation in males and females), reduced body weight gain (females), reduced food consumption (females) and alteration of blood parameters (reduced number of red blood cells, haemoglobin and haematocrit more severely in males) at ≥5.27 mg/kg bw/day.

It should be noted that no adverse effects on organ weight (absolute and relative) and histopathological findings in ovaries and uterus were observed in this study up to the highest dose tested.

A statistically significant dose-dependent decrease of absolute testes weight in the mid (9.7 mg/kg bw/day) and high (14.2 mg/kg bw/day) dose group (30.3% and 38.1%, respectively) was reported in the **second 90 days dog study** (*RAR B.6.3.2.3. Study 2, 1999*). However, no histopathological changes were observed in these groups. Histopathological examination revealed some changes in prostate: prostate hypoplasia (0/4, 1/4, 1/4 and 2/4) was reported in the 0, 4.9, 9.7 and 14.2 mg/kg bw/day dose groups, respectively. No histopathological findings were reported in epididymis in the control and the highest dose groups tested. At 9.7mg/kg bw/day the MTD might have been reached for males. The NOAEL for males is proposed at 4.9 mg/kg bw/day based on clinical observations ('weakness'), loss body weight gain, reduced food consumption and histological findings in the thymus at ≥9.7 mg/kg bw/day.

The **first 1 year dog dietary study** (*RAR B.6.3.3.1., 1994)* does not fulfil the requirements of the OECD TG 452 (2018). Considering the top dose selection, the amount of compound administered was not high enough to elicit evidence of toxicity with respect to findings on male reproductive organs weights. A non-statistically significant and non-dose-dependent decrease of absolute and relative testes weight in the high dose (5.7 mg/kg bw/day) group (10.1% and 19.2%, respectively) was reported; however, no histopathological changes testes were observed in this group. Histopathological examination revealed prostate benign adenoma (0/4, 0/4, 0/4 and 1/4) and mild periarteritis in epididymis (0/4, 0/4, 0/4 and 1/4) in this group. Effects on testes and epididymis found in a 90 days study on dogs (at 10.56 mg/kg bw/day) [*RAR B.6.3.2.3. Study 1, 1993*] could not be confirmed in this 1 year dog study due to low amount administered: the highest dose administered was much lower than the "effect dose" in the 90 days study (10.56 mg/kg bw/day). The NOAEL is proposed at 3.0 mg/kg bw/day based on alterations of haematological parameters (reduced RBC and Hb), alterations of clinical chemistry (reduced potassium) and bilateral cataract in males at 5.7 mg/kg bw/day. The MTD has not been reached at the highest dose level.

The **second 1 year dog study** (*RAR B.6.3.3.2., 2003*) does not fulfil the requirements of the OECD TG 452 (2018). Considering the top dose selection, the amount of compound administered was not high enough to elicit clear evidence of toxicity. The MTD have not been clear reached for males at the highest dose. From week 5 until study termination, body weight of males at highest dose (5.6 mg/kg bw/day) was approximately less 10-14% than the control; however, these values did not attain statistical significance. No test article related statistically significant effects on testes/epididymis weights (relative and absolute) were apparent at any concentration. With respect to pathological findings on male reproductive organs, macroscopic examination exhibited a reduced size of testis and/or flaccid testis in one the same animal of the high dose group as well as reduced size of epididymis in the same male and thickened epididymis in another animal in the high dose group. Regarding histopathological

changes, there was an apparent trend in the incidence of atrophy of testes, with two (out of four) and three males (out of four) in 2.8 and 5.6 mg/kg bw/day dose groups, respectively, showing from minimal to severe grade in the absence of such findings in control animals. The histological findings in the epididymis were bilateral seminiferous cell debris (minimal) and unilateral atrophy (moderate) with aspermia of one animal of the high dose group each. Based on some scientific data (*Michael J. Goedken et al., 2008*) atrophy/hypoplasia of seminiferous tubules is decreasing with age to 14%-17% in dogs twelve to thirty-six months old and dogs less than eight months of age have lower testicular weights, incomplete filling of epididymal tails with sperm and abnormal epididymal content. Furthermore, atrophy/ hypoplasia was found in 7.4% of dogs and findings in epididymis consisted mainly of reduced spermatids (5.6%), based on the RMS selected 54 dogs over one year of age from background control data provided by the Applicant. It means that changes in testes/epididymis of this study are outside the range of background pathology in Beagle dogs of this age. Based on the study results and some scientific data, the RMS considers that a treatment related effect of cymoxanil to testes at two high doses could not be excluded as well as effect to epididymis at the highest dose. The NOAEL is proposed at 1.3 mg/kg bw/day based on histological findings in the testes (minimal/slight bilateral atrophy) at 2.8 mg/kg bw/day with an apparent trend in the incidence and severity.

It should be noted that no adverse effects on weight (absolute and relative) of ovaries and uterus were observed in this study up to the highest dose tested. The macroscopic examination and histological evaluation provided no effects in ovaries, uterus, vagina and mammary glands too.

2.6.6.1.2 Comparison with the CLP criteria regarding adverse effects on sexual function and fertility

In one two generation study *(RAR B.6.6.1.2., 1993)* no adverse effects on fertility parameters were reported, whereas in other two generation study *(RAR B.6.6.1.1., 2001)* minor effects on fertility parameters were reported in the F1 generation. For the F1 generation parents (0, 10.5, 31.6, 94.0 mg/kg bw/day for males and 0, 14.9, 42.8 and 116.3 mg/kg bw/day for females), there were a statistically significant (*) decrease in mean litter size (10.8, 11, 11, , 8.6*, respectively), reduction in the percentage of live pups born (90.1, 91.0, 88.8, 81.0*, respectively) together with a reduced mean number of corpora lutea (14.3, 14.1, 13.8, 12.2*, respectively) and reduced mean number of implantations (11.7, 12.0, 12.0, 10.1*, respectively) as well as an increased percentage of post-implantation loss (9.9, 9.0, 11.2, 19.0*, respectively) in the high dose only. Slight maternal effects at the high dose female parents of the F1 were observed: reduced initial body weight that persisted about 10% for the remainder of the study; reduced body weight gain by 20% during gestation and reduced food consumption by 8-26% during all phases. It should be noted that according to the *Guidance on the Application of the CLP Criteria* (2017) point 3.7.2.2.1. adverse effects on fertility and reproductive performance seen at dose levels causing less marked systemic toxicity (e.g. than lethality, dramatic reduction in absolute body weight, coma) are not a secondary consequence of this toxicity. The NOAEL for parental toxicity was set at 450 ppm (equal to 31.6 mg/kg bw/day for males and 42.8 mg/kg bw/day for females) based on reduced bodyweight gain in the F0 and F1 parental animals; reduced food consumption in the F1 males; reduced food consumption in the F0 and F1 females at the high dose level.

In the one generation study *(RAR B.6.6.1.3., 1998)* minor effects on fertility parameters were reported: the statistically significantly reduced the female fertility index (%), reduced numbers of corpora lutea, increased pre-implantation and post-implantation loss, reduced mean number of implantations as well as reduced mean litter size at 3000 ppm (equal to 240.8 mg/kg bw/day for females). The body weight gain of females was statistically significantly reduced by 12% and 43% (respectively) during gestation (0 – 20 days) at the mid and high doses levels (equal 136.1 mg/kg bw/day and 240.8 mg/kg bw/day); food consumption was statistically significantly reduced (>10%) during all gestation and all lactation period from the mid dose of 1500 ppm (equal 136.1 mg/kg bw/day). Food consumption was statistically significantly reduced (12.9 – 28.4%) at 3000 ppm in parental females throughout all premating period. It is noteworthy that statistically significantly decreased maternal body weight gain during gestation (0 – 20 days) and food consumption during all gestation / lactation period at high doses level could be due to the dramatically reduced mean litter size at LD1 (10.2 in control and 4.5 in high dose group). However, at the mid dose level (136.1 mg/kg bw/day) this is not the case as mean litter size and mean weight of pups (combined, male and female) was not statistically significantly changed compared to control. Furthermore, although there were some differences in sex ratio at birth in all three tested groups compared to control (43.6; 46.8; 51.1 and 48.5 males % at 0, 75.1, 136.1, 240.8 mg/kg bw/day for females, respectively; no statistics regarding this parameter is available), there were not statistically significant differences in mean weight of male pups and female pups in all groups at LD1.

Furthermore, at necropsy 5 parent males in the 3000 ppm (equal to 226.2 mg/kg bw/day) group had bilateral small and flaccid testes, rendering them infertile. However, microscopic examination of testes was not performed.

Several repeated dose toxicity studies in rats, mice and dogs have been performed with cymoxanil. In these studies statistically significant adverse effects on testes and epididymis were reported in all three species. These effects in testes included elongate spermatid degeneration, atrophy of the seminiferous tubules, atrophy of testes, aspermatogenesis and multinucleate spermatids which was secondary to the elongate spermatid abnormality;

while the effects in epididymis included seminiferous cell debris, multinucleated spermatids, sperm granuloma, atrophy, hypospermia, oligospermia and aspermia. Histological changes in rat testes started from about 50 mg/kg bw/day and in epididymis started from 224 mg/kg bw/day (the MTD) in the 90 days rat oral study. Histological changes in dog testes started from about 11 mg/kg bw/day and in epididymis started from about 23 mg/kg bw/day in the 90 days dog oral study and/or extrapolated to 90 day dog exposure assay. In a 2-year study in rats and the one year interim sacrifice study (*RAR B.6.5.1.1., 1994a*) a dose related increase in elongated spermatid degeneration from 30.3 mg/kg bw/day and 32.8 mg/kg bw/day (both were also the MTD) were reported as well as an increase in multinucleated spermatids at 90 mg/kg bw/day and multinucleated spermatids in epididymis at 97.4 mg/kg bw/day, respectively. The effects on male reproductive organs in mice were reported at higher doses than effects reported in rats and dogs: tubular dilatation, aggregate lymphoid and sperm cysts/cystic dilation of epididymis were increased from 42.0 mg/kg bw/day, whereas tubular atrophy of testes was observed from the dose 446 mg/kg bw/day (216 mg/kg bw/day was the MTD) in carcinogenicity study of 18 months (*RAR B.6.5.2.1., 1994b*).

In repeated dose toxicity studies in rats, mice and dogs, studies were also reported that induced minor or no effects on male reproductive organs. However, it should be noted that in some studies neither weight of epididymis/prostate/seminal vesicles with coagulating glands was recorded nor histopathological examination of these organs/tissues was performed (e.g. *RAR B.6.3.2.1. Study 2, 1999b; RAR B.6.3.2.2., 1999b*); whereas in one study histological examination of epididymis was not specified (*RAR B.6.3.3.2., 2003*). It is noteworthy that some studies had several methodological limitations: despite the fact that male reproductive organs (testes and epididymis) were target tissues of cymoxanil and these tissues showed treatment-related changes in the high dose group, no histopathological examination was performed from all animals in all dose groups and no statistical analysis has been performed (e.g. *RAR B.6.5.1.2., 2003*). Additionally, regarding the dose level spacing selected, in some studies wide intervals were used, instead of recommended shorter intervals for setting the descending dose levels (e.g. *RAR B.6.5.1.2., 2003; RAR B.6.5.1.1., 1994a; RAR B.6.5.2.1., 1994b*). Furthermore, considering the top dose selection, not high enough dose levels were selected in some studies (e.g. *RAR B.6.3.3.1., 1994; RAR B.6.3.3.2., 2003; RAR B.6.5.2.2., 2002*). For example, effects on testes/epididymis found in studies on dogs (at 10.56 mg/kg bw/ day, *RAR B.6.3.2.3. Study 1, 1993*) could not be confirmed in other dog study due to low amount of substance administered (highest dose applied was 5.7 mg/kg bw/ day, *RAR B.6.3.3.1., 1994*). The difference in the results in the rat and mouse studies could also have been due to difference in the rat (Crl:CDBR and HsdCpb:WU) or mouse (Crl:CD-1®BR and HsdOla:MF 1) strains used in the various studies.

In a weight of evidence analysis, the effects on male reproductive organs observed in rats are considered most relevant for classification; furthermore, the effects on dog male reproductive organs observed can be also used. These included in a 90 day rat study (*RAR B.6.3.2.1. Study 1, 1993*) a dose related increase in bilateral spermatid degeneration in testes from 47.6 mg/kg bw/day (the incidence and severity increasing with dosage). The effects on male reproductive organs in dogs not always have been related to marked body weight loss resulting in delayed puberty. Indeed, aspermatogenesis in the dog testes at 10.56 mg/kg bw/day were observed in the presence of general toxicity (clinical signs, reduced body weight, loss body weight gain and reduced mean food consumption) and at the MTD in 90 days dog study (*RAR B.6.3.2.3. Study 1, 1993*). However, it is noteworthy that an apparent trend in the incidence of atrophy of testes from 2.8 mg/kg bw/day dose and the histological findings in the epididymis (bilateral seminiferous cell debris and unilateral atrophy with aspermia) at 5.6 mg/kg bw/day dose were observed in the absent of general toxicity in 1 year dog oral study (*RAR B.6.3.3.2., 2003*).

According to RAC Opinion (2012): "*The absence of effects on male fertility parameters in the 2-generation studies are not considered contradictory to or inconsistent with the testis and epididymis toxicity reported in several animal species in repeated dose toxicity studies. It is well known that as to male reproduction in animal species the most sensitive endpoint is histopathology of the testis which has a higher sensitivity compared to fertility parameters (Magelsdorf et al., 2003). This is related to the fact that rats have a high sperm reserve; they are still fertile after a reduction in sperm counts up to around 90%. In contrast, human fertility may already be affected by a small reduction in sperm count. Therefore, the toxicity on testes and epididymis are considered more relevant for humans than the minor effects on fertility parameters reported in the two 2-generation studies in rats regarding male reproductive organ toxicity.* "

The effects on testis and epididymis are in accordance with the CLP classification criteria for effects on sexual function and fertility. Adverse effects on sexual function and fertility are defined (Annex I: 3.7.1.3) as any effect of a substance that has the potential to interfere with sexual function and fertility including, but not limited to, alterations to the female and male reproductive system, adverse effects on onset of puberty, gamete production and transport, reproductive cycle normality, sexual behaviour, fertility, parturition, pregnancy outcomes, premature reproductive senescence, or modifications in other functions that are dependent on the integrity of the reproductive system.

In Annex I section 3.7.2.5.3 of the CLP Regulation, it is further described that *"Adverse effects or changes, seen in short- or long-term repeated dose toxicity studies, which are judged likely to impair reproductive function and*

which occur in the absence of significant generalised toxicity, may be used as a basis for classification, e.g. histopathological changes in the gonads". In the related CLP Guidance (2017) the use of data from repeated dose toxicity studies is further discussed in Section 3.7.2.3.1 under the heading fertility effects as follows: *"Toxicological effects, including marked effects, observed in a standard repeated dose study could be considered valid for the pre-mating phase for adult females and the pre- and post-mating phase for adult males. However in case of contradictions between the standard repeat dose studies and reproductive studies, the result from the latter should be considered more relevant."*

Furthermore, in CLP Annex I section 3.7.2.3.1 it is described that both positive and negative results are assembled together into a weight of evidence determination. A single positive study performed according to good scientific principles and with statistically or biologically significant positive results may justify classification.

In one of the 2-generation reproductive toxicity studies minor effects on fertility parameters were reported in the F1 generation in the high dose group (116.0 mg/kg bw/day for females) including decrease in mean litter size, the percentage of live pups born, together with a reduced mean number of corpora lutea and mean number of implantations as well as an increased percentage of post-implantation loss in the absence of marked systemic maternal toxicity. In the repeated dose toxicity studies in rats and dogs clear evidence of toxic effects on testes were reported starting around 50 mg/kg bw/day and around 11 mg/kg bw/day, respectively. The effects on rat and dog male reproductive organs were supported by some evidence of effects on mouse male reproductive organs in repeated dose toxicity studies.

As no evidence from humans are available a classification in Repr. 1A according to Regulation (EC) No 1272/2008 is not considered appropriate. Since the effects on male reproductive organs were not consistent in all repeated dose toxicity studies in rats, dogs and mice and since no effects on male reproductive organs were reported in the two 2-generation studies, the evidence is not sufficient to classify cymoxanil in Repr. 1B, H360F according to Regulation (EC) No 1272/2008.

In a weight of evidence analysis both positive and negative results are assembled together and a single positive study performed according to good scientific principles and with statistically or biologically significant positive results may justify classification. Clear effects were reported in repeated dose toxicity studies in rats, mice and dogs. However, in repeated dose toxicity studies in rats, mice and dogs, studies were also reported that induced minor or no effects on male reproductive organs.

Since there is some evidence from animal studies of an adverse effect on sexual function and fertility, cymoxanil should be classified as **Repr. 2, H361f** according to Regulation (EC) No 1272/2008.

2.6.6.2 Adverse effects on development [equivalent to section 10.10.4 of the CLH report template]

For more detailed data on effects on development please refer to RAR Volume 3CA B-6, section B.6.6. and section B.6.7.1.2.
For more detailed data on effects regarding two 2-generation reproduction toxicity studies in rats, one reduced-size 1-generation study in rats and one developmental neurotoxicity rat study please refer to Section 2.6.6.1. and Table 34.

Table 35: Summary table of animal studies on adverse effects on development

Method, guideline, deviations[1] if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results** **- NOAEL/LOAEL (for parent, offspring and for developmental effects)** **- target tissue/organ** **- critical effects at the LOAEL**	**Reference**
Two-generation reproduction toxicity study OECD 416 (1983) GLP Rat, Hsd Cpb: WU 30/sex/group Acceptable	Cymoxanil 0972 and 498VF973, 98.8% 0, 150, 450, 1350 ppm equal to 0, 10.5, 31.6, 94.0 mg/kg bw/day (F0 males) and 0, 14.9, 42.8, 116.3 mg/kg bw/day (F0 females premating, gestation and lactation) Oral: diet	**Parental** NOAEL: 31.6 (♂) – 42.8 (♀) mg/kg bw/day LOAEL: 94.0 (♂) – 116.3 (♀) mg/kg bw/ day based on ↓bw gain premating (F0 males: 17%, F1 males: 14%, F0 female: 10%), gestation (F1 female: 20%), lactation (F0 female: 78%) ↓FC premating (F1 males: 10%, F0/F1 female: 9%), gestation (F0/F1 female: 11%/8%), lactation (F0/F1 females: 33%/26%) **Offspring** NOAEL: 10.5 (♂) – 14.9 (♀) mg/kg bw/day	RAR B.6.6.1.1., 2001

Method, guideline, deviations[1] if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results** **- NOAEL/LOAEL (for parent, offspring and for developmental effects)** **- target tissue/organ** **- critical effects at the LOAEL**	**Reference**
	Approximate number of dose weeks: mating and throughout the mating period: males F0/F1 (14/18 weeks), females F0/F1 (10/14 weeks) females F0/F1: gestation (21 days) and lactation (21 days)	LOAEL: 31.6 (♂) – 42.8 (♀) mg/kg bw/day based on ↓bw (both sexes combined F1 pups Days 14 and 21: >10%; F2 pups Days 7, 14 and 21: >8%)	
Two-generation reproduction toxicity study OECD 416 (1983) GLP Rat, Crl:CD®BR 30/sex/group Acceptable	Cymoxanil, DPX-T3217-113, 97.8% 0, 100, 500, 1500 ppm equal to 0, 6.5, 32.1, 97.9 mg/kg bw/day (F0 males) 0, 6.65, 34.7, 103.0 mg/kg bw/day (F0 females gestation) Oral: diet Approximate number of dose weeks: mating and throughout the mating period: males F0/F1 (16/32 weeks), females F0/F1 (10/15 weeks) females F0/F1: gestation (21 days) and lactation (21 days)	**Parental** NOAEL: 32.1 – 34.7 mg/kg bw/day LOAEL: 97.9 – 103 mg/kg bw/day based on clinical signs (end of tail missing, necrotic tip of tail, sore), ↓bw (>10%) premating (F0 males, F0 female), gestation (F0 female) ↓bw gain premating (F0 males: 18%, F1 males: 14%, F0 female: 23%, F1 female: 14%), gestation (F0 female: 12%) ↓FC (>10%) premating (F0/F1 males, F1 female), 1st gestation (F1 female) **Offspring** NOAEL: 6.5 – 6.65 mg/kg bw/day LOAEL: 32.1 – 34.7 mg/kg bw/day based on ↓viability index (%) of F1 pups (days 1-4) ↓bw (both sexes combined F2B pups, Days 4, 7, 14 and 21: 13-18%)	RAR B.6.6.1.2., 1993
One generation reproduction toxicity study OECD 415 (1983) GLP Rat, Hsd Cpb: WU 15/sex/group Supportive only A range finding study	Cymoxanil 0972, 98.8% 0, 750, 1500 and 3000 ppm equal to 0, 57.7, 114.3, 226.2 mg/kg bw/day (males premating) and 0, 75.1, 136.1, 240.8 mg/kg bw/day (females premating, gestation and lactation) 0, 68.4, 127.7, 235.2 mg/kg bw/day (combined) Oral: diet Approximate number of dose weeks: mating and throughout the mating period: males (15 weeks), females (10 weeks) females: gestation (21 days) and lactation (21 days)	**Parental** NOAEL: 68.4 (combined) mg/kg bw/day LOAEL: 127.7 (combined) mg/kg bw/day based on ↓bw gain female (gestation 12%) ↓FC female: all premating (9%), all gestation (≥10%), all lactation (≥20%) periods **Offspring** LOAEL: 68.4 (combined) mg/kg bw/day based on ↓bw (both sexes combined pups Days 14 and 21: >10%)	RAR B.6.6.1.3., 1998
Developmental neurotoxicity study	Cymoxanil DPX-T3217-113; 97.8 %	Neurodevelopmental NOAEL: ≥ 100 mg/kg bw/day	RAR B.6.7.1.2.,

Method, guideline, deviations[1] if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results** **- NOAEL/LOAEL (for parent, offspring and for developmental effects)** **- target tissue/organ** **- critical effects at the LOAEL**	**Reference**
in rats US EPA OPPTS 870.6300 [complies generally with OECD 426 (2007)] GLP Rat CD®(SD)IGS VA/Plus® 25 females/dose Acceptable	0, 5, 50, 100 mg/kg bw/day administered from GD 6 to lactation day 21, gavage	Neurodevelopmental LOAEL: Not obtained. No developmental neurotoxic effects up to the highest dose level tested Maternal NOAEL: 5 mg/kg bw/day Systemic LOAEL: 50 mg/kg bw /day based on ↓body weight gain at GD 6 – 9 (49.1%) ↓FC (13.2%) Developmental NOAEL = 50 mg/kg bw/day Developmental LOAEL = 100 mg/kg bw/day based on ↑pup mortality Days 2-5/9-12 post-partum ↓viability index on day 5, ↓lactation indexes on day 12/22, ↓average number of live pups/litter, ↓ live litter size, clinical observations in pups (cold to the touch, not nursing and not nesting),	2001
Developmental toxicity (teratogenicity) study OECD 414 (1981) GLP Rat, Crl:CD BR 25 females/dose group Acceptable	Cymoxanil DPX-T3217-113, 97.8% 0, 10, 25, 75, 150 mg/kg bw/day Days 7-16 of gestation, gavage	**Maternal:** NOEL: 10 mg/kg bw/day LOAEL: 25 mg/kg bw/day based on ↓ body weight gain (45*%) over 7-9 days of gestation ↓adjusted body weight gain at 1-22 days of gestation (3.8%) and at 7-22 days (6.8%) ↓ food consumption (12%) over 7-9 days of gestation **at 75mg/kg bw/day** ↓adjusted body weight gain at 1-22 days of gestation (20.8*%) and at 7-22 days (25.1*%) ↓ body weight gain (9.3*%) over 17-22 days of gestation ↓ body weight (4.9*%) at 22 day ↓adjusted body weight (4.1*%) at 22 day **at 150 mg/kg bw/day** ↓adjusted body weight gain at 1-22 days of gestation (25.3*%) and at 7-22 days (36.2*%) ↓ body weight gain (13*%) over 17-22 days of gestation ↓ body weight (10.1*%) at 22 day ↓adjusted body weight (5.5*%) at 22 day **Developmental:** NOAEL: 10 mg/kg bw/day LOAEL: 25 mg/kg bw/day based on ↑incidence of skeletal variations and delayed ossification Foetal toxicity at higher dose levels: ↑ incidence of skeletal malformations (hemi vertebra at ≥75 mg/kg/day; exencephalic head and fused ribs at 150 mg/kg/day) ↑incidence of skeletal variations (partially ossified sternebra, unossified sternebra, wavy ribs and partially ossified pelvis at 150 mg/kg/day)	RAR B.6.6.2.1., 1993
Developmental toxicity (teratogenicity)	Cymoxanil 0972, 98.8 %	**Maternal:** NOEL: 60 mg/kg bw/day LOAEL: 120 mg/kg bw/day based on	RAR B.6.6.2.2., 1998

Method, guideline, deviations[1] if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results** **- NOAEL/LOAEL (for parent, offspring and for developmental effects)** **- target tissue/organ** **- critical effects at the LOAEL**	**Reference**
study OECD 414 (1981) GLP Wistar rats, 27 females/dose group Acceptable	0, 30, 60, 120 mg/kg bw/day Days 6-15 of gestation, gavage	↓ body weight gain (50%/20%) over 6-15/0-20 days of gestation ↓ food consumption (25%/13%) over 6-15/0-20 days of gestation *Information on corrected maternal body weight is not available* **Developmental:** NOAEL cannot be established LOAEL: 30 mg/kg bw/day based on ↑incidence of skeletal minor anomalies (dumb-bell shaped thoracic vertebra 6/13) Foetal toxicity at higher dose level: ↑incidence of skeletal variations: delayed ossification (servical vertebra: 7/7 and supraoccipital) and minor anomalies (dumb-bell shaped thoracic vertebra 6/13, hypoplasia of sternum: sternebra no. 1/2 and rudimentary 14th rib) at ≥60 mg/kg bw/day ↑incidence of skeletal variations: delayed ossification (sternum, vertebra, phalanges and supraoccipital) and minor anomalies (dumb-bell shaped thoracic vertebra 6/13, hypoplasia of sternum: sternebra no. 1/2, rudimentary 14th rib and vertebra) at ≥120 mg/kg bw/day	
Developmental toxicity (teratogenicity) study OECD 414 (1981) GLP New Zealand White rabbits 15 females/dose group Supportive only (too low number females with implantations and no maternal toxicity demonstrated)	Cymoxanil, 7800-20-C, 94.2% 0, 4, 8, 16 mg/kg bw/day Days 6-18 of gestation, gavage	**Maternal:** NOAEL: ≥ 16 mg/kg bw/day LOAEL cannot be established No effects even at the highest dose tested **Developmental:** NOAEL ≥ 16 mg/kg bw/day LOAEL cannot be established No effects even at the highest dose tested	RAR B.6.6.2.3., 1980
Developmental toxicity (teratogenicity) study OECD 414 (1981) GLP New Zealand White rabbits 15 females/dose group	Cymoxanil, 7800-20-C, 94.2% 0, 8, 16, 32 mg/kg bw/day Days 6-18 of gestation, gavage	**Maternal:** NOEL: 8 mg/kg bw/day LOAEL: 16 mg/kg bw/day based on clinical observations (anorexia/ reduced faecal output) *Information on corrected maternal body weight is not available* **Developmental:** NOAEL: 16 mg/kg bw/day LOAEL: 32 mg/kg bw/day based on ↑incidences of skeletal malformations (vertebra and/or rib alterations linked with scoliosis)	RAR B.6.6.2.4., 1981

Method, guideline, deviations[1] if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results** **- NOAEL/LOAEL (for parent, offspring and for developmental effects)** **- target tissue/organ** **- critical effects at the LOAEL**	**Reference**
Acceptable			
Developmental toxicity (teratogenicity) study OECD 414 (1981) GLP New Zealand White rabbits 17-20 females/dose group Acceptable	Cymoxanil, INT-3217-90, 95.8% 0, 1, 4, 8, 32 mg/kg bw/day Days 6-18 of gestation, gavage	**Maternal:** NOAEL: ≥ 32 mg/kg bw/day LOAEL cannot be established No effects even at the highest dose tested *Information on corrected maternal body weight is not available* **Developmental:** NOAEL: 8 mg/kg bw/day LOAEL: 32 mg/kg bw/day based on ↑incidences of visceral malformations (cleft palate and hydrocephaly)	RAR B.6.6.2.5., 1982
Developmental toxicity (teratogenicity) study OECD 414 (1981) GLP New Zealand White rabbits 17 females/dose group Acceptable	Cymoxanil 0972, 98.8 % 0, 5, 15, 25 mg/kg bw/day Days 6-18 of gestation, gavage	**Maternal:** NOEL: 15 mg/kg bw/day LOAEL: 25 mg/kg bw/day based on ↓body weight gain (160%) over 6-18 days of gestation ↓food consumption (17%) over 6-19 days of gestation *Information on corrected maternal body weight is not available* **Developmental:** NOAEL: 15 mg/kg bw/day LOAEL: 25 mg/kg bw/day based on ↑incidences of visceral malformations (dilation of heart ventricles), visceral variants (slight renal pelvis dilation), skeletal variants (incomplete/poor ossification of fore limb), skeletal minor anomalies (accessory floating rib no. 13)	RAR B.6.6.2.6., 1999

Table 36: Summarized fetal findings from available developmental studies (*for more detailed data on effects please refer to RAR Volume 3CA B-6, section B.6.6.2.*)

Study (owner, author)	**Species, Strain**	**Purity (%)**	**Dose levels mg/kg bw/day**	**NOAEL**	**Relevant fetal findings (malformations in bold) at**
Developmental toxicity (teratogenicity) study in rats (*RAR B.6.6.2.1., 1993)*	Rat Crl:CD®BR	97.8	0, 10, 25, 75, 150	10 (maternal) 10 (developmental)	25 mg/kg: incidence of skeletal variations and delayed ossification (partially ossified vertebra and partially ossified skull)↑ * **75 mg/kg**: incidence of skeletal variations and delayed ossification (partially ossified vertebra, partially ossified skull and partially ossified sternebra)↑ * **skeletal malformations: number of fetuses with hemivertebra↑ ***** **150 mg/kg**: incidence of skeletal variations and delayed ossification (partially ossified sternebra, unossified sternebra, wavy ribs and partially ossified pelvis) ↑ ***

Study (owner, author)	Species, Strain	Purity (%)	Dose levels mg/kg bw/day	NOAEL	Relevant fetal findings (malformations in bold) at
					skeletal malformations: number of fetuses with hemivertebra↑ * **number of fetuses with exencephalic head and fused ribs↑ ***
Developmental toxicity (teratogenicity) study in rats *(RAR B.6.6.2.2., 1998)*	Wistar art	98.8	0, 30, 60, 120	60 (maternal) **30 (developmental LOAEL)**	30 mg/kg: delayed or incomplete ossification (e.g. sternum, scull)↑ ** incidence of minor skeletal anomalies (dumb-bell shaped thoracic vertebra 6/13)↑ *** 60 mg/kg : delayed or incomplete ossification (e.g. vertebra, supraoccipital)↑ *** incidence of minor skeletal anomalies (dumb-bell shaped thoracic vertebra 6/13, hypoplasia of sternum: sternebra no. 1/2 and rudimentary 14th rib)↑ *** 120 mg/kg: delayed or incomplete ossification (e.g. sternum, vertebra, phalanges, supraoccipital)↑ *** incidence of minor skeletal anomalies (dumb-bell shaped thoracic vertebra 6/13, hypoplasia of sternum: sternebra no. 1/2, rudimentary 14th rib and vertebra)↑ ***
Developmental toxicity (teratogenicity) study in rabbits *(RAR B.6.6.2.3., 1980)*	New Zealand White rabbit	94.2	0, 4, 8, 16	> 16 (maternal) > 16 (developmental)	No adverse foetal findings but study regarded as supplementary information only (too low number females with implantations and no maternal toxicity demonstrated)
Developmental toxicity (teratogenicity) study in rabbits *(RAR B.6.6.2.4., 1981)*	New Zealand white rabbit	94.2	0, 8, 16, 32	8 (maternal) 16 (developmental)	**32 mg/kg: incidence of vertebra and rib alterations↑ #** *(including hemivertebra, absent or fused vertebrae, misaligned vertebral centra/arches, fused/absent ribs, and various degrees of resulting scoliosis)*
Developmental toxicity (teratogenicity) study in rabbits *(RAR B.6.6.2.5., 1982)*	New Zealand White rabbit	95.8	0, 1, 4, 8, 32	> 32 (maternal) 8 (developmental)	**32 mg/kg**: incidence of vertebra and rib alterations↑ ** **Visceral malformations: hydrocephaly (2/117 [1.7%] fetuses) #** **cleft palate (2/117 [1.7%] fetuses)***
Developmental toxicity (teratogenicity) study in rabbits *(RAR B.6.6.2.6., 1999)*	New Zealand White rabbit	98.8	0, 5, 15, 25	15 (maternal) 15 (developmental)	**25 mg/kg**: incidence of skeletal variants [incomplete/poor ossification of fore limb (middle phalange: 1/5)]↑ * incidence of visceral variants (slight renal pelvis dilation)↑ * incidence of skeletal minor anomaly (accessory floating 13th rib)↑ *** **Visceral malformations: incidence of dilation of heart ventricles↑ ***

Studies and results which were relevant for classification as Repr. 2, H361d only are highlighted in grey.

* *statistically significant*
\# *not statistically significant but above historical range; regarded as relevant*
** *statistically significant but within historical range*
*** *statistically significant and above historical range*

No human data on adverse effects on development are available. No other studies relevant for developmental toxicity are available.

2.6.6.2.1 Short summary and overall relevance of the provided information on adverse effects on development

This section is represented by two 2-generation reproduction toxicity studies in rats, one reduced-size 1-generation study in rats, one developmental neurotoxicity rat study, two developmental toxicity studies in rats and four developmental toxicity studies in rabbits. These data except a new reduced-size one-generation study were evaluated during the first Annex I review of cymoxanil and were presented in the monograph (Vol.3, Annex B, Section B6, Point B.6.6, June 2007) and addendum to the monograph (April 2008). All these studies have been re-evaluated by the RMS under the renewal programme. The results of two-/one-generation reproduction (the parental and offspring effects presented only) and developmental toxicity studies are summarised in the Table above.

Worthy of notice that these six developmental toxicity studies do not appear to comply with the updated OECD TG 414 (2018) as the following deviations were identified: the dosing period covered solely the period of major organogenesis (i.e. days 6-15 / 7-16 in the rat and days 6-18 in the rabbit), no endocrine sensitive end-points (e.g. thyroid weight and histopathology, hormonal analysis of T3, T4, TSH and anogenital distance etc) were evaluated, some groups were with fewer than 16 animals with implantation sites at necropsy (rabbit studies), information on HCD provided didn't meet all requirements as set out in Commission Regulation (EU) No 283/2013 (section 5; 5.6. Reproductive toxicity) and were quite limited. One rabbit developmental toxicity study is considered of limited validity for the assessment of developmental effects, because of too low number females with implantations and no maternal toxicity was demonstrated in all dose levels tested.

For more detailed data on effects regarding two 2-generation reproduction toxicity studies in rats, one reduced-size 1-generation study in rats and one developmental neurotoxicity rat study please refer to Section 2.6.6.1.

In the **first two-generation study** (*RAR B.6.6.1.1.*, 2001), the NOAEL concerning systemic toxicity for parental animals in the 2-generation study is proposed at 450 ppm (equal to 31.6 mg/kg bw/day for males and 42.8 mg/kg bw/day for females) based on reduced bodyweight gain in the F0 and F1 males and in the F0 females (during premating and lactation) and F1 females (during gestation); reduced food consumption in the F1 generation males and in the F0 and F1 females (during all phases) at the high dose of 1350 ppm (equal to 94.0 mg/kg bw/day for males and 116.3 mg/kg bw/day for females). It was considered inappropriate to establish the LOAEL for parental toxicity at 450 ppm (equal to 31.6 mg/kg bw/day for males and 42.8 mg/kg bw/day for females) based on reduced initial body weight in the F1 generation (11% for males and 9% for females) in the absence of changes in body weight gain after administration of cymoxanil, slight reduced food consumption (7% - 9%) in F0 females (during premating and gestation) and reduced body weight gain (23.7%) in the F1 females during the first five days of gestation only. These findings without any other associated adverse effects were considered insufficiently relevant for setting the LOAEL at 450 ppm. Organs (testes, seminal vesicles with coagulating gland, epididymis, prostate, uterus (with cervix), and ovaries) were not weighed but were subject to gross pathology and microscopic examinations; there were no unusual gross necropsy or microscopic findings in males and females of either parental generation.

With respect to offspring toxicity, statistically significant reductions have been observed for number of the F1 litter pups (both sexes combined) alive on day 14 and 21 and the respective survival indices of the high dose (1350 ppm) group animals. These findings are associated with the statistically significantly increased number of pups dead/cannibalised on days 8-14 and days 15-21. At the highest dose (1350 ppm) group of the F2 litters, the statistically significantly lower mean litter size including the mean viable litter size was reported and was likely a consequence of the lower number of corpora lutea, an increased percentage of post-implantation loss and a reduced mean number of implantations of the F1 dams. The number of F2 pups (both sexes combined) alive (pre-cull) on day 4 and number of pups alive (post-cull) on day 4 were found to be statistically significantly decreased for the high dose (1350 ppm) group animals. There was again a reduced number of F2 pups alive on day 21 (including the day 21 survival index) related to the increased pups found dead/cannibalised on day 15 – 21 for the high dose group animals. Although some of above mentioned findings were within the range of the HCD declared, due to limited information provided by the Applicant the assessment of their relevance was impossible. Concerning pup development, statistically significantly decreased (10 - 46%) body weights (both sexes combined) for F1 pups were reported from day 4 until day 21 at the high dose level of 1350 ppm. Pup body weights were also statistically significantly reduced (>10%) in the mid-dose group on days 14 and 21. Amongst the F2 pups statistically significantly decreased (19.5 - 38%) body weights were reported from day 7 until day 21

at the high dose level of 1350 ppm. Pup body weights were also statistically significantly slight reduced (8.4-9.1%) in the mid dose group on days 7, 14 and 21.

The NOAEL for parental toxicity is proposed at 450 ppm (equal to 31.6 mg/kg bw/day for males and 42.8 mg/kg bw/day for females) based on reduced bodyweight gain in the F0 and F1 parental animals; reduced food consumption in the F1 males; reduced food consumption in the F0 and F1 females at the high dose level. The NOAEL for offspring toxicity was set at 150 ppm (equal to 10.5 mg/kg bw/day for males and 14.9 mg/kg bw/day for females) based on reduced body weight of F1 pups (both sexes combined) on days 14 and 21 as well as reduced body weight of F2 pups (both sexes combined) on days 7, 14 and 21.

In the **second two-generation study** (*RAR B.6.6.1.2.,* 1993), the F1 parental animals at high dose of 1500 ppm (equal to 97.9 mg/kg bw/day for males and 103 mg/kg bw/day for females) showed a statistically significant increase of clinical observations: males showed a statistically significant increase of "end of tail missing", "necrotic tip of tail" as well as "sore"; females of this group showed statistically significant increase of "sore" (premating until lactation), "end of tail missing", "necrotic tip of tail", "stained fur" and "masses". Body weight gain was statistically significantly impaired (>10%) at 1500 ppm in parental males of the F0/F1 generations as well as in females of the F0 generation (premating and gestation) and F1 generation (premating). The body weight of F0 males and females (premating and gestation) at the top dose groups was statistically significantly reduced (10 - 13%) throughout a long observation period. Meanwhile the initial mean body weights of F1 males and females at high dose were substantially and statistically significantly below the concurrent control values (~30%), that persisted for the remainder of the study (>10% reduction). Food consumption was statistically significantly reduced (>10%) at 1500 ppm in parental males of the F0/F1 generations as well as in females of the F1 generation (premating and 1st gestation). There were no adverse effects (decrease ≥10%) on body weight, body weight gain and food consumption of F0/F1 generations of both sexes at the mid dose level and lower.

With respect to offspring toxicity, the viability index of F1 pups during early lactation (days 1-4) was statistically significantly reduced at 500 ppm and 1500 ppm and this finding was not evident at both F2 generations. The litter survival (%) (Number of litters weaned/ Number of viable litters delivered x 100) for F_1 pups at 1500 ppm was also statistically significantly lower than the respective control. There was a statistically significant increase in the total number of affected litters with respect to clinical observations in pups of the highest dose of 1500 ppm group in the F1, F2A and F2B generations: relevant clinical observations comprise "gasping", "no milkspot", "subcutaneous haemorrhage" and "weak". Concerning pup weight (both sexes combined), statistically significant reductions (11-40%) were evident at 1500 ppm (all generations) and also at the mid dose level of 500 ppm for the F2B generation (reduction 13-18%).

Based on these findings, the NOAEL for parental toxicity is proposed at 500 ppm (equal to 32.1 mg/kg bw/day in males and 34.7 mg/kg bw/day in females). The NOAEL for offspring toxicity was set at 100 ppm (equal to 6.5 mg/kg bw/day in males and 6.65 mg/kg bw/day in females).

The additional **reduced-size one generation study in rats** (*RAR B.6.6.1.3., 1998*) is considered acceptable as a range finding study and supportive only. There were no clinical signs observed, no pre-terminal mortality, and no treatment related ophthalmological findings. The body weight of males at the top dose of 3000 ppm (equal 226.2 mg/kg bw/day for males) was statistically significantly reduced (10 - 13%) throughout the observation period; overall body weight gain (0 – 15 weeks) was statistically significantly decreased by 18%. Food consumption was statistically significantly reduced (10 – 27.6%) at 3000 ppm in parental males throughout the first 6 weeks based on the originally study report. The body weight of females was reduced at the top dose of 3000 ppm marginally, whereas the body weight gain was statistically significantly reduced by 12% and 43% (respectively) during gestation (0 – 20 days) at the mid and high doses levels (equal 136.1 mg/kg bw/day and 240.8 mg/kg bw/day for females). Based on the originally study report, food consumption was statistically significantly reduced (12.9 – 28.4%) at 3000 ppm in parental females throughout all premating period, whereas food consumption was statistically significantly reduced (>10%) during all gestation and all lactation period from the mid dose of 1500 ppm (equal 136.1 mg/kg bw/day).

With respect to offspring toxicity, statistically significant reductions have been observed for number of pups (both sexes combined) alive on LD 1 and LD 4 and the respective LD 4 survival index (%) of the high dose (3000 ppm) group animals. These findings are associated with the statistically significantly increased number of pups dead at birth/on day 1 and dead/cannibalised up to day 4. Additionally, the statistically significantly lower mean live litter size including lower live birth index (%) was reported the high dose. Concerning pup development, statistically significantly decreased (>11%) body weight (both sexes combined) for pups were reported from second week of lactation at 750 ppm, statistically significant reduction (19.6 – 31.2%) of body weight was observed from LD 7 at 1500 ppm and decreased (15.1 – 46.7%) body weight for pups were reported from LD 4 at 3000 ppm.

Based on these findings, the NOAEL for parental toxicity is proposed at 750 ppm (equal to 68.4 mg/kg bw/day combined). The LOAEL for offspring toxicity was set at 750 ppm (equal to 68.4 mg/kg bw/day combined).

In the **developmental neurotoxicity rat study** (*RAR B.6.7.1.2., 2001*), there was no evidence of neurotoxicity up to the highest-dose tested in a battery of functional tests; furthermore, examination of the nervous system tissues revealed no unusual findings. Maternal toxicity was characterised by reductions in body weight gain and food consumption at gestation days 6 – 9 at 50 mg/kg bw/day. In pups of the highest dose (100 mg/kg bw/day) group, following treatment-related statistically significant changes were observed: statistically significantly increased number of pups (%) found dead or presumed cannibalized ((13.2% and 2.3% on Days 2-5/9-12, respectively), reduced viability index (number of live pups on day 5 post-partum/number of live born pups on day 1 post-partum), reduced lactation indexes (number of live pups on day 12 post-partum/number of live pups on day 5 post-partum; number of live pups on day 22 post-partum (weaning)/number of live pups in subsets 2 – 4 on day 12 post-partum), lower number of surviving pups/litter (from Day 5 post-partum to Day 22 post-partum) and live litter size (Days 12/18/22 post-partum). The average number of pups delivered, live born and stillborn pups as well as sex ratio remained unaffected. Regarding clinical signs, in the pre-weaning period in top dose (100 mg/kg bw/day) group there were four pups that were cold to the touch, two that were not nursing, two not nesting, one dehydrated and one emaciated pup. In the gross pathology examination on the pups that died prior to weaning the only finding noted was a dose-related increase in pups with no milk in their stomachs (see the table below). 33% of dead pups had no milk in their stomachs at high dose of 100 mg/kg bw/day; however, in percent values it was not dose-related. Body weight, body weight gain and food consumption were not significantly affected by treatment in any dose group. Cymoxanil had no effect on the sex ratio of pups or the timing of sexual maturation.

A NOAEL for maternal toxicity of 5 mg/kg bw/d, a NOAEL for offspring toxicity of 50 mg/kg bw/d is therefore proposed and a NOAEL for developmental neurotoxicity was ≥100 mg/kg bw/day.

In the **first developmental rat study** (*RAR B.6.6.2.1., 1993*) maternal toxicity occurred at 25 mg/kg bw/day and above. There were reductions in mean body weight gain by 44.6% and food consumption by 11.7% at 25 mg/kg bw/day dose over the first few days of dosing (7 -9 days of gestation). It is noteworthy that litter size and/or body weight of pups could not have influence on this maternal toxicity parameter at 25 and 75 mg/kg bw/day dose levels: there was not effect on the mean number of implants, total live foetuses per litter and mean fetal weight (total, males and females) in these dose groups. According to Regulation (EC) No 1272/2008 (Annex I: point 3.7.2.4.4.) the calculation of an adjusted (corrected) mean maternal body weight change, may indicate whether the effect is maternal or intrauterine. Maternal adjusted body weight gains were statistically significantly reduced (>20%) at 1-22 days and 7-22 days from 75 mg/kg bw/day only. Mean maternal body weights were significantly reduced (9 - 11%) from day 9 of gestation until the end of the observation period (day 22 of gestation) at the high dose level (150 mg/kg bw/day). Meanwhile, there were no toxicologically relevant changes in corrected body weight when compared to controls: corrected body weights were statistically significantly reduced 4.1% and 5.5 % only at day 22 at the upper two dose levels. There were no test substance-related effects with respect to mortality in all dose groups.

There was a statistically significant decrease in the number of male viable foetuses per litter from 75 mg/kg bw/day dose. In the high dose group there was a statistically significant increase in the total number of resorptions/litter, an increase in the number of early resorptions/litter, a reduction in the total number of viable foetuses per litter and a reduction by 16.7% in the mean foetal body weight.

Concerning teratogenic effects, increased incidences of treatment related skeletal variations (mean percent of affected foetuses per litter and number of foetuses affected) as well as incidences of total variations (mean percent of affected foetuses per litter) were observed from 25 mg/kg bw/day dose level. The skeletal variations included partially ossified skull, partially ossified/unossified sternebra, partially ossified vertebra, wavy ribs, unossified hyoid and partially ossified pelvis and these variations indicated retarded/ skeletal development/delay ossification.

It should be noted that detailed information on the HCD for the study under evaluation was not available at the time of evaluation (identification of species and strain; name of the supplier; name of the laboratory; the dates when the study was performed etc) and, therefore, the assessment of their relevance was impossible. Since the HCD provided did not meet all requirements as set out in Commission Regulation (EU) No 283/2013 (section 5; 5.6. Reproductive toxicity), additional clarification by the Applicant was requested. The applicant subsequently (14.02.2020) commented that *"The BCD were from the same strain and supplier, from 35 studies conducted in the same laboratory over a 10-year period bracketing the study in question. Incidences in control groups, by year of study conduct, are shown in the accompanying document."*. It should be noted that the HCD presented has been compiles from 35 studies which were carried out between the year 1988 and 1997. Therefore, the studies cover a longer period than the requested five-year period. Furthermore, no summary data on the HCD are available (e.g. mean, median and standard deviation) as is required. Therefore, the HCD previously submitted for the study under evaluation should be used with caution and acknowledgement of its lower relevance and reliability as HCD cover a longer period than the requested and absolute values only are available. For more detailed information on HCD please refer to RAR Volume 3CA B-6, section B.6.6.2.1.

The skeletal variations showed a treatment relationship, although not a clear dose relationship, they were

observed in the presence of maternal toxicity, were within/ above the range of HCD declared and some of them were quite marked at the highest dose only, consistent with the observed reduction in fetal weight at this dose level. The variations, i.e. partially ossified pelvis, partially ossified sternebra, unossified sternebra and wavy ribs, at the highest dose group (150 mg/kg bw/day) tested are considered to be treatment related and of toxicological relevance.

The percentage of affected foetuses per litter with any malformations (external, visceral or skeletal) as well as the total number of foetuses affected was significantly increased from 25 mg/kg bw/day. Increased incidences of skeletal malformations such as hemi vertebra were reported from 75 mg/kg bw/day and such malformations as exencephalic head and fused ribs at 150 mg/kg bw/day. These findings were above the range of historical control values declared, however, the assessment of their relevance was impossible. Although incidences of these malformations observed were low, treatment-relation with respect to these findings cannot be excluded. Cleft sternebrae occurred at the upper two dose levels and the incidence was within the HCD. These effects were observed in the presence of maternal toxicity evident as statistically significant reduced maternal body weight gain (from 25 mg/kg bw/day) and/or adjusted body weight gain (from 75 mg/kg bw/day). However, it should be noted that according to Regulation (EC) No 1272/2008 (Annex I: point 3.7.2.4.2.) "*Developmental effects which occur even in the presence of maternal toxicity are considered to be evidence of developmental toxicity, unless it can be unequivocally demonstrated on a case-by-case basis that the developmental effects are secondary to maternal toxicity. Moreover, classification shall be considered where there is a significant toxic effect in the offspring, e.g. irreversible effects such as structural malformations, embryo/foetal lethality, significant post-natal functional deficiencies.".*

Based on reduced body weight gain and food consumption observed at 25 mg/kg bw/day, the maternal NOAEL was set at 10 mg/kg bw/day. The developmental NOAEL was set at 10 mg/kg bw/day, based on increased incidences of skeletal variations. It should be noted that increased incidences of some malformations were reported from 75 mg/kg bw/day.

There were no treatment-related maternal deaths in the **second developmental rat study** (*RAR B.6.6.2.2., 1998*). Maternal toxicity occurred at 120 mg/kg bw/day. There were reductions in mean body weight gain by 50%, food consumption by 25% over the first days of dosing (6 -15 days of gestation). Furthermore, mean body weight gain and food consumption was shown to be reduced (20 and 13%, respectively) for the high dose group throughout gestation (day 0 – 20) too. It should be noted that information on corrected maternal body weight and body weight gain is not available for this study. However, it is noteworthy that mean number of implants, litter size and/or sex ratio could not have influence on the maternal toxicity parameters as there were not effects on these parameters at all dose levels.

In the high dose (120 mg/kg bw/day) group there was a non-statistically significant but marked increase in the number of late resorptions and post-implantation loss as well as a statistically significant reduction by 11% in the mean male foetal body weight.

Concerning foetal skeletal alterations, there were no major skeletal malformations, whereas incidences for minor anomalies (dumb-bell shaped thoracic vertebra 6/13) were shown to be statistically significantly increased and above the historical control data declared even at the lowest dose tested (30 mg/kg bw/day). These alterations are demonstrating an impact of the test material to the development of foetuses. Meanwhile several skeletal variations comprising incidences of delayed ossification (cervical vertebra: 7/7 and supraoccipital) and some related minor anomalies (hypoplasia of sternum: sternebra no. 1/2 and rudimentary 14th rib) were statistically significantly increased and were above the range of HCD from 60 mg/kg bw/day. Many skeletal variations comprising incidences of delayed ossification (sternum, vertebra, phalanges and supraoccipital) and related minor anomalies (dumb-bell shaped thoracic vertebra 6/13, hypoplasia of sternum: sternebra no. 1/2, rudimentary 14th rib and vertebra) were statistically significantly increased and were above the range of HCD at 120 mg/kg bw/day.

The HCD presented for the study under evaluation has been compiles from 21 studies which were carried out between the year 1990 and 1998, therefore, the studies cover a longer period than the requested five-year period. Additionally, the HCD provided did not meet other requirements as set out in Commission Regulation (EU) No 283/2013 (section 5; 5.6. Reproductive toxicity): information on HCD provided didn't include identification of strain of species, name of the supplier, approximate age of the control animals etc. Therefore, it was not possible to conclude if the data submitted meet the requirement of being strain-specific and from the laboratory which carried out the index study. Additional clarification by the Applicant was requested and it subsequently (14.02.2020) commented that *"Expanded BCD information is not available at present. The applicant will apply to the laboratory concerned for the requested further information, with a view to presenting it if necessary during the commenting period of the peer review.".* Therefore, the HCD currently submitted for the study under evaluation should be used with caution and acknowledgement of its lower relevance and reliability because it does not meet all the requirements. For more detailed information on HCD please refer to RAR Volume 3CA B-6, section B.6.6.2.2.

The NOAEL for maternal toxicity can be established at 60 mg/kg bw/day, based on the findings with respect to body weight gain and food consumption at the highest dose level tested (120 mg/kg bw/day). Since incidences for skeletal minor anomalies (dumb-bell shaped thoracic vertebra 6/13) were shown to be statistically significantly increased and above the HCD even at the lowest dose tested (30 mg/kg bw/day), the developmental NOAEL cannot be established. Only the developmental LOAEL of 30 mg/kg bw/day can be derived in this study.

The **first developmental rabbit study** (*RAR B.6.6.2.3., 1980*) is considered as additional information only: the study is considered of limited validity for the assessment of developmental effects, because of too low number females with implantations and no maternal toxicity was demonstrated in all dose levels tested. Mean maternal body weights were not altered for all dose groups tested and no gross pathological changes were found attributed to treatment. No test substance related effects with respect to clinical observations were observed. Regarding a maternal mortality, no dose relationship was evident and was not considered to be treatment related.

There were no significant alterations and dose-related effects on the pregnancy parameters (e.g. number of implantations, number of corpora lutea, pre- or post-implantation loss and litter size) and on foetal weight. There were no meaningful intergroup differences in the incidences of either major malformations or minor abnormalities/variations in either viscera or skeleton.

The maternal and development NOAEL is above the highest dose level tested (16 mg/kg bw/day). No treatment related effects regarding maternal toxicity, pregnancy parameters and foetal toxicity (malformations or variations) were observed at any dose level.

No test substance related effect with respect to mortality was observed in the **second developmental rabbit study** (*RAR B.6.6.2.4., 1981*). There was a dose related incidence of anorexia/reduced faecal output among the dams at 16 and 32 mg/kg bw/day during the dosing period. Additionally, statistically significant increase of incidence of "cold ears" for the high dose animals was observed. The body weight gain was dose dependent reduced with statistical significance during dosing days 6 – 10 and 6 – 19 for the high dose (32 mg/kg bw/day) animals (84.7% and 39.3%, respectively). A finding "body weight loss of > 50 g" was dose dependent and significant at 32 mg/kg bw/day, however, no statistical analysis was performed. The body weight gain was reduced during dosing days 6 – 10 and 6 – 19 for the mid dose (16 mg/kg bw/day) animals (11% and 6.2%, respectively), however, these values didn't attain statistical significance and were considered not adverse. It should be noted that data on corrected maternal body weight and body weight gain are not available for this study. However, it is noteworthy that there were no effects on mean number of implants, litter size, fetal sex ratio and/or mean fetal body weight at all dose levels. The litter mean body weight was reduced (16.1%), however, this value didn't attain statistical significance and was not considered as adverse. On the other hand, according to Regulation (EC) No 1272/2008 (Annex I: point 3.7.2.4.4.) in rabbits, the body weight gain may not be useful indicators of maternal toxicity because of normal fluctuations in body weight during pregnancy.

There was no effect of cymoxanil on the pregnancy parameters (e.g. number of implantations, number of corpora lutea, pre- or post-implantation losses) or on foetal weight.

For the skeletal malformations, there were increases in the incidence of a vertebral and/or rib alterations, including hemivertebra, absent or fused vertebrae, misaligned vertebral centra/arches, fused/absent ribs, and various degrees of resulting scoliosis at the high dose of 32 mg/kg bw/day. Although these findings were not statistically significant, the percentage of foetuses with these malformations in the high dose group was above the HCD range submitted. These effects were observed in the presence of clear maternal toxicity.

The HCD presented for the study under evaluation has been compiles from 10 studies which were carried out between the year 1980 and 1981 using the same strain and supplier, conducted in the same laboratory. Therefore, the studies cover two-year period instead of the requested five-year period. Although the ranges of values of combined data were available, other values (mean, median and SD) were not provided. Additionally, information on HCD provided didn't include approximate age of the control animals etc. requested according to Commission Regulation (EU) No 283/2013 (section 5; 5.6. Reproductive toxicity). Additional clarification by the Applicant was requested and it subsequently (14.02.2020) commented that *"Detailed data from a broader period of time (1978-1983) from 104 studies are not immediately available, although relevant outcomes were shown graphically in the data summary…"*. Therefore, the HCD currently submitted for the study under evaluation should be used with caution and acknowledgement of its lower relevance and reliability because it does not meet all the requirements. For more detailed information on HCD please refer to RAR Volume 3CA B-6, section B.6.6.2.4.

Although other finding as "vertebral and other changes between upper cervical and mid-thoracic regions" was not statistically significant, the percentage of foetuses affected in the mid and high dose groups was clearly above the HCD range declared. However, these all skeletal alterations include malformations and other alterations that were classified as falling into a "borderline area" between malformation and anomaly allocating them into the category malformation. Diagnostic criteria for malformations and variations were not available in the study summary. The

glossary of terminology under development by the International Federation of Teratology Societies was not considered by the Applicant. Since malformations and variations should be reported separately according to the Regulation (EU) No 283/2013, skeletal abnormality as "vertebral and other changes between upper cervical and mid-thoracic regions" was not considered as relevant.

Based on clinical observations (anorexia/reduced faecal output) at 16 mg/kg bw/day, the maternal NOAEL is set at 8 mg/kg bw/day. Concerning developmental adverse effects, the developmental NOAEL can be established at 16 mg/kg bw/day based on increased incidences of skeletal malformations which were above the historical control data at the highest dose level tested (32 mg/kg bw/day).

No maternal toxicity occurred, even at the highest dose, in the **third developmental rabbit study** (*RAR B.6.6.2.5., 1982*). Mean maternal body weights or body weight gains were not significantly reduced at all observation periods. No test substance related effect with respect to mortality was observed. There were not treatment related clinical signs: anorexia observed in some animals across the groups during treatment was not dose related. It should be noted that data on corrected maternal body weight and body weight gain are not available for this study. However, it is noteworthy that there was no effect on mean number of implants, litter size and/or mean body weight of fetuses at all dose levels. On the other hand, according to Regulation (EC) No 1272/2008 (Annex I: point 3.7.2.4.4.) in rabbits, the body weight gain may not be useful indicators of maternal toxicity because of normal fluctuations in body weight during pregnancy.

There was no effect of cymoxanil on the pregnancy parameters (e.g. number of implantations, number of corpora lutea, resorption, abortion and litter size) or foetal viability and foetal body weight.

The HCD presented for the study under evaluation has been compiles from 20 studies which were carried out between the year 1980 and 1984 using the same strain and supplier, conducted in the same laboratory. Therefore, the studies cover five--year period requested. The historical control is presented on a study by study basis giving absolute values (incidences) only and no combined or summary data are available, i.e. the ranges of values (in percentages), the means, the median and the SD. Additionally, information on HCD provided didn't include approximate age of the control animals etc. requested according to Commission Regulation (EU) No 283/2013 (section 5; 5.6. Reproductive toxicity). Additional clarification by the Applicant was requested and it subsequently (14.02.2020) commented that *"Comparing results in terms of actual incidences, rather than percentages, is valid. Data for a broader range of the abnormalities from these background studies is provided in the accompanying document."*. No summary data on the HCD were submitted (e.g. ranges of values (in percentages), mean, median and standard deviation) as is required. Therefore, the HCD currently submitted for the study under evaluation should be used with caution and acknowledgement of its lower relevance and reliability because it does not meet all the requirements. For more detailed information on HCD please refer to RAR Volume 3CA B-6, section B.6.6.2.5.

Visceral malformation such as *hydrocephaly* was found in two foetuses of the highest dose group (32 mg/kg bw/day); the increased number of foetuses affected was without statistical significance but clearly above the range of historical control data. In addition, incidences of foetuses with *cleft palates* were found in the highest dose tested, the increased number of foetuses affected showed statistical significance and was above the range of historical control. The two latter malformations (cleft palate and hydrocephaly) occurring in the highest dose group tested were found in two foetuses (i.e. each foetus with hydrocephaly and cleft palate) from dams that lost weight during the dosing period (i.e. 0.44 and 0.16 kg during 6-18 days) and showed anorexia (5 and 2 days during 12-19 days of gestation) indicating maternal toxicity. Hence, these visceral malformations (cleft palate and hydrocephaly) are considered to be of toxicological relevance and treatment related.

Skeletal malformations like *vertebra and/or rib alterations* (malformed and absent vertebra, fused vertebra, hemivertebra, branched and fused ribs) were shown to be dose related increased; the increase of incidences for the two highest dose groups were of statistical significance but within the range of historical control. The number and incidence of foetuses with variations (external, visceral and skeletal variations; variations due to retarded development) was considered to be comparable with the concurrent control and revealed no statistical significance.

The maternal NOAEL of this study is above the highest dose level tested (32 mg/kg bw/day). Concerning developmental adverse effects, the developmental NOAEL can be established at 8 mg/kg bw/day based on increased incidences of visceral malformations (cleft palate and hydrocephaly) which were above the historical control data at the highest dose level tested (32 mg/kg bw/day).

In the **fourth developmental rabbit study** (*RAR B.6.6.2.6., 1999*), mean maternal body weights were not significantly altered; while body weight gain at the highest dose and food consumption at the mid and high dose were statistically significantly reduced (160%, 18.3% and 17.3%, respectively) during the treatment period (6 – 18/19 days of gestation). There were no clinical signs attributed to treatment with the test substance. No test substance related effects on mortality could be observed for all dose groups. It should be noted that data on corrected maternal body weight and body weight gain are not available for this study. However, it is noteworthy that there

were not effects on the parameters (mean number of implants, litter size, fetal sex ratio and/or mean fetal body weight) which could have influence on the above mentioned maternal toxicity.

The reproductive parameter investigated (number of corpora lutea, number of implantation, early resorptions, late resorptions, pre-implantation loss, post-implantation loss and dams with any resorptions) did not show treatment-related effects at any dose level. The number of litters, the total number of foetuses, the mean litter size, the number of dead foetuses, the number of live foetuses, the sex ratio as well as the foetus weights did not show any treatment related changes.

The HCD presented for the study under evaluation has been compiles from 14 studies which were carried out between the year 1990 and 1998 using the same strain and supplier, conducted in the same laboratory. Therefore, the studies cover a longer period than the five-year period requested. Additionally, information on HCD provided didn't include approximate age of the control animals etc. requested according to Commission Regulation (EU) No 283/2013 (section 5; 5.6. Reproductive toxicity). Therefore, the HCD submitted for the study under evaluation should be used with caution and acknowledgement of its lower relevance and reliability because it does not meet all the requirements. For more detailed information on HCD please refer to RAR Volume 3CA B-6, section B.6.6.2.6.

With respect to the different types of alterations (malformations and variations) some observations have been reported. Visceral variants such as *slight renal pelvis dilation* were statistically significantly increased for the high dose (25 mg/kg bw/day) foetuses showing a clear dose relationship. The incidence of *dilation of heart ventricles* was statistically significantly increased in the high dose animals and was above the historical control data. As dilation of heart ventricles must be classified as a structural change that could impair foetal survival, development or function, this alteration should be indicated as major visceral malformations rather than minor anomalies.

Regarding the skeletal alterations, the incidences of skeletal variants as *incomplete/poor ossification of fore limb (middle phalange: 1/5)* as well as skeletal minor anomalies as *accessory floating rib no. 13* were also shown to be relevant at maternal toxic dose levels (25 mg/kg bw/day).

Based on decreased body weight gain and food consumption at 25 mg/kg bw/day, the maternal NOAEL was set at 15 mg/kg bw/day. The developmental NOAEL can be established at 15 mg/kg bw/day based on increased incidences of visceral malformations (dilation of heart ventricles), visceral variants (slight renal pelvis dilation), skeletal variants (incomplete/poor ossification of fore limb) and skeletal minor anomalies (accessory floating rib no. 13) which were above the historical control data and/or were statistically significantly increased at the highest dose level tested (25 mg/kg bw/day).

2.6.6.2.2 Comparison with the CLP criteria regarding adverse effects on development

Taking into account the results of the developmental studies available as well as the 2-generation study, there is reasonable evidence that cymoxanil can impair foetal development producing also malformations (demonstrated in two developmental toxicity studies in rats and in three out of four studies in rabbits). The resulting classification is available in Commission Regulation (EU) No 605/2014 (6th adaptation to technical and scientific progress of Regulation (EC) No 1272/2008) and the classification for adverse effects on development is Repr. 2, H361d "Suspected of damaging the unborn child".

- It should be noted that according to Regulation (EC) No 1272/2008 (Annex I: point 3.7.2.4.2.) "*Developmental effects which occur even in the presence of maternal toxicity are considered to be evidence of developmental toxicity, unless it can be unequivocally demonstrated on a case-by-case basis that the developmental effects are secondary to maternal toxicity. Moreover, classification shall be considered where there is a significant toxic effect in the offspring, e.g. irreversible effects such as structural malformations, embryo/foetal lethality, significant post-natal functional deficiencies.".*
- In one 2-generation study *(RAR B.6.6.1.1., 2001)*, effects on development were reported. These included a statistically significant increase in post-implantation loss in the high dose F1 generation (equal to 116.0 mg/kg bw/day for females). In F1 and F2 pups, a statistically significant decrease in body weight was reported from the mid dose (94.0 mg/kg bw/day) and above. During gestation in F1 parental females there was a 20% reduction in body weight gain during gestation at the high dose with an 8% reduction in food intake.
- In the first rat developmental toxicity study *(RAR B.6.6.2.1., 1993)* increased incidences of skeletal malformations such as hemi vertebra were reported from 75 mg/kg bw/day and such malformations as exencephalic head and fused ribs were reported at 150 mg/kg bw/day. These findings were above the HCD range declared, however, the HCD previously submitted for the study under evaluation should be used with caution and acknowledgement of its lower relevance and reliability as HCD cover a longer period than the requested and absolute values only are available. . These effects were observed in the presence of maternal toxicity evident as statistically significant reduced maternal body weight gain (from 25 mg/kg bw/day) and/or adjusted body weight gain (from 75 mg/kg bw/day).

- In the second rat developmental toxicity study *(RAR B.6.6.2.2., 1998),* increased incidences of minor anomalies (dumb-bell shaped thoracic vertebra 6/13) from 30 mg/kg bw/day (they were above the HCD range declared). Meanwhile several skeletal variations comprising incidences of delayed ossification (cervical vertebra: 7/7 and supraoccipital) and some related minor anomalies (hypoplasia of sternum: sternebra no. 1/2 and rudimentary 14th rib) were statistically significantly increased and were above the range of HCD from 60 mg/kg bw/day. The HCD currently submitted for the study under evaluation should be used with caution and acknowledgement of its lower relevance and reliability because it does not meet all the requirements (cover a longer period than requested, no information on strain of species, name of the supplier, approximate age of the control animals etc). These anomalies at non-maternally toxic dose levels indicate the potential of cymoxanil to disturb the development of foetuses. There was a non-statistically significant but marked increase post-implantation loss as well as a statistically significant reduction by 11% in the mean male foetal body weight at 120 mg/kg bw/day (at the LOAEL for maternal toxicity).
- In the first (relevant) rabbit developmental toxicity study *(RAR B.6.6.2.4., 1981),* there were increases in the incidence of the skeletal malformations such as a vertebral and/or rib alterations (including hemivertebra, absent or fused vertebrae, misaligned vertebral centra/arches, fused/absent ribs, and various degrees of resulting scoliosis) at the high dose of 32 mg/kg bw/day. Although these findings were not statistically significant, the percentage of foetuses with these malformations in this high dose group was above the HCD range submitted. However, the HCD currently submitted for the study under evaluation should be used with caution and acknowledgement of its lower relevance and reliability because it does not meet all the requirements (data cover a shorter period than the requested etc). These effects were observed in the presence of maternal toxicity evident as statistically significant clinical observations (anorexia/ reduced faecal output) and reduced maternal body weight gain.
- In the second rabbit developmental toxicity study *(RAR B.6.6.2.5., 1982),* increased incidences of visceral malformations (hydrocephaly and cleft palates) occurred at the highest dose tested (32 mg/kg bw/day). Hydrocephaly was not statistically significantly increased but was clearly above the range of HCD, while cleft palates was statistically significantly increased and was above the range of HCD. The HCD submitted for the study under evaluation should be used with caution and acknowledgement of its lower relevance and reliability because it does not meet all the requirements (no combined or summary data are available). The two malformations occurring in the highest dose group tested were found in two foetuses from dams that lost weight during the dosing period and showed anorexia, however, the overall maternal LOAEL was not established in this study.
- Finally the incidence of visceral malformations such as dilation of heart ventricles of a third developmental toxicity study in rabbits *(RAR B.6.6.2.6., 1999)* was statistically significantly increased in the high dose animals (25 mg/kg bw/day) and was above the historical control data. The HCD submitted for the study under evaluation should be used with caution and acknowledgement of its lower relevance and reliability as HCD cover a longer period than the requested. These effects were observed in the presence of maternal toxicity evident as statistically significant reduced maternal body weight gain.

In section 3.7.2.4.2 of Annex I to Regulation (EC) No 1272/2008 it is clearly stated that *"developmental effects which occur even in the presence of maternal toxicity are considered to be evidence of developmental toxicity, unless it can be unequivocally demonstrated on a case-by-case basis that the developmental effects are secondary to maternal toxicity. Moreover, classification shall be considered where there is a significant toxic effect in the offspring, e.g. irreversible effects such as structural malformations, embryo/foetal lethality, significant post-natal functional deficiencies".*

For the purpose of classification for reproductive toxicity, substances are allocated to one of two categories. The following criteria for classification for adverse effects on development are given in CLP regulation:

Classification in reproductive toxicity Category 1A is reserved for substances known to be reproductive toxicants in humans.

Classification in reproductive toxicity Category 1B is reserved for substances that are presumed to be developmental toxicants in humans, and is largely based on data from animal studies where there is clear evidence of an adverse effect on development in the absence of other toxic effects, or not occur as a secondary non-specific consequence of other toxic effects.

Classification in reproductive toxicity Category 2 is reserved for substances that are suspected to be reproductive toxicants in humans, and where there is some evidence from experimental animals of an adverse effect on development but where the evidence is not sufficiently convincing to place the substance in Category 1. The adverse effect on reproduction is considered not to be a secondary non-specific consequence of the other toxic effects.

The available data on developmental toxicity reported in rats and rabbits did not show a clear and consistent

pattern regarding developmental toxicity following exposure to cymoxanil. However, marked effects were reported in five developmental toxicity studies as well as post-implantation loss in a 2-generation study. As no evidence from humans is available, classification in CLP Repr. 1A is not considered appropriate. Since the developmental toxicity reported in rats and mice was not consistently observed in the studies, a classification according to CLP in Repr.1B H360D is not considered appropriate. Based on the evaluated data, a classification according to CLP in **Repr. Cat 2 H361d** is warranted.

2.6.6.3 Adverse effects on or via lactation [equivalent to section 10.10.7 of the CLH report template]

For more detailed data on effects regarding two 2-generation reproduction toxicity studies in rats, one reduced-size 1-generation study in rats and one developmental neurotoxicity rat study please refer to Section 2.6.6.1.

Table 37: Summary table of animal studies on effects on or via lactation

Method, guideline, deviations[1] if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results** **- NOAEL/LOAEL** **- target tissue/organ** **- critical effects at the LOAEL**	**Reference**
Two-generation reproduction toxicity study OECD 416 (1983) GLP Rat, Hsd Cpb: WU 30/sex/group Acceptable	Cymoxanil 0972 and 498VF973, 98.8% 0, 150, 450, 1350 ppm equal to 0, 10.5, 31.6, 94.0 mg/kg bw/day (F0 males) and 0, 14.9, 42.8, 116.3 mg/kg bw/day (F0 females premating, gestation and lactation) 0, 11.6, 35.1, 11.4 mg/kg bw/day (F1 males) and 0, 15.0, 45.1, 132.4 mg/kg bw/day (F1 females premating, gestation and lactation) Oral: diet Approximate number of dose weeks: mating and throughout the mating period: males F0/F1 (14/18 weeks), females F0/F1 (10/14 weeks) females F0/F1: gestation (21 days) and lactation (21 days)	Please refer to Section 2.6.6.1., Table 34	RAR B.6.6.1.1., 2001
Two-generation reproduction toxicity study OECD 416 (1983) GLP Rat, Crl:CD®BR 30/sex/group Acceptable	Cymoxanil, DPX-T3217-113, 97.8% 0, 100, 500, 1500 ppm equal to 0, 6.5, 32.1, 97.9 mg/kg bw/day (F0 males) 0, 6.65, 34.7, 103.0 mg/kg bw/day (F0 females gestation) Oral: diet Approximate number of dose weeks: mating and throughout the mating period: males F0/F1 (16/32 weeks), females F0/F1 (10/15 weeks) females F0/F1: gestation (21 days) and lactation (21 days)	Please refer to Section 2.6.6.1., Table 34	RAR B.6.6.1.2., 1993
One generation reproduction toxicity	Cymoxanil	Please refer to Section 2.6.6.1., Table 34	RAR B.6.6.1.3., 1998

Method, guideline, deviations[1] if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results - NOAEL/LOAEL - target tissue/organ - critical effects at the LOAEL	Reference
study OECD 415 (1983) GLP Rat, Hsd Cpb: WU 15/sex/group Supportive only A range finding study	0972, 98.8% 0, 750, 1500 and 3000 ppm equal to 0, 57.7, 114.3, 226.2 mg/kg bw/day (males premating) and 0, 75.1, 136.1, 240.8 mg/kg bw/day (females premating, gestation and lactation) 0, 68.4, 127.7, 235.2 mg/kg bw/day (combined) Oral: diet Approximate number of dose weeks: mating and throughout the mating period: males (15 weeks), females (10 weeks) females: gestation (21 days) and lactation (21 days)		
Developmental neurotoxicity study in rats US EPA OPPTS 870.6300 [complies generally with OECD 426 (2007)] GLP Rat CD®(SD)IGS VA/Plus® 25 females/dose Acceptable	Cymoxanil DPX-T3217-113; 97.8 % 0, 5, 50, 100 mg/kg bw/day administered from GD 6 to lactation day 21, gavage	Please refer to Section 2.6.6.1., Table 34	RAR B.6.7.1.2., 2001

No human data on effects on or via lactation are available. No other studies relevant for effects on or via lactation are available.

2.6.6.3.1 Short summary and overall relevance of the provided information on effects on or via lactation

In the **first two-generation study** (*RAR B.6.6.1.1., 2001*), statistically significant reductions have been observed for number of the F1 litter pups (both sexes combined) alive on day 14 and 21 and the respective survival indices of the high dose (1350 ppm) group animals. These findings are associated with the statistically significantly increased number of pups dead/cannibalised on days 8-14 and days 15-21. At the highest dose (1350 ppm) group of the F2 litters, the statistically significantly lower mean litter size including the mean viable litter size was reported and was likely a consequence of the lower number of corpora lutea, an increased percentage of post-implantation loss and a reduced mean number of implantations of the F1 dams. The number of F2 pups (both sexes combined) alive (pre-cull) on day 4 and number of pups alive (post-cull) on day 4 were found to be statistically significantly decreased for the high dose (1350 ppm) group animals. There was again a reduced number of F2 pups alive on day 21 (including the day 21 survival index) related to the increased pups found dead/cannibalised on day 15 – 21 for the high dose group animals. Although some of above mentioned findings were within the range of the HCD declared, due to limited information provided by the Applicant the assessment of their relevance was impossible. Concerning pup development, statistically significantly decreased (10 - 46%) body weights (both sexes combined) for F1 pups were reported from day 4 until day 21 at the high dose level of 1350 ppm. Pup body weights were also statistically significantly reduced (>10%) at the mid-dose of 450 ppm on days 14 and 21. Amongst the F2 pups statistically significantly decreased (19.5 - 38%) body weights were

reported from day 7 until day 21 at the high dose level of 1350 ppm. Pup body weights were also statistically significantly slight reduced (8.4-9.1%) in the mid dose group on days 7, 14 and 21.

It should be noted, that although body weight gain was statistically significantly decreased (78%) in females of the F0 generation during the lactation (1-21 days); however, the mean body weight was statistically significantly reduced at 21 day only (13%) at the high dose of 1350 ppm (equal to 116.3 mg/kg bw/day). It is noteworthy that the initial mean body weights of F1 parental females at the mid and high dose were statistically significantly below the concurrent control values and that persisted for the remainder of the study in high dose group only, whereas the body weight gain in F1 females during lactation was comparable to control. The NOAEL for parental toxicity is proposed at 450 ppm (equal to 31.6 mg/kg bw/day for males and 42.8 mg/kg bw/day for females) based on reduced bodyweight gain in the F0 and F1 parental animals; reduced food consumption in the F1 males; reduced food consumption in the F0 and F1 females at the high dose level. The NOAEL for offspring toxicity was set at 150 ppm (equal to 10.5 mg/kg bw/day for males and 14.9 mg/kg bw/day for females) based on reduced body weight of F1 pups (both sexes combined) on days 14 and 21 as well as reduced body weight of F2 pups (both sexes combined) on days 7, 14 and 21.

In the **second two-generation study** (*RAR B.6.6.1.2., 1993*), the viability index of F1 pups during early lactation (days 1-4) was statistically significantly reduced at 500 ppm and 1500 ppm and this finding was not evident at both F2 generations. The litter survival (%) (Number of litters weaned/ Number of viable litters delivered x 100) for F_1 pups at 1500 ppm was also statistically significantly lower than the respective control. There was a statistically significant increase in the total number of affected litters with respect to clinical observations in pups of the highest dose of 1500 ppm group in the F1, F2A and F2B generations: relevant clinical observations comprise “gasping”, “no milkspot”, “subcutaneous haemorrhage” and “weak”. Concerning pup weight (both sexes combined), statistically significant reductions (11-40%) were evident at 1500 ppm (all generations) and also at the mid dose level of 500 ppm for the F2B generation (reduction 13-18%).

It is noteworthy that there were no adverse effects on the body weight and body weight gain in females of the F0 and F1 generation during the lactation. Based on these findings, the NOAEL for parental toxicity is proposed at 500 ppm (equal to 32.1 mg/kg bw/day in males and 34.7 mg/kg bw/day in females). The NOAEL for offspring toxicity was set at 100 ppm (equal to 6.5 mg/kg bw/day in males and 6.65 mg/kg bw/day in females).

In the additional **reduced-size one generation study in rats** (*RAR B.6.6.1.3., 1998*), statistically significant reductions have been observed for number of pups (both sexes combined) alive on LD 1 and LD 4 and the respective LD 4 survival index (%) of the high dose (3000 ppm) group animals. These findings are associated with the statistically significantly increased number of pups dead at birth/on day 1 and dead/cannibalised up to day 4. Additionally, the statistically significantly lower mean live litter size including lower live birth index (%) was reported the high dose. Concerning pup development, statistically significantly decreased (>11%) body weight (both sexes combined) for pups were reported from second week of lactation at 750 ppm, statistically significant reduction (19.6 – 31.2%) of body weight was observed from LD 7 at 1500 ppm and decreased (15.1 – 46.7%) body weight for pups were reported from LD 4 at 3000 ppm.

It should be noted that there were no clear dose dependant changes on the body weight/body weight gain of females during the lactation period. Based on these findings, the NOAEL for parental toxicity is proposed at 750 ppm (equal to 68.4 mg/kg bw/day combined). The LOAEL for offspring toxicity was set at 750 ppm (equal to 68.4 mg/kg bw/day combined).

In the **developmental neurotoxicity study** (*RAR B.6.7.1.2., 2001*), there was no evidence of neurotoxicity up to the highest-dose tested in a battery of functional tests; furthermore, examination of the nervous system tissues revealed no unusual findings. Maternal toxicity was characterised by reductions in body weight gain and food consumption at gestation days 6 – 9 at 50 mg/kg bw/day. In pups of the highest dose (100 mg/kg bw/day) group, following treatment-related statistically significant changes were observed: statistically significantly increased number of pups (%) found dead or presumed cannibalized ((13.2% and 2.3% on Days 2-5/9-12, respectively), reduced viability index (number of live pups on day 5 post-partum/number of live born pups on day 1 post-partum), reduced lactation indexes (number of live pups on day 12 post-partum/number of live pups on day 5 post-partum; number of live pups on day 22 post-partum (weaning)/number of live pups in subsets 2 – 4 on day 12 post-partum), lower number of surviving pups/litter (from Day 5 post-partum to Day 22 post-partum) and live litter size (Days 12/18/22 post-partum). The average number of pups delivered, live born and stillborn pups as well as sex ratio remained unaffected. Regarding clinical signs, in the pre-weaning period in top dose (100 mg/kg bw/day) group there were four pups that were cold to the touch, two that were not nursing, two not nesting, one dehydrated and one emaciated pup. In the gross pathology examination on the pups that died prior to weaning the only finding noted was a dose-related increase in pups with no milk in their stomachs. 33% of dead pups had no milk in their stomachs at high dose of 100 mg/kg bw/day; however, in percent values it was not dose-related. Body weight, body weight gain and food consumption were not significantly affected by treatment in any dose group. Cymoxanil had no effect on the sex ratio of pups or the timing of sexual maturation.

A NOAEL for maternal toxicity of 5 mg/kg bw/d, a NOAEL for offspring toxicity of 50 mg/kg bw/d is therefore proposed and a NOAEL for developmental neurotoxicity was ≥100 mg/kg bw/day.

In conclusion, it should be noted that there was no effect on pup body weight at birth in three reproductive studies. At birth, the mean body weight of F1 and F2 pups in all treated groups was equivalent (<10%) to that of the controls so that there was no in-utero effect exerted by treatment with cymoxanil in two-generation studies (highest doses were 1350 ppm, equal to 116.0 mg/kg bw/day and 1500 ppm, equal to 103.0 mg/kg bw/day for females), one generation study (highest dose was 3000 ppm, equal to 240.8 mg/kg bw/day) and developmental neurotoxicity study (highest dose was 100 mg/kg bw/day). This was partly supported by results from the two rat developmental toxicity studies: mean foetal body weight at the time of laprohysterectomy (GD 21) was reduced by 16.7% to that of the controls at the highest dose level only (150 mg/kg bw/day). Based on the results of three reproductive studies, from LD4 onwards until LD21 mean body weight for all F1 pups was statistically significantly decreased (≥10% up to 46%) at the highest dose levels tested (1350 ppm, 1500 ppm and 3000 ppm, respectively), i.e. growth retardation with decreased post-natal body weight at all measured time points during and at the end of the lactation period. In two-generation studies the similar effects were reported also for F2 pups except one study (*RAR B.6.6.1.1., 2001*), where mean body weight for F2 pups was decreased (≥10%) at the highest dose levels tested from LD7 onwards. Regarding the lower doses tested (450 ppm, 500 ppm and 1500/750 ppm, respectively), the effect on mean body weight was not so obvious: body weights were reduced for F1 pups from LD14 (*RAR B.6.6.1.1., 2001*) at 450 ppm, for F2B pup from LD4 (*RAR B.6.6.1.2., 1993*) at 500 ppm and for F1 pups from LD4 at 1500 ppm/ from LD14 at 750 ppm (*RAR B.6.6.1.3., 1993*). Hence, significant effects on mean body weight of pups were observed during that time in which the only nutritional source for the pups was via maternal lactation (until LD14). The effects were consistent, across two generations and biologically and statistically significant. It should be noted that such effect on the mean body weight for pups was not observed in the developmental neurotoxicity study even at the highest dose (*RAR B.6.7.1.2., 2001*).

Furthermore, the effects on body weight reduction were accompanied by a small but significantly reduced survival index in the offspring. The survival of pups at the top doses was significantly reduced under certain conditions - F1 pups (sexes pooled: 94.6 % survival index, day 14; 92.4 % survival index, day 21 (*RAR B.6.6.1.1., 2001*); 85.3% viability, 0-4 day (*RAR B.6.6.1.2., 1993*); 81.5% survival index, day 4 (*RAR B.6.6.1.3., 1993*) and F2 pups (sexes pooled: 94.8 % survival index, day 21 (*RAR B.6.6.1.1., 2001*)). Additionally, the survival of F1 pups at the mid dose (500 ppm) was also significantly reduced in the second two-generation study: 97.7% viability on 0-4 day was observed (*RAR B.6.6.1.2., 1993*). Additionally, in the developmental neurotoxicity study (*RAR B.6.7.1.2., 2001*), there was increased pup mortality during lactation (Days 2-5 and 9-12), reduced viability index (on day 5 post-partum), reduced lactation indexes (on day 12 and 22 post-partum), lower number of surviving pups/litter (from Day 5 post-partum to Day 22 post-partum) and live litter size (Days 12/18/22 post-partum) at 100 mg/kg bw/day.

There is no indication of behavioural changes in dams that could have affected body weight of the pups. As mentioned above, there were no clear adverse effects on the body weight and body weight gain in females of the F0 and/or F1 generation during the lactation 1-14 days in three reproductive studies submitted. Therefore, some decreases in maternal body weight/ body weight gain likely didn't have a strong bearing on the reductions in post-natal (1-14 days) pup body weight. In the developmental neurotoxicity study, there were no adverse effects on maternal body weight, body weight gain and food consumption during lactation at high dose and lower dose levels.

In one two-generation study (*RAR B.6.6.1.2., 1993*) and in the developmental neurotoxicity study (*RAR B.6.7.1.2., 2001*), there was some information that likely is relevant to effects on or via lactation. In the first study, there was a statistically significant increase in the total number of affected litters with respect to clinical observations (relevant to effects on or via lactation) in pups of the highest dose of 1500 ppm group in the F1, F2A and F2B generations: relevant clinical observations comprise “gasping”, “no milkspot” and “weak”. In the developmental neurotoxicity study, in the gross pathology examination on the pups that died prior to weaning the only finding noted was a dose-related increase in pups with no milk in their stomachs.

2.6.6.3.2 Comparison with the CLP criteria regarding effects on or via lactation

Adverse effects on or via lactation are included under reproductive toxicity, but for classification purposes such effects are treated separately (section 3.7.1.5 of Annex I of the CLP Regulation). The classification of a substance is derived from the hazard categories in the following order of precedence: Category 1A, Category 1B, Category 2 and the additional Category for effects on or via lactation. Classification in the additional category for effects on or via lactation will be considered irrespective of a classification into Category 1A, Category 1B or Category 2.

In accordance with the CLP Regulation (Section 3.7.1.2 of Annex I), for the purpose of classification the hazard class Reproductive Toxicity is differentiated into adverse effects on sexual function and fertility or on

development as well as into effects on or via lactation.

Effects on or via lactation are allocated to a separate single category. It is recognised that for many substances there is no information on the potential to cause adverse effects on the offspring via lactation. However, substances which are absorbed by women and have been shown to interfere with lactation, or which may be present (including metabolites) in breast milk in amounts sufficient to cause concern for the health of a breastfed child, shall be classified and labelled to indicate this property hazardous to breastfed babies. This classification can be assigned on the: (a) human evidence indicating a hazard to babies during the lactation period; and/or (b) results of one or two generation studies in animals which provide clear evidence of adverse effect in the offspring due to transfer in the milk or adverse effect on the quality of the milk; and/or (c) absorption, metabolism, distribution and excretion studies that indicate the likelihood that the substance is present in potentially toxic levels in breast milk (Section 3.7.2, Table 3.7.1(b) of Annex I of the CLP Regulation).

Regarding the *(a) human evidence indicating a hazard to babies during the lactation period…*

No such data from humans are available for cymoxanil.

Regarding the (*b) results of one or two generation studies in animals which provide clear evidence of adverse effect in the offspring due to transfer in the milk or adverse effect on the quality of the milk…*

No specific information is available on the quality of the milk produced by the dams, nor was the rat milk analysed for the presence of cymoxanil or its metabolites. Some consideration only can be presented by the RMS. In one two-generation study (*RAR B.6.6.1.2., 1993*) and in the developmental neurotoxicity study (*RAR B.6.7.1.2., 2001*), there was some information that likely is relevant to effects on or via lactation. In the first study, there was a statistically significant increase in the total number of affected litters with respect to clinical observations in pups of the highest dose of 1500 ppm group in the F1, F2A and F2B generations: relevant clinical observations comprise “no milkspot”, “gasping” and “weak”. In the developmental neurotoxicity study, in the gross pathology examination on the pups that died prior to weaning the only finding noted was a dose-related increase in pups with no milk in their stomachs.

On the other hand, the RAR study in ruminants (please see RAR Volume 3CA B-7, point B.7.2.3, *<confidential>, 1996*) showed cymoxanil/its metabolites are excreted in milk to some extent (in goats 2.64% of the totally administered dose; in total, 68.8% of the dose was recovered). Given these results, it seems likely that cymoxanil/its metabolites could be transferred into the milk of rats; however an effect through lactation is plausible as a consequence of the very high dose employed in the top dose group only. Relative to the doses given to rats, the dose in the ruminant study was low (one goat was dosed daily by gavage for 3 days a gelatine capsule, equivalent to a concentration as 0.35 mg/kg bw /day of [2-^{14}C]cymoxanil).

Regarding the *(c) absorption, metabolism, distribution and excretion studies that indicate the likelihood that the substance is present in potentially toxic levels in breast milk…*

The information available regarding this point is limited. The ADME studies in rats clearly show that cymoxanil is rapidly eliminated via mainly the urine, faeces and then via bile. The log P_{OW} of 0.67 at neutral pH indicates a low potential of bioaccumulation and limited lipophilicity. There is no data to indicate how much cymoxanil or its metabolites would be transferred into the milk of rats.

Therefore, the cause of the reduced body weights in pups during the lactation period is not entirely clear. On the other hand, although the two-generation studies in animals provide some evidence of a treatment related adverse effect in the offspring possibly due to transfer of the active substance in milk at the high dose levels, while clear analytical evidence regarding the presence of cymoxanil in milk is absence. The effects on pup body weight reduction are statistically significant, equal or greater than 20%, occur at LD7 and LD14 and throughout the remainder of the lactation period, and are consistent across generations. The significant adverse effect on rat F1 and F2 postnatal pup body weight seen in the 2-generation studies may be viewed in the context of an important postnatal growth delay but without significant impact on later maturation and fertility.

The effects on body weight reduction were accompanied by a small but significantly reduced survival index in the offspring (F1, F2 pups) in the reproductive studies. Additionally, in the developmental neurotoxicity study, there was increased pup mortality during lactation, reduced viability index, reduced lactation indexes, lower number of surviving pups/litter and live litter size at 100 mg/kg bw/day. However, there is no information as to the cause of the reduction in these parameters. It is noteworthy that variations and malformations above the historical control values were demonstrated in two developmental toxicity studies in rats from 30 mg/kg bw/day and from 75 mg/kg bw/day, respectively.

It should be noted that there were no clear adverse effects on the body weight and body weight gain in females of the F0 and/or F1 generation during the lactation period 1-14 days in three studies submitted. Therefore, some decreases in maternal body weight/ body weight gain likely didn't have a strong bearing on the reductions in post-natal (1-14 days) pup body weight. In the developmental neurotoxicity study, there were no adverse effects on

maternal body weight, body weight gain and food consumption during lactation at high dose and lower dose levels. Therefore, the available data do not allow linking directly the effects in the post-natal rat pup to lactation. Other possibilities include a direct effect of pups consuming treated diet (or avoiding it, because of palatability reasons), as solid food intake starts from around LD14. While this may contribute to the reduced body weight development during the later phase of the lactation period in reproductive studies, it does not explain the effect seen at LD4-14 when the pups are suckle-fed only.

There was only some evidence that treatment with cymoxanil affected nursing behaviour (some clinical signs such as 'not nursing' in the developmental neurotoxicity study) and there was some indirect indications of a lack of milk delivery to pups during the lactation phase: there was an increase in the total number of affected litters with respect to clinical observations in pups of the highest dose group in the F1and F2 generations such "no milkspot"(second two-generation study) and the pups that died prior to weaning the only finding noted was a dose-related increase in pups with no milk in their stomachs (developmental neurotoxicity study).

Since there was no clear evidence of a treatment related adverse effect on the offspring due to transfer of cymoxanil in milk or due to effects of cymoxanil on lactation, no classification for adverse effects on or via lactation is proposed.

2.6.6.4 Conclusion on classification and labelling for reproductive toxicity

The adverse effects on testes and/or epididymis in repeated dose toxicity studies in rats, mice and dogs favour a classification of cymoxanil for effects on sexual function and fertility. There was clear evidence of adverse effects on male reproductive organs (testes) in a 90 day repeated dose toxicity study in rats (from 50 mg/kg bw/day) and 1 year dog study (from 2.8 mg/kg bw/day dose) as well as clear evidence of adverse effects on male reproductive organs (epididymis) in a carcinogenicity study in mice (from 42.0 mg/kg bw/day). Since these effects on rat, dog and mouse male reproductive organs are observed in the absent of general toxicity, it is not considered to be a secondary consequence of other toxic effects. In repeated dose toxicity studies in rats, mice and dogs, studies were also reported that induced minor or no effects on male reproductive organs. However, it is noteworthy that some studies had several methodological limitations and again the difference in the results in the rat and mouse studies could also have been due to difference in the strains used in the various studies. In one of the 2-generation reproductive toxicity studies minor effects on fertility parameters were reported in the F1 generation in the high dose group (equal to 116.0 mg/kg bw/day for females). These included a statistically significant decrease in mean litter size, the percentage of live pups born, together with a reduced mean number of corpora lutea and mean number of implantations as well as an increased percentage of post-implantation loss at reduced bodyweight gain in the F1 females during gestation. Since there is some evidence from animal studies of an adverse effect on sexual function and fertility, cymoxanil should be classified as **Repr. 2, H361f** according to Regulation (EC) No 1272/2008.

The available data shows that there is reasonable evidence that cymoxanil can impair foetal development. Variations and malformations above the historical control values were demonstrated in two developmental toxicity studies in rats (variations from 30 mg/kg bw/day and malformations from 75 mg/kg bw/day) and in three out of four developmental toxicity studies in rabbits (variations and malformations from 25 mg/kg bw/day). In a 2-generation study in rats increases in post-implantation loss were reported (at 116.0 mg/kg bw/day for females). These effects were considered not to be related to marked maternal toxicity. Based on the impaired foetal development following exposure to cymoxanil it is considered that cymoxanil should be classified as **Repr. 2, H361d** according to Regulation (EC) No 1272/2008.

The effects noted in the two-generation rat studies as well as one generation rat study with cymoxanil were not sufficient to trigger a proposal for classification for the additional category for effects on or via lactation according to Regulation (EC) No 1272/2008.

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

Fertility

For the evaluation of reproductive toxicity, two multigenerational studies in rats were reviewed. The DS noted that some endocrine activity parameters required by the updated OECD TG 416 (2001) were not investigated in these studies, which were compliant with OECD TG 416 from 1983. An additional, reduced-size one generation study for range

finding in rats that was submitted for renewal was considered as supportive only and had no measurements of endocrine activity parameters either. Furthermore, several repeated dose studies in rats, mice and dogs contained information on fertility and sexual function and were also evaluated. It should be noted that the majority of these repeated dose studies have limitations due to missing macroscopic/histopathological investigations.

Generation studies

In the **first two-generation study in rats** (RAR B.6.6.1.1., 2001), the F1 generation showed statistically significant reductions of mean number of corpora lutea, mean number of implantations, mean litter size and live born pups as well as a statistically significantly increased post-implantation loss at top dose.

In the **second two-generation study in rats** (RAR B.6.6.1.2., 1993), the mean relative testes weight of F0 parental males was statistically significantly increased at the mid and high doses, whereas the absolute weight of the testes in F1 males was reduced at high dose only. Nevertheless, no histopathological correlates were found. Thus, these effects on testes were not considered adverse.

In the **additional reduced-size one generation study in rats** (RAR B.6.6.1.3., 1998), the parental animals had a reduced food consumption during all phases at the highest dose tested (females: 240.8 mg/kg bw/d; males 226.2 mg/kg bw/d). Furthermore, there was a statistically significant reduction of body weight gain in females (during gestation) at mid and high dose and in males at high dose only. Concerning reproductive toxicity, statistically significant reductions of female fertility index, mean number of corpora lutea, mean number of implantations and mean litter size as well as an increase in pre-implantation loss and post-implantation loss were identified at the high dose. Additionally, 5 males showed bilateral small and flaccid testes at the top dose. The bodyweight of the offspring was statistically significantly reduced from the mid dose (females: 136.1 mg/kg bw/d; males 114.3 mg/kg bw/d). At the top dose, a statistically significantly lower mean live litter size including lower live birth index was reported.

Repeated dose studies

Rats

In the **28-day dietary study in rats** (RAR B.6.3.1.1., 1999a), a statistically significant reduction of testes weight as well as increase of epididymis weight were observed in the animals of the two highest dose levels. The changes in organ weight were considered to be linked to the reduction in body weight and body weight gain, that occurred in these dose levels. However, no histopathology has been performed in this study.

In the **first 90 -day dietary rat study** (RAR B.6.3.2.1. Study 1, 1993), male animals of the top dose had a statistically significantly reduced body weight, body weight gain and overall food conversion efficiency. With respect to fertility and sexual function, there were dose-related increases in mean relative testis weights at the mid and two high dose groups. Histological changes like bilateral elongated spermatids, multinucleated spermatids, cell debris, and hypospermia were also observed.

Neither changes in the weight of testes nor macroscopic/histopathological effects/changes in the testes were observed in the **second 90-day dietary rat study** (RAR B.6.3.2.1. Study 2, 1999b) up to the highest dose tested (174.3 mg/kg bw/d).

In the **first 2-year dietary rat study** (RAR B.6.5.1.1., 1994a), a statistically significant elongated spermatid degeneration and an increase in relative testes weight accompanied

by a statistically significant increase of multinucleate spermatids were observed.

In the **second 2-year dietary rat study** (RAR B.6.5.1.2., 2003), the incidence of mild-to-moderate seminiferous tubule atrophy in the testis was statistically significantly increased.

Mice

No effects on testes caused by cymoxanil were evident in a **28-day dietary study** (RAR B.6.3.1.2., 1999a) or a **90-day dietary study** (RAR B.6.3.2.2., 1999b) **in mice** up to a dose of 624.4 mg/kg bw/d. No histology had been performed in the shorter study.

In the **first 18-month dietary mice study** (RAR B.6.5.2.1., 1994b), tubular dilatation, aggregate lymphoid and sperm cysts/cystic dilation of the epididymis were statistically significantly increased in a dose-dependent manner from the mid-dose. A statistically significantly increased unilateral and bilateral oligospermia and sperm granuloma in the epididymis were reported from the second highest dose. The animals of the top dose showed a statistically significant increase in the incidences of small and "soft" testes, tubular atrophy of testes and reduction in the absolute testes weight.

No effects on testes/epididymis caused by cymoxanil were evident in the **second 18-month dietary mice study** (RAR B.6.5.2.2., 2002), up to the highest dose tested (178.3 mg/kg bw/d). However, the weights of the testes were not recorded in this study.

Dogs

In the **first 90-day dog study** (RAR B.6.3.2.3. Study 1, 1993), small testes in one male, aspermatogenesis in the testes of two animals as well as a statistically significant decrease of relative and absolute epididymis weight were observed in the highest dose group (10.56 mg/kg bw/d). However, no histopathological findings were reported in any of the groups tested.

In the **second 90-day dog study** (RAR B.6.3.2.3. Study 2, 1999), the males of mid and high dose showed a statistically significant dose-dependent decrease in absolute testes weight. However, no histopathological changes were observed in these groups.

No effects on testes/epididymis caused by cymoxanil technical were evident in the **first 1-year dog dietary study** (RAR B.6.3.3.1., 1994), up to the highest dose tested (5.7 mg/kg bw /d).

In the **second 1-year dog study** (RAR B.6.3.3.2., 2003), histological changes in the testes (minimal/slight bilateral atrophy) with an apparent trend in the incidence and severity were determined. The epididymis of one animal showed bilateral seminiferous cell debris and unilateral atrophy with aspermia at the top dose. Furthermore, the size of the testes and epididymis were reduced in one male, and a thickened epididymis was reported in another male at this dose.

<u>Conclusion on classification</u>

Based on some adverse effects on testes and epididymis in rats, mice, and dogs that were not consistently reported in the repeated dose toxicity and multi-generational studies, the DS proposed a classification of cymoxanil with **Repr. 2, H361f** according to Regulation (EC) No 1272/2008.

Development

For the renewal, two 2-generation reproduction toxicity studies in rats, one

developmental neurotoxicity rat study, two developmental toxicity studies in rats and four developmental toxicity studies in rabbits were re-evaluated. Additionally, one reduced-size one-generation study in rats was reviewed. It must be pointed out, that the developmental toxicity studies were not compliant with the updated OECD TG 414 (2018) due to the following identified deviations: dosing period covered solely the period of major organogenesis, no evaluation of endocrine sensitive end-points, limited information on the HCD, and some groups had fewer than 16 animals with implantation sites at necropsy. Furthermore, the validity of one rabbit developmental toxicity study was limited for the assessment of developmental effects, based on the insufficient number of females with implantations and no maternal toxicity reported at any dose levels tested.

Generation studies

The two 2-generation reproduction toxicity studies in rats, and one reduced-size one-generation study in rats have been described in the fertility section. Developmental effects comprised reduced offspring body weight, viability index, and percentage of live pups born. Additionally, there was a statistically significant increase in clinical observations in one of the studies (e.g. gasping, no milk spot, subcutaneous haemorrhage, and weakness) in all generations at the high dose.

Developmental studies

It should be noted that the HCD provided for the following studies have clear limitations: no detailed information (e.g. identification of species and strain, name of the supplier, and laboratory, dates when the studies were performed), and differences in the covered period (shorter/longer than the requested five-year-period).

Rats

The females of the **first developmental rat study** (RAR B.6.6.2.1., 1993) showed maternal toxicity, evidenced by statistically significant reductions of mean body weight, body weight gain, and food consumption from the mid-dose. In addition, foetotoxicity was evident as increased incidences of skeletal variations (partially ossified skull, partially ossified/unossified sternebra, partially ossified vertebra, wavy ribs, unossified hyoid and partially ossified pelvis) as well as malformations (external, visceral or skeletal) from this dose level. Some malformations as hemi vertebra occurred only in the higher dose groups accompanied by maternal toxicity.

In the **second developmental rat study** (RAR B.6.6.2.2., 1998), maternal toxicity was evident from the reduced mean body weight gain and food consumption at the highest dose tested. With respect to foetotoxicity, statistically significantly increased minor anomalies (dumb-bell shaped thoracic vertebra 6/13) occurred already from the low dose and were above the HCD range. From the mid-dose, incidences of several skeletal variations as delayed ossification (cervical vertebra and supraoccipital), and some related minor anomalies (hypoplasia of sternebra no. 1/2, and rudimentary 14th rib) were statistically significantly increased above the range of the HCD. Further statistically significantly increased incidences of delayed ossification (sternum, phalanges) and minor anomalies (vertebra) were reported at the high dose, and were also above the HCD range.

In the **developmental neurotoxicity study in rats** (RAR B.6.7.1.2., 2001), no developmental neurotoxic effects were evident up to the highest dose level tested (100 mg/kg bw/d). Maternal toxicity was characterised by reduced body weight gain and food consumption at high dose. With respect to the developmental toxicity, the offspring

showed statistically significant reductions in viability index, lactation index, number of live pups/litter and live litter size as well as increases in pup mortality and incidences of clinical observations (cold to the touch, not nursing and not nesting) at 100 mg/kg bw/d.

Rabbits

Based on the limited validity in assessing developmental effects, the **first developmental rabbit study** (RAR B.6.6.2.3., 1980) was considered supportive only. No test compound related effects as maternal toxicity, gross pathological changes, changes in pregnancy parameters and foetal parameters were reported at all dose levels tested (highest dose: 16 mg/kg bw/d).

In the **second developmental rabbit study** (RAR B.6.6.2.4., 1981), statistically significantly increased incidences of anorexia and reduced faecal output were observed from 16 mg/kg bw/d. A statistically significantly reduced maternal body weight gain occurred at the high dose only (32 mg/kg bw/d). Concerning developmental toxicity, incidences of skeletal malformations (vertebral and/or rib alterations, including hemi vertebra, absent or fused vertebrae, misaligned vertebral centra/arches, fused/absent ribs, and various degrees of resulting scoliosis) were not statistically significantly increased at the high dose, but were above the range of the HCD.

In the **third developmental rabbit study** (RAR B.6.6.2.5., 1982), no maternal toxicity was reported up to the highest dose (32 mg/kg bw/d). Regarding foetal findings, visceral malformations (cleft palate and hydrocephaly) were found in two foetuses each of the highest dose group (32 mg/kg bw/d). The incidences of hydrocephaly were not statistically significantly increased, but were above the range of HCD. The foetuses with cleft palate were from dams that showed anorexia. The incidence of this effect was statistically significantly increased and above the HCD.

In the **fourth developmental rabbit study** (RAR B.6.6.2.6., 1999), maternal toxicity was characterised by statistically significantly reduced body weight gain at the top dose (25 mg/kg bw/d) and statistically significantly reduced food consumption from the mid dose (15 mg/kg bw/d). The foetuses showed statistically significantly increased incidences of dilated heart ventricles at the highest dose, which were above the range of HCD. Furthermore, visceral variants (slight renal pelvis dilation), skeletal alterations (incomplete/poor ossification of fore limb - middle phalange: 1/5) and skeletal minor anomalies (accessory floating rib no. 13) were statistically significantly increased at the high dose.

Conclusion on classification

The DS summarised that the available data on developmental toxicity reported in rats and rabbits did not show a clear and consistent pattern regarding developmental toxicity following exposure to cymoxanil. However, effects were reported in five developmental toxicity studies as well as post-implantation loss in a 2-generation study. Thus, the DS proposed classification according to CLP as **Repr. 2, H361d**.

Effects on or via lactation

The DS described some effects on pup body weight and litter survival from the generational studies as well as some pups found without milk spot or milk in the stomach in the developmental neurotoxicity study. In the latter a reduced lactation index was also reported in the high dose group. In none of the four studies clear effects on body weight were reported for F0 and/or F1 females during the lactation period. However, no information on the quality or quantity of milk produced by the dams and no analytical

data on potential transfer to rat milk were available. Moreover, ADME studies showed that cymoxanil is rapidly eliminated mainly via the urine, faeces, and bile. Physico-chemical properties indicate a low potential for bioaccumulation and a limited lipophilicity. Therefore, the DS argued that it was not possible to link effects seen in pups to a transfer of cymoxanil to the milk or an effect of cymoxanil on lactation. Thus, they concluded that **no classification for effects on or via lactation** is warranted.

Comments received during consultation

One Member State Competent Authority (MSCA) commented on this hazard class and stated that multiple malformations were seen in development studies:

- In the first rat study (RAR B.6.6.2.1., 1993), an increased incidence in malformations was observed; these findings showed a low incidence but were above the HCD range and showed a dose-response relationship.
- In the second rat study (RAR B.6.6.2.2., 1998), one foetus was found with a cleft palate at 120 mg/kg bw/d. Increased incidences of variants and minor anomalies were shown to be statistically significantly increased and above the HCD.
- In one rabbit study (RAR B.6.6.2.4., 1981), increased incidences of skeletal malformations associated with scoliosis, such as "vertebra and/or rib alterations" and "vertebral and other changes between upper cervical and mid-thoracic regions /or rib alterations" were observed above the HCD range at 32 mg/kg bw/d.
- In a further rabbit study (RAR B.6.6.2.5., 1982) statistically significantly increased incidences (above the range of HCD) of major malformations [(hydrocephaly (2 foetuses), cleft palates (2 foetuses)] occurred at 32 mg/kg bw/d.
- Finally, incidence of dilated heart ventricles in a third study in rabbits (RAR B.6.6.2.6., 1999) increased from 15 mg/kg bw/d, reaching statistical significance only at 25 mg/kg bw/d. However, they acknowledged that even the control value for this malformation was above the range of HCD in this study. In addition, the incidences of visceral and skeletal variants as well as skeletal anomalies were shown to be relevant.

The MSCA concluded that some major malformations were found at low dose levels and above the range of HCD range for both tested species cannot be attributed to maternal toxicity or to a mechanism of action not relevant for humans. Thus, they proposed a classification as Repr. 1B, H360Df.

The DS argued that the effects were inconsistently observed. Furthermore, the DS noted that the HCD submitted for all studies under evaluation should be used with caution due to their low reliability (differences in the covered period, no correct summary data, missing information). In addition, in the third rabbit study (RAR B.6.6.2.6., 1999), the incidence of dilated heart ventricles was statistically significantly increased in the high dose animals only. Moreover, there was high percentage of this finding (15.2%) outside the HCD range in the control group.

Assessment and comparison with the classification criteria

Fertility

No human data on adverse effects of cymoxanil on sexual function and fertility are available.

Studies available for evaluation of this endpoint were already considered in the 2012 RAC opinion on cymoxanil except for a reduced-size one-generation study in rats that was submitted in the renewal process. In their last opinion, RAC concluded that there was some evidence of adverse effects on sexual function and fertility from the two, two-generation studies as well as several repeated dose toxicity studies (reduced number of corpora lutea and mean number of implantation sites in one of the two-generation studies, and effects on testes and epididymis in several but not all repeated dose toxicity studies). Thus, taking into account both positive and negative results, RAC concurred with the DS at that time (Austria) and proposed classification as Repr. 2, H361f.

According to CLP Guidance (section 3.7.2.3.1), regarding effects on fertility, "*in case of contradictions between the standard repeat dose studies and reproductive studies, the result from the latter should be considered more relevant*", and "*appropriate classification will always depend on an integrated assessment of all available data and their interrelationship using a weight of evidence approach*". Therefore, all available data regarding effects on sexual function and fertility were compiled in the table below to assist re-evaluation.

Table: *Effects of cymoxanil on sexual function and fertility reported in generational studies and repeat dose toxicity studies in rats, mice, and dogs, including studies with no effects, and limitations in reporting. Doses are rounded to whole numbers. Reductions in body weights are given as compared to controls unless otherwise stated.*

Study	**Effects on male fertility**	**Effects on female fertility**	**Limitations/ Remarks**
2-generation study (2001) in rats (HsdCpb:WU)	*No effects up to 94 mg/kg bw/d*	Decreased number of corpora lutea, mean number of implantations, mean litter size, percentage of live pups born + increased post-implantation loss in high dose F1 (116 mg/kg bw/d); in these dams body weight (bw) and food consumption (fc) was reduced stat sign. weeks 0-14 premating/ days 0-20 gestation (uncorrected)/ days 1-21 lactation: week 14 bw -8%, fc -9% GD20 bw -12%, fc -8% LD0 bw -12%, fc -26%	Number of corpora lutea and number of implantations were reduced compared to F1 controls but not compared to F0 controls; reproductive organs were not weighed but subjected to gross and microscopic pathology; no examination of sperm parameters, oestrus cyclicity, and sexual maturation; no corrected body weights available
2-generation study (1993) in rats (Crl:CD BR)	*No biologically relevant effect up to 98 mg/kg bw/d*	*No effects up to 103 mg/kg bw/d*	Reproductive organs (other than testes) were not weighed but subjected to gross and microscopic pathology; no examination of sperm parameters, oestrus cyclicity, and sexual maturation
reduced-size one-generation	Bilateral and small flaccid testes in 5/15 at high dose (226 mg/kg	Decreased fertility index (-53%), mean number of corpora lutea, mean	Only 15 animals/sex/dose; lower mean litter size

study (1998) in rats (HsdCpb:WU)	bw/d); in high dose males body weight was reduced compared to controls stat sign. weeks 1-15: -10% week 1 -15% week 3 -11% week 10 -12% week 15	number of implantations, mean litter size; increased pre- and post-implantation loss (by 12.9% and 30.5%) in high dose group (241 mg/kg bw/d); in high dose females body weight was reduced stat. sign. weeks 1-3: -11% week 1 -8% week 3 -5% week 10	presumably due to reductions in other parameters; high proportion of post-implantation loss compared to control due to low number of implantations and pregnant females (7/15); stat. sign. reduced body weight gain in high dose females during gestation (by 43%), body weight gain in high dose males reduced by 18% no corrected body weights available
28-d dietary study (1999a) in rats (HsdCpb:WU)	Decreased absolute testis weights (by 15% and 30%); increased relative epididymis weights (by 27% and 43%) in two highest dose groups (260 and 400 mg/kg bw/d); body weight reduced stat. sign, weeks 1-4 in two high doses: -17%/-26% week 1 -28%/-41% week 4	Decreased absolute weight of ovaries in high dose group (416 mg/kg bw/d); in high dose females body weight was reduced week 1-4 (stat sign week 4): -26% week 1 -28% week 4	Weight of prostate and seminal vesicles not recorded; no histopathology; reduction in body weight gains (by 45% and 66%) and mean food consumption (by 13% and 20%) in males of the two highest dose groups, and females (bw by 54%, fc by 21%) of high dose group
28-d dietary study (1999a) in mice (Swiss albino)	*No effects up to 303 mg/kg bw/d (mid dose group)*	Decreased absolute weight of ovaries in low and mid dose group (179 and 330 mg/kg bw/d)	Weight of prostate, epididymis, seminal vesicles, and uterus not recorded; no histopathology; no weight gain in second highest dose groups and weight reduction in the highest dose groups of both males and females throughout study period
90-d dietary study (1993) in rats (Crl:CD BR)	increased relative testis weights, dose-related increase in bilateral elongated spermatid degeneration from 48 mg/kg bw/d; cell debris and bilateral hypospermia in epididymis in high dose group (224 mg/kg bw/d); body weights were reduced D97 in two high doses (stat. sign. in highest dose): -5%/-15%	*no toxicologically relevant macroscopic or histopathological changes in ovaries, uterus, cervix, and vagina up to 333 mg/kg bw/d*	weight of prostate, epididymis, and seminal vesicles not recorded; sperm morphology not examined; stat. sign. reduced body weight gain (by 22%) in high dose group males no effect on food consumption
90-d dietary study (1999b)	*No changes in testis weight and no*	*No changes in weight of ovaries and no*	Weight of prostate, epididymis, and seminal

in rats (HsdCpb:WU Wistar)	*macroscopic or histopathologic changes in testis up to 174 mg/kg bw/d;* small sized and flabby testes with atrophy/calcification of seminiferous tubules in 1/10 males of recovery group; body weight was reduced stat. sign. weeks 1-17 in recovery group: -12% week 1 -12% week 17	*macroscopic or histopathologic changes in ovaries and uterus up to 188 mg/kg bw/d*	vesicles not recorded, and no histopathology of these organs was performed; food consumption stat. sign. reduced in recovery group weeks 1-13 (by 27% week 1, by 8% week 13)
90-d dietary study (1999b) in mice (Swiss albino)	*No changes in testis weight and no macroscopic changes in testis up to 267 mg/kg bw/d;* decreased spermatogenesis in 1/10 males of high dose group°; body weight was reduced weeks 1-13 (stat. sign. week 2) in high dose: -7% week 1 -6% week 2 -7.5% week 13	*No changes in weight of ovaries and no macroscopic or histopathologic changes in ovaries and uterus up to 303 mg/kg bw/d*	Weight of prostate, epididymis, and seminal vesicles not recorded and no histopathology of these organs was performed; °the DS reported this as an effect in the recovery group but in table 6.3.2.2-5 provided with the study summary it is attributed to the high dose group of the main study; stat. sign. reduction in body weight gain in high dose males (by 21%) and recovery group males (by 15%) food consumption reduced stat. sign. weeks 1, 3, 4 in high dose, and weeks 1, 2, 8 in recovery males
90-d study (1993) in dogs	Decrease of relative (24.1%) and absolute (48.4%) epididymis weight, decreased relative and absolute testis weight at high dose (11 mg/kg bw/d); small testes with aspermatogenesis and minimal or no spermatid formation in 2/4 males of high dose (11 mg/kg bw/d); in high dose males body weight was reduced weeks 1-13 (stat sign week 13): Week 1: -1% vs ctrl/ -2.8% vs week 0 Week 13: -31.6% vs	Increased absolute and relative weight of ovaries in low dose (3 mg/kg bw/d); in low dose females, body weight was reduced weeks 1-13: Week 1: -1% (-6%) Week 13: -4.7% (-12.5%) One ctrl female had particularly high bw and was excluded for comparison in study summary (when using mean for all ctrl females)	No histopathological findings in ovaries and uterus; body weight loss in males of high dose and females of mid and high dose groups over the whole study period; reduced body weight gain in females of low dose group (by 51%); food consumption reduced in high dose males weeks 1-13 (stat. sign. weeks 1-8; no data for weeks 9-12) by 51 to 69%, and in low dose females (stat. sign. weeks 1, 8) by 16 to 28%

	ctrl/ -20% vs week 0		
90-d dietary study (1999) in dogs	Decreased absolute testes weight in mid and high dose groups (10 and 15 mg/kg bw/d); reduced body weight weeks 2-13 in mid and high dose: Week 2: -7.3%/ -12% vs ctrl -6%/ -9.8% vs week 0 Week 13: -16%/ -31% vs ctrl -12%/ -28% vs week 0	Decreased absolute and relative uterus weight and weight of ovaries in high dose; reduced body weights weeks 1-13 in high dose: Week 1: -6.7% vs ctrl -6.7% vs week 0 Week 13: -30.8% vs ctrl -29% vs week 0	No histopathological correlates; body weight loss in males and females of mid and high dose groups and no weight gain in males and females of low dose groups over the whole study period (however, only slight weight gain in control females: 0.2 kg); reduced food consumption in males and females of all dose groups (stat. sign. for males high dose weeks 1-13, mid dose weeks 1-8, low dose weeks 4, 5, and females high dose weeks 1-4, 8)
1-year study (1994) in dogs	Non-significant decrease in absolute and relative testis weight in high dose (6 mg/kg bw/d); reduced body weights weeks 1-13 in high dose: -8.6% week 1 -15% week 8 -10% week 13 Increased body weights weeks 26, 39, 52 (other weeks not available): +3% week 26 +9% week 39 +9.6% week 52	*No histopathological findings in vagina (other organs not included in study summary available to RAC) up to 6 mg/kg bw/d*	No histopathological correlates; weights of ovaries and uterus not recorded; no histopathological examination of seminal vesicles and cervix; stat sign. reduced food consumption in high dose males weeks 1, 2
1-year study (2003) in dogs	*No effects on testis and epididymis weights up to 6 mg/kg bw/d;* unilaterally reduced testis and epididymis size in 1/4 and thickened epididymis in another male of high dose group (6 mg/kg bw/d); testis atrophy in 2/4 and 3/4 males of mid (3 mg/kg bw/d) and high dose groups; bilateral seminiferous cell debris in 1/4 and unilateral epididymis atrophy with aspermia in another male of high dose group; reduced body weights weeks 2-53 in mid and	*No toxicologically relevant changes in weights and histopathology of ovaries and uterus up to 3 mg/kg bw/d*	Histopathological examination of epididymis not specified; stat. sign. but not dose-dependently reduced body weight gain in females of all treated groups (by 11-18%), male controls did not gain weight from acclimatisation period to end of study, weight loss in males of mid and high dose groups up to week 39, some weight gain in weeks 39-53 (but animals did not regain their initial weight); reduced food consumption in mid and high dose males weeks 1-6 (stat. sign. for high

	high dose: Week 2: -3.3% / -6.6% vs ctrl -2% / -4.8% vs week 0 Week 53: -5.3% / -14.4% vs ctrl -2% / -11% vs week 0		dose) by 5 to 42%
18-months dietary study (1994b) in mice (Crl:CD-1 BR)#	Reduced absolute testis weight, increased incidence of small testis and tubular atrophy in high dose (446 mg/kg bw/d); dose-related increase of tubular dilatation, aggregate lymphoid and sperm cysts/cystic dilation of epididymis from 42 mg/kg bw/d (mid dose); increased incidence unilateral and bilateral oligospermia and sperm granuloma in epididymis from 216 mg/kg bw/d (second highest dose); body weight reduced after 18 months (no other data available; stat. sign. for 2 high doses): -1.2%, -3.4%, -7.4%, -11%	*No changes in weights and histopathology of reproductive organs up to 582 mg/kg bw/d*	Stat. sign. reduction in body weight gain for both males and females of the two highest dose groups (by 15-48%); no effect on mean food consumption;
18-months dietary study (2002) in mice (Swiss albino)#	*No effects on testes and epididymis up to 178 mg/kg bw/d*	Increased incidence of follicular cysts in ovaries in 4/50 females of high dose group; significantly decreased body weight in high dose females weeks 2 and 25 (no values were available to RAC)	No histopathology of cervix and vagina
2-year dietary study (1994a) in rats (Sprague Dawley)#	Increased relative testis weight at high dose (90 mg/kg bw/d); elongate spermatid degeneration from second highest dose (30 mg/kg bw/d) at interim sacrifice and termination; increased incidence of multinucleated spermatid in epididymis in high dose (97 mg/kg bw/d) at interim sacrifice; study summary provides body weight data after 23 month only: stat.	*No adverse effects on histopathology of ovaries, uterus, vagina, and mammary glands and no effect on weight of ovaries up to 134 mg/kg bw/d*	Weights of epididymis and ovaries not recorded; histopathological examination of cervix not specified; stat. sign. reduction of body weight gain for males of two highest dose (by 22 and 36%) and females of high dose groups (by 26%); poor survival in all groups including controls (45-21%) at 23 months, study terminated early

	sign. reduction in 2 high doses: -15% /-28%		
2-year dietary study (2003) in rats (HsdCpb:WU)[#]	Mild to moderate atrophy of seminiferous tubules in 12/50 males of high dose group (59 mg/kg bw/d); increased combined incidence of epididymal oligospermia with aspermia (a low sperm count and the complete lack of semen) at high dose only at terminal sacrifice; stat. sign. reduction in body weight (by 6 and 13%) for mid and high dose males	*No effects on organ weights or histopathology up to 67 mg/kg bw/d*	Organ weights recorded only for 10 animals per group; histopathological examination of testis and epididymis only for controls and high dose; no histopathological examination of vagina; cervix not specified; stat. sign. reduced body weight gain (by 7 and 15%) for males of mid and high dose; no effect on food consumption

neoplastic effects discussed in section on carcinogenicity

Overall, effects on male and female fertility parameters and reproductive organs were observed in several studies in rats, mice, and dogs with oral exposure to cymoxanil. However, these effects were accompanied by reductions in body weights and body weight gain as compared to controls or sometimes body weight loss in the affected groups, occurred at single incidences only, or were without histopathological correlates.

Conclusion on classification

Thus, RAC concurs with the DS that **Repr. 2, H361f** is the most appropriate classification based on a weight of evidence approach evaluating effects on male and female fertility observed in generational and repeat dose toxicity studies presented in the CLH dossier.

Development

No new developmental toxicity studies have been submitted since the first assessment of this endpoint by RAC in 2012. However, the developmental neurotoxicity study was not mentioned in the reproductive toxicity section of the 2012 opinion (but rather in the section on other effects) and its results were seemingly not considered for this endpoint. In their evaluation at that time, RAC concluded the "*the available data on developmental toxicity reported in rats and rabbits did not show a clear and consistent pattern regarding developmental toxicity following exposure to cymoxanil*" but that effects observed in two rat developmental toxicity studies, three out of four rabbit developmental toxicity studies, and one of the two 2-generation studies in rats warranted classification as Repr. 2, H361d (as was proposed by the previous DS Austria).

Effects relevant for classification regarding developmental toxicity are compiled in the table below. It should be noted that none of the studies, except the developmental neurotoxicity study, was compliant with the respective current guidelines (shorter exposure periods).

***Table**: Effects observed in reproductive toxicity studies relevant for classification for developmental*

toxicity (modified from table 36 of the CLH report).

Study	Effects on offspring	Effects on maternal animals	Limitations/ Remarks
2-generation study (2001) in rats (HsdCpb:WU) males (F0): 0,10.5, 31.6, 94 mg/kg bw/d females (F0): 0, 14.9, 42.8, 116.3 mg/kg bw/d via diet	F1: decreased survival indices at PND 14 (94.6% vs. 100% in ctrl.) and 21 (92.4% vs. 100% in ctrl) in high dose; F2: increased post-implantation loss (19% vs. 9% in ctrl.), decreased percentage of live pups born (81% vs. 90.1 in ctrl.) and number of pups alive on PND 4 (229 vs. 267 in ctrl.) in high dose	Decreased number of corpora lutea, mean number of implantations, mean litter size in high dose F1; F1: body weight reduced stat sign. weeks 0-14 premating (uncorrected)/ days 0-20 gestation/ days 1-21 lactation: GD20 -12% LD1 -12% LD21 -12%	Number of corpora lutea and number of implantations were reduced compared to F1 controls but not compared to F0 controls; no corrected body weights available; decreased body weight gain in both F0 and F1 high dose dams during lactation (by 78% and 20%, respectively); F0 mid and high dose dams: stat. sign. reduced food consumption during premating (-7% and -9%), during gestation (-9% and -11%), and high dose during lactation (-33%); F1 high dose dams: stat. sign. reduced food consumption during premating (-9%), gestation (-8%), and lactation (-26%)
2-generation study (1993) in rats (Crl:CD BR) males (F0): 0, 6.5, 32.1, 97.9 mg/kg bw/d females (F0): 0, 6.65, 34.7, 103 mg/kg bw/d via diet	F1: decreased viability PND 1-4 (85.3% vs. 100% in ctrl)) and lactation indices (84% vs. 100% in ctrl.), consistently lower body weights during lactation (by 32% on PND 21) in high dose F2A: consistently lower body weights during lactation (by 40% on PND 21) in high dose F2B: consistently lower body weights during lactation (by 37% on PND 21)	F0 high dose: body weight reduced stat. sign. d0-70 premating/ days 0-21 gestation (uncorrected)/ days 0-14 lactation PMD70 -11%, GD0 -9%, GD21 -12%, LD0 -9%. LD14 -8% F1 high dose: body weight reduced stat. sign. d7-105 premating/ days 0-21 gestation (uncorrected)/ days 0-21 lactation PMD105 -17%, GD0 -13/16%, GD21 -12/15%, LD0 -15/17%,	No corrected body weights available; decreased body weight gain in F0 and F1 high dose dams during premating (by 23% and 14%), and in F0 high dose dams during gestation (by 12%); no effects on food consumption for F0 dams; F1 dams: stat. sign. reduced food consumption during premating (-7%) and 1st gestation (-10%); no food consumption data for dams during

		LD21 -14/10% F1 mid dose: body weights stat. sign reduced days 0-7 premating/ day 0-7 2nd gestation (uncorrected)/ days 0-7 2nd lactation	lactation available
Reduced-size one-generation study (1998) in rats (HsdCpb:WU) males: 0, 57.7, 114.3, 226.2 mg/kg bw/d females: 0, 75.1, 136.1, 240.8 mg/kg bw/d via diet	Increased pre- and post-implantation loss (by 12.9% and 30.5%) in high dose group	Decreased fertility index (-53%), mean number of corpora lutea, mean number of implantations, mean litter size in high dose; Body weights reduced week 1-10 (stat sign. 1-3) in high dose: -11% week 1 -8% week 3 -5% week 10	Only 15 animals/sex/dose; only 7 females pregnant, only 6 delivering live litters in high dose; lower mean litter size presumably due to reductions in other parameters; high proportion of post-implantation loss compared to control due to low number of implantations and pregnant females; no corrected weights available; significantly reduced body weight gain in high dose females during gestation (by 43%); no food consumption data available
Developmental neurotoxicity study (2001) in rats (CD (SD)IGS VA Plus) 0, 5, 50, 100 mg/kg bw/d gavage	Decreased viability index (85.6% vs. 98.7% in ctrl.) and lactation index (95.4% vs. 100% in ctrl.) at high dose; single incidences of pups that were cold to touch (4), not nursing (2), not nesting (2), dehydrated (1), and emaciated (1) in high dose	Body weights reduced GD6-21 (stat. sign. from GD7; uncorrected)/ LD1-19 (stat sign. LD2-7) in high dose: GD7 -3.7% GD21 -5.7% LD1 -4.3% LD19 -1% LD22 +2%	*No neurotoxic effects;* no corrected body weight available; stat. sign. reduced food consumption and body weight gain in high dose dams (by 9% and 15%, respectively) during gestation and first days of lactation
Developmental toxicity study (1993) in rats (Crl:CD BR) 0, 10, 25, 75, 150 mg/kg bw/d	<u>*Control:*</u> *partially ossified vertebra [12.8%], partially ossified skull [2.7%], partially ossified sternebra [4%]* <u>25 mg/kg</u>: incidence of	Initial body weight loss, reduced (uncorrected) body weights (by 5% and 10% on GD21) throughout gestation in two highest dose groups	Dosing: GD 7-16; one litter in the mid dose group (25 mg/kg bw/d) showed various malformations and variations that were not present in other dose

gavage	skeletal variations and delayed ossification (partially ossified vertebra [33%] and partially ossified skull [6.7%]) ↑* 75 mg/kg: incidence of skeletal variations and delayed ossification (partially ossified vertebra [44.9%], partially ossified skull [9.7%] and partially ossified sternebra [5.4%]) ↑* skeletal malformations: number of foetuses with hemivertebra (2 from 2 litters) ↑* 150 mg/kg: incidence of skeletal variations and delayed ossification (partially ossified sternebra [21.6%], unossified sternebra [2.1%], wavy ribs and partially ossified pelvis [3.1%]) ↑* skeletal malformations: number of foetuses with hemivertebra and fused ribs (3 from 2 litters) ↑* number of foetuses with exencephalic head (1) ↑*		groups; foetus with exencephaly was overall small and showed several malformations of different organs; no corrected body weights available; reduced body weight gain GD 1-11 and GD 17-22 (by 9-15%) in two highest dose groups; reduced food consumption in dams GD 7-9 from 25 mg/kg bw/d by 12, 30, 58%; consistently reduced food consumption until GD17 in two highest dose groups compared to controls (but increased over time)
Developmental toxicity study (1998) in rats (Wistar) 0, 30, 60, 120 mg/kg bw/d gavage	30 mg/kg: delayed or incomplete ossification (e.g. sternum, scull) ↑* incidence of minor skeletal anomalies (dumb-bell shaped thoracic vertebra 6/13) ↑* 60 mg/kg: delayed or incomplete ossification (e.g. vertebra, supraoccipital) ↑* incidence of minor skeletal anomalies (dumb-bell shaped thoracic vertebra 6/13,	(Uncorrected) body weights reduced GD15-20 in mid and high dose (stat sign. GD 15 high dose), [data for GD0, 6, 15, 20 only]: GD15 -3.5%/ -7% GD20 -4.3%/ -6.9%	Dosing GD 6-15; no corrected body weights available; reduced body weight gain on GD 6-15 in mid and high dose groups (by 25 and 50%) and GD 0-20 in high dose group (by 20%); reduced food consumption on GD 6-16 in mid and high dose groups (by 9 and 25%) and GD 0-20 in high dose group (by 13%)

	hypoplasia of sternum: sternebra no. 1/2 and rudimentary 14th rib) ↑* 120 mg/kg: delayed or incomplete ossification (e.g. sternum, vertebra, phalanges, supraoccipital) ↑* incidence of minor skeletal anomalies (dumb-bell shaped thoracic vertebra 6/13, hypoplasia of sternum: sternebra no. 1/2, rudimentary 14th rib and vertebra) ↑*		
Developmental toxicity study (1980) in rabbits (NZW) 0, 4, 8, 16 mg/kg bw/d gavage	*No adverse foetal findings*	*No maternal toxicity*	Dosing GD 6-18; low number of females with implantations
Developmental toxicity study (1981) in rabbits (NZW) 0, 8, 16, 32 mg/kg bw/d	32 mg/kg: incidence of vertebra and rib alterations [8 in 2 litters] ↑ (including hemivertebra, absent or fused vertebrae, misaligned vertebral centra/arches, fused/absent ribs, and various degrees of resulting scoliosis)	Although body weight gain was increased by 74% compared to controls on GD 19-23, (uncorrected) body weight of dams remained lower throughout gestation (by 6% on GD 29) in high dose group; anorexia and reduced faecal output in 5/15 and 10/15 does of mid and high dose groups	Dosing GD 6-18; only 13-15 pregnant does per group; maternal toxicity findings inconsistent with 1982 study (see below); 6/8 foetuses with vertebra and rib alterations were in one litter (all foetuses of that litter); no corrected body weights available; reduced body weight gain in mid and high dose groups on GD 6-10 (by 11 and 85%) and on GD 6-19 in high dose group (by 39%)
Developmental toxicity study (1982) in rabbits (NZW) 0, 1, 4, 8, 32 mg/kg bw/d	32 mg/kg: incidence of vertebra and rib alterations [2 foetuses of 2 litters] ↑* Visceral malformations: hydrocephaly (2/117	*No maternal toxicity*	Dosing only GD 6-18; maternal toxicity findings inconsistent with 1981 study; no visceral malformations observed

gavage	[1.7%] foetuses) cleft palate (2/117 [1.7%] foetuses) *		in 1981 study at same dose (see above); hydrocephaly occurred also in 1 foetus of the control group; hydrocephaly and cleft palate in high dose occurred in the same 2 foetuses
Developmental toxicity study (1999) in rabbits (NZW) 0, 5, 15, 25 mg/kg bw/d gavage	25 mg/kg: incidence of skeletal variants [incomplete/poor ossification of fore limb (middle phalange: 1/5)] ↑* incidence of visceral variants (slight renal pelvis dilation) [7.8%]↑* incidence of skeletal minor anomaly (accessory floating 13th rib) ↑* Visceral malformations: incidence of dilation of heart ventricles [31%] ↑*	Minimal body weight loss (30 g) and stat. sign. reduced food consumption on GD 6-18 in high dose group (-17%); body weight values (uncorrected) were only available for GD 0, 6, 18, 29, reduced body weight GD18, 29: GD18 -2.8% vs ctrl - 1% vs GD6 GD29 -2% vs ctrl	Dosing GD 6-18; no corrected body weights available; dilation of heart ventricles considered of doubtful biological significance by study authors (likely dependent on phase of heart contraction in which foetus was killed by sodium thiopentone injection); incidence of dilated heart ventricles was considerably above HCD range in all groups including controls (15, 13, 18% in control, low, and mid dose, respectively; HCD: 0-8.6%);

* statistically significant

Conclusion on classification

According to the CLP criteria, a classification of a substance in Category 1B is based on data that provide *clear* evidence of an adverse effect on development in the absence of other toxic effect, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effects.

Substances are classified in Category 2 for reproductive toxicity when there is *some* evidence from human or experimental animals of an adverse effect on development, and where the evidence is not sufficiently convincing to place the substance in Category 1.

No human data are available on reproductive toxicity of cymoxanil. There was some indication from animal studies that cymoxanil has the potential to induce adverse effects on the development of the offspring in rats and rabbits. Although some of the effects in the developmental toxicity studies were outside the range of the provided historical control data, these data did not meet the requirements set out in Commission Regulation (EU) No. 283/2013 in terms of length of the period covered, information on the used

strain and/or age of animals, and provided descriptive statistics such as ranges, means and/or standard deviations.

Malformations observed were namely:

- An exencephalic head in one foetus, and hemivertebra and fused ribs in 3 foetuses of 2 litters of the highest dose group were observed in the first rat developmental toxicity study. Dams of the high dose lost weight until GD9 and did not regain weight until GD15. Hemivertebra and fused ribs were also observed in 2 foetuses of 2 litters in the second highest dose.
- Hemivertebra and absent or fused vertebrae were reported in all six foetuses of one litter and two foetuses of another litter in the highest dose group (32 mg/kg bw/d) of the second rabbit developmental toxicity study. In this dose group, maternal toxicity occurred as anorexia and reduced faecal output in 10/15 dams, a reduced body weight gain (-85% GD6-10 and -39% GD6-19 as compared to controls), and reduced body weights throughout gestation as compared to controls (-6% at GD29).
- Hydrocephaly and cleft palate were observed in 2 foetuses of 117 in the highest dose group in the third rabbit developmental toxicity study. One foetus with hydrocephaly was also found in the control group in this study. In contrast to the second rabbit study, in this study no maternal toxicity was reported although the highest dose group was also 32 mg/kg bw/d.
- A statistically significantly increased incidence of dilated heart ventricles was reported in the fourth rabbit developmental toxicity study in the highest dose group (25 mg/kg bw/d) which was not observed in any other study. Dilated heart ventricles were also observed at high incidences in all other dose groups of this study, including controls.

Overall, malformations were not consistently observed throughout the studies (even at similar dose levels in the same species) and occurred at low or single incidences in litters/foetuses affected by several effects. The effects observed were not considered to be related to maternal toxicity (weight loss during gestation or reduced body weight gain).

Therefore, RAC concurs with the DS that classification as **Repr. 2, H361d** is appropriate.

Effects on or via lactation

Some effects on pup survival and body weight as well as incidences of pups without milk in the stomach or without milk spot were noted in the generational and developmental neurotoxicity studies. However, no clear link could be established to cymoxanil transfer to the milk or adverse effects of the substance on lactation performance of the dams. No information is available on the quality and quantity of milk, and physico-chemical properties of the substance do not necessarily indicate a potential to accumulate in the milk. Thus, RAC concurs with the DS that **no classification** for effects on or via lactation is warranted.

These recommendations on sexual function and fertility, development and lactation are in line with RAC's previous evaluation (RAC, 2012) of Cymoxanil.

2.6.7 Summary of neurotoxicity

The neurotoxic potential of cymoxanil has been investigated in a combined neurotoxicity study with the 90-day dietary study (complies with OECD TG 424, 1997) and a developmental neurotoxicity (gavage) study (complies with OECD 426); both were conducted in rats.

In a **90-day neurotoxicity study** (*RAR B.6.7.1.1., 1993*), after 90-days dietary exposure in rats, there was no evidence of a neurotoxic effect in the FOB or motor activity assessments and there were no lesions noted in any examined tissues that would indicate a specific effect on the nervous system. Histology did not show any statistical significant difference between control and the animals of the highest dose group tested. In the absence of specific evidence of neurotoxicity, the NOAEL for subchronic neurotoxicity was ≥3000 ppm for both sexes (equal to 224 mg/kg bw /day for males and 333 mg/kg bw/day for females), the highest tested dose. Based on decreased body weight and/or body weight gain of this sub-study, the NOAEL for systemic toxicity could be set at 1500 ppm (equal to 102 mg/kg bw /day for males and 137 mg/kg bw/day for females). However, it is noteworthy that based on reduced relative testes weight and correlated histopathological findings in testes at 750 ppm (equal to 47.6 mg/kg bw) and above, the overall NOAEL was set at 100 ppm (equal to 6.54 mg/kg bw/day/day) in 90 days rat oral sub-study.

In the **developmental neurotoxicity study** (*RAR B.6.7.1.2., 2001*), there was no evidence of neurotoxicity up to the highest-dose tested in a battery of functional tests; furthermore, examination of the nervous system tissues revealed no unusual findings. Maternal toxicity was characterised by reductions in body weight gain and food consumption at gestation days 6 – 9 at 50 mg/kg bw/day. In pups of the highest dose (100 mg/kg bw/day) group, following treatment-related statistically significant changes were observed: statistically significantly increased number of pups (%) found dead or presumed cannibalized ((13.2% and 2.3% on Days 2-5/9-12, respectively), reduced viability index (number of live pups on day 5 post-partum/number of live born pups on day 1 post-partum), reduced lactation indexes (number of live pups on day 12 post-partum/number of live pups on day 5 post-partum; number of live pups on day 22 post-partum (weaning)/number of live pups in subsets 2 – 4 on day 12 post-partum), lower number of surviving pups/litter (from Day 5 post-partum to Day 22 post-partum) and live litter size (Days 12/18/22 post-partum). The average number of pups delivered, live born and stillborn pups as well as sex ratio remained unaffected. Regarding clinical signs, in the pre-weaning period in top dose (100 mg/kg bw/day) group there were four pups that were cold to the touch, two that were not nursing, two not nesting, one dehydrated and one emaciated pup. In the gross pathology examination on the pups that died prior to weaning the only finding noted was a dose-related increase in pups with no milk in their stomachs (see the table below). Body weight, body weight gain and food consumption were not significantly affected by treatment in any dose group. Cymoxanil had no effect on the sex ratio of pups or the timing of sexual maturation.

A NOAEL for maternal toxicity of 5 mg/kg bw/d, a NOAEL for offspring toxicity of 50 mg/kg bw/d is therefore proposed and a NOAEL for developmental neurotoxicity was ≥100 mg/kg bw/day.

Overall, there is no evidence that cymoxanil has the potential to affect the nervous system or the developing nervous system in two well-conducted, guideline-compliant rat studies. The findings of a 90-day neurotoxicity study and a developmental neurotoxicity study are summarised in the table below.

Table 38: Summary table of animal studies on neurotoxicity

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results: - NOAEL/LOAEL - target tissue/organ -critical effect at LOAEL**	**Reference**
Combined neurotoxicity study with the 90-day study (oral, dietary) OECD 424 (1997) GLP Sprague-Dawley rat (Crl:CD®BR) 10/sex/dose in sub-studies	Cymoxanil DPX-T3217-107, 96.8% 0, 100, 750, 1500, 3000 ppm equal to 0, 6.54, 47.6, 102, 224 mg/kg bw /day (males) 0, 8.00, 59.9, 137, 333 mg/kg bw /day (females)	Neurotoxicity NOAEL: ≥ 224 mg/kg bw/day Neurotoxicity LOAEL: Not obtained. No signs of neurotoxicity observed at highest dose tested Systemic NOAEL of neurotoxicity sub-study: 102 mg/kg bw/day Systemic LOAEL of neurotoxicity sub-study: 224 mg/kg bw /day based on ↓body weight (♂12.9%), ↓body weight gain (♂17.5% & ♀16.9%) Systemic NOAEL of 90 days rat oral sub-study: 6.54 (♂) mg/kg bw/day Systemic LOAEL of 90 days rat oral sub-study: 47.6 (♂) based on histopathological findings in testes (bilateral elongate spermatid degeneration)	RAR B.6.7.1.1., 1993

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results: - NOAEL/LOAEL - target tissue/organ -critical effect at LOAEL**	**Reference**
Acceptable		and ↑ relative testes weight (10%)	
Developmental neurotoxicity study in rats US EPA OPPTS 870.6300 [complies generally with OECD 426 (2007)] GLP Rat CD®(SD)IGS VA/Plus® 25 females/dose Acceptable	Cymoxanil DPX-T3217-113; 97.8 % 0, 5, 50, 100 mg/kg bw/day administered from GD 6 to lactation day 21, gavage	Neurodevelopmental NOAEL: ≥ 100 mg/kg bw/day Neurodevelopmental LOAEL: Not obtained. No developmental neurotoxic effects up to the highest dose level tested Maternal NOAEL: 5 mg/kg bw/day Systemic LOAEL: 50 mg/kg bw /day based on ↓body weight gain at GD 6 – 9 (49.1%) ↓FC (13.2%) Developmental NOAEL = 50 mg/kg bw/day Developmental LOAEL = 100 mg/kg bw/day based on ↑pup mortality Days 2-5/9-12 post-partum ↓viability index on day 5, ↓lactation indexes on day 12/22, ↓average number of live pups/litter, ↓ live litter size, clinical observations in pups (cold to the touch, not nursing and not nesting),	RAR B.6.7.1.2., 2001

2.6.8 Summary of other toxicological studies

2.6.8.1 Toxicity studies of metabolites and impurities

Metabolites. As demonstrated in ADME studies in the rat, cymoxanil is subject to extensive degradation, generating a polar fraction containing mainly bound glycine. Major metabolite IN-W3595 and several further metabolites (IN-U3204, Metabolite 4, Metabolite A and IN-R3274) identified in low or very low amounts are intermediates leading to the formation of glycine used for incorporation and conjugation. In addition, some metabolites were seen in comparative *in vitro* metabolism studies only, e.g. IN-JX915, IN-T4226, IN-R3273, IN-KP533, IN-KQ960 and IN-M2417.

Most of these metabolites (except Metabolite 4, Metabolite A and IN-M2417) are the degradation products in the environment (soil/water/water sediment) and/or in plants (grapes/lettuce) too. In addition, metabolite M-5 was found in crops (lettuce) and water sediments only.

Some studies on metabolites (IN-U3204, Metabolite 4 and IN-KP533) have been evaluated in the original DAR (2007) and Addendum (2008). Re-evaluation of these studies has been performed by the RMS.

For purpose of renewal the applicants provided new acute oral toxicity and/or genotoxicity data on metabolites IN-KP533, M-5, IN-R3274 and IN-KQ960. Additionally, 10 metabolites (IN-JX915, Metabolite 4, IN-U3204, IN-KP533, M-5, IN-KQ960, IN-T4226, IN-M2417, IN-R3273, Metabolite A) were also subject to *in silico* analysis based on different versions of (Q)SAR models such as DEREK Nexus, OECD Toolbox, US EPA T.E.S.T, ToxTree and VEGA. Based on these models the different toxicological endpoints were searched (e.g. acute toxicity, mutagenicity, carcinogenicity, developmental/reproductive toxicity, endocrine disruption, organ toxicity). The evaluation of these (Q)SAR reports has been performed by the RMS. Details of the analysis such as the applicability domain of the respective models used and/or reliability of the models predictions were reported in new studies submitted if applicable. However, the QSAR predictions obtained were limited in reliability as many of the structures evaluated were not in the prediction domain. Thus, given the structural relationship of the metabolites evaluated *inter alia* and in relation to the parent molecule cymoxanil, the predicted alerts were compared to those for the parent.

The toxicity studies on all these metabolites, the summaries and conclusions on toxicity of these metabolites are presented in RAR Volume 3CA B-6, point B.6.8.1. Additionally, an overview of the metabolites of Cymoxanil, the summaries of the results for genotoxicity and the general toxicity studies on metabolites are given in Tables B.6.8.1-1, 6.8.1-2 and 6.8.1-3 of RAR Volume 3CA B-6, point B.6.8.1., respectively.

A brief overview of the metabolites toxicity is given below.

Impurities. The issue the potential toxicity of impurities in the technical specification was evaluated at length in the confidential part (for more detailed data please refer to Volumes 4). The impurity profile remains confidential, therefore this information is presented in the confidential parts only.

Toxicological evaluation of Metabolite 4 (INT-3204)

Metabolite 4 was seen in ADME studies only (*RAR B.6.1.1.6., 2003*). The Metabolite 4 was found in rat urine in very low amounts (< 1 % of the urine radioactivity).

The submitted acute oral toxicity study (*RAR B.6.8.1.1. Study 1, 1975*) on this metabolite was considered unacceptable due to substantial deficiencies from current test guidelines.

The metabolite of cymoxanil Metabolite 4 (INT-3204 or IN-T3204) was evaluated for mammalian toxicity by *in-silico* modelling tools using QSAR software including Derek Nexus (v.6.01/Nexus v.2.2.1 (Jan 2018)), OECD Toolbox (v.4.1) and VEGA (v.1.1.3). The assessment relied mainly on CMR (carcinogenicity, mutagenicity and reproductive/developmental toxicity) and endocrine effects. Due to limitations of the documentation of (Q)SAR data the study is considered as supportive only. The toxicological profile of the Metabolite 4 does not differ significantly from the active substance cymoxanil according to this assessment; however, some important aspects should be taken into account. Fertility or overall reproductive performance is not suspected to be impaired by Metabolite 4, similarly to cymoxanil based on the results of this assessment. However, (Q)SAR prediction regarding reproductive and development toxic potential of the metabolite is wrong according to the experimental results for the parent cymoxanil and its CLP classification (Repr. 2, H361fd). Therefore, reproductive toxicity hazard classification could be assigned to this metabolite too. The long-term toxicological profile of the Metabolite 4 is very similar to cymoxanil technical grade, showing no carcinogenic potential. Based on (Q)SAR prediction provided, the Metabolite 4 is unlikely to have genotoxic potential.

The second QSAR analysis evaluated the Metabolite 4 for potential genotoxicity by *in-silico* modelling tools using QSAR software including DEREK Nexus (v.6.01/Nexus v.2.2.1, Jan 2018), OECD QSAR Toolbox (v.4.3) and OASIS TIMES V2.29.1. Based on this QSAR analysis Metabolite 4 was predicted as '*Equivocal'* by *in vitro* bacteria mutagenicity models from Derek Nexus with the alert '*049 Oxime*' and as positive *in vitro* chromosomal aberration with S9 activation by OASIS TIMES. Therefore, for the Metabolite 4 final conclusion on the genotoxic potential cannot be reached reliably as positive predicted outcome was elicited unlike cymoxanil. This positive predicted outcome cannot be entirely disregarded.

A final conclusion on the genotoxicity and the general toxicity cannot be drawn for this metabolite as no other data were provided. Since there is no data on repeated toxicity potency of Metabolite 4 a conclusion if the metabolite is of lower, equal or higher toxicity than the parent cannot be reached.

Toxicological evaluation of metabolite IN-U3204 (INU-3204)

Metabolite IN-U3204 was seen in ADME studies: it was found in rat urine in very low amounts (< 1 % of the urine radioactivity) (*RAR B.6.1.1.1., 1995; RAR B.6.1.1.2., 1995 and 1997 (supplement)*). Additionally, this metabolite was detected in mouse, rat, rabbit, dog and human microsomes incubation samples and in rat hepatocytes in low amounts (< 10% of the sample radioactivity) in comparative *in vitro* metabolism study (*RAR B.6.1.3.2., 2018*).

IN-U3204 is a postulated metabolite in the crops (grapes, lettuce), soil (aerobic) and water sediment systems.

The submitted acute oral toxicity study (*RAR B.6.8.1.2. Study 1, 1976*) on this metabolite was considered unacceptable due to substantial deficiencies from current test guidelines.

The metabolite of cymoxanil IN-U3204 was evaluated for mammalian toxicity by *in-silico* modelling tools using QSAR software including Derek Nexus (v.6.01/Nexus v.2.2.1 (Jan 2018)), OECD Toolbox (v.4.1) and VEGA (v.1.1.3). The assessment relied mainly on CMR (carcinogenicity, mutagenicity and reproductive/developmental toxicity) and endocrine effects. Due to limitations of the assessment and documentation of (Q)SAR data the study is considered as supportive only. The toxicological profile of the metabolite does not differ significantly from the active substance cymoxanil according to this assessment; however, some important aspects should be taken into account. Fertility or overall reproductive performance is not suspected to be impaired by IN-U3204, similarly to cymoxanil based on the results of this assessment. However, (Q)SAR prediction regarding reproductive and development toxic potential of the metabolite is wrong according to the experimental results for the parent cymoxanil and its CLP classification (Repr. 2, H361fd). Therefore, reproductive toxicity hazard classification could be assigned to this metabolite too. The long-term toxicological profile of the IN-U3204 is very similar to cymoxanil technical grade, showing no carcinogenic potential. The data provided do not allow final conclusion to be reached on the genotoxic potential of the metabolite.

A conclusion on the genotoxicity and the general toxicity cannot be drawn for this metabolite as no data were provided. Since there is no data on repeated toxicity potency of IN-U3204 a conclusion if the metabolite is of lower, equal or higher toxicity than the parent cannot be reached.

Toxicological evaluation of metabolite IN-KP533

Metabolite IN-KP533 was seen in comparative *in vitro* metabolism study (*RAR B.6.1.3.2., 2018*). This metabolite was detected in mouse, rat, rabbit, dog and human microsomes incubation samples in low amounts (< 8% of the sample radioactivity).

IN-KP533 is a postulated metabolite in the crops (grapes, lettuce) and water sediment systems.

Under the conditions of two acute oral studies on mice and rats, the acute oral LD_{50} of IN-KP533 was found to be greater than 2000 mg/kg bw. Consequently, the available data on general toxicity (acute toxicity) demonstrated that the compound might be considered less toxic than the parent substance.

IN-KP533 was tested in *S. typhimurium* and *E. coli* in the presence and absence of S9-mix and did not induce point mutations in any of bacterial tested strains (2 Ames tests). Additionally, IN-KP533 was tested in two *in vitro* mammalian chromosome aberration test using human peripheral blood lymphocytes (HPBL) in the absence and presence of a metabolic activation system. However, one mammalian chromosome aberration study was regarded as supportive only. Based on the findings of these studies, IN-KP533 was negative for the induction of chromosome aberrations *in vitro*. No evidence for a genotoxic potential was identified in the submitted two types of studies, however, neither aneugenicity nor gene mutation in mammalian cells were properly investigated. It should be noted that in order to predict the genotoxic potential of the metabolite IN-KP533, complete standard test battery should be used.

QSAR prediction for genotoxicity and many other endpoints was provided, however, a structural activity relationship analysis was performed for IN-KP533 using only one (and quite old) a knowledge base expert system for the prediction of toxicological hazard [DEREK Nexus (2006) and DEREK Nexus (version 12, 2009)]. It was declared that DEREK Nexus produced no alerts for possible toxicities of IN-KP533. However, this QSAR prediction submitted was considered unacceptable.

A final conclusion on the genotoxic potential cannot be drawn for the metabolite IN-KP533 based on data provided. The available data on acute toxicity demonstrated that metabolite IN-KP533 might be considered less toxic than the parent substance. However, since there is no data on repeated toxicity potency of IN-KP533 a conclusion if the metabolite is of lower, equal or higher toxicity than the parent cannot be reached.

Toxicological evaluation of metabolite M-5 (HHAC-010 or AS999)

Metabolite M-5 is a postulated metabolite in the crops (lettuce) and water sediment systems only.

Metabolite M-5 was found to be of low acute oral toxicity LD_{50} >2000 mg/kg bw for rats (*RAR B.6.8.1.4. Study 1, 2010b*). Consequently, the available data on general toxicity (acute toxicity) demonstrated that the compound might be considered less toxic than the parent substance.

No evidence of point mutations was seen in the Bacterial Reverse Mutation assay (Ames test) (*RAR B.6.8.1.4. Study 2, 2010c*). In order to predict the genotoxic potential of the metabolite M-5, complete standard test battery should be used.

QSAR prediction for many endpoints (including genotoxicity, chromosome damage and others) was provided, however, a structural activity relationship analysis was performed for M-5 using only one (and quite old) a knowledge base expert system for the prediction of toxicological hazard [DEREK ,version 12.0.0, 2009]. It was declared that DEREK Nexus produced no alerts for possible toxicities of M-5. However, this QSAR prediction submitted was considered unacceptable.

Metabolite M-5 was evaluated for mammalian toxicity by *in-silico* modelling tools by using QSAR software including DEREK Nexus (v.6.01/Nexus v.2.2.1, Jan 2018), OECD Toolbox (v.4.1), US EPA T.E.S.T (version 4.2.1), ToxTree (v. 2.6.13) and VEGA (v.1.1.3). Due to limitations of the assessment and documentation of (Q)SAR data the study is considered as supportive only. Based on this evaluation, the toxicological profile of the metabolite M-5 does not differ significantly from the active substance cymoxanil; however, some important aspects should be taken into account. According to this evaluation, the metabolite M-5 has low acute oral toxicity based on the lowest estimated LC_{50} value (rat) of 1671.30 mg/kg bw and this result classifies the metabolite M-5 in a comparable acute toxic hazard category to cymoxanil (Acute Tox. 4). Since the results from QSAR skin sensitisation models revealed that this metabolite of cymoxanil shared structural similarities for the protein binding reactive domains with cymoxanil, skin sensitisation hazard classification should be assigned to it too. On

the other hand fertility or overall reproductive performance is not suspected to be impaired by M-5, similarly to cymoxanil based on the results of this assessment. However, (Q)SAR prediction regarding reproductive and development toxic potential of the metabolite is wrong according to the experimental results for the parent cymoxanil and its CLP classification (Repr. 2, H361fd). Therefore, reproductive toxicity hazard classification could be assigned to these metabolites too. The data provided do not allow final conclusion to be reached on the genotoxic potential of this metabolite.

A final conclusion on the genotoxic potential of metabolite M-5 cannot be drawn based on data provided. Since there is no data on repeated toxicity potency of M-5 a conclusion if the metabolite is of lower, equal or higher toxicity than the parent cannot be reached.

Toxicological evaluation of metabolite IN-R3274 (HHAC-009)

Metabolite IN-R3274 was seen in ADME study: it was found in trace amounts in rat urine (*RAR B.6.1.1.5., 2001*). Additionally, this metabolite was detected in mouse, rat, rabbit, dog and human liver microsomes in low amounts (<8% of the sample radioactivity) in comparative *in vitro* metabolism study (*RAR B.6.1.3.2., 2018*).

Metabolite IN-R3274 is a postulated metabolite in the soil and water (aerobic mineralisation) only.

Under the conditions of acute oral study (*RAR B.6.8.1.5. Study 1, 2009*) in female rats, the LD_{50} for IN-R3274 was below 2000 mg/kg bw (between 500 and 1000 mg/kg bw). Based on this oral LD_{50} value, the substance should be classified as Acute Tox. 4; H302 according to CLP Regulation (EC) No. 1272/2008. This is equivalent to the parent molecule of cymoxanil for which the same harmonised classification exists (Acute Tox. 4). Consequently, the available data on general toxicity (acute toxicity) demonstrated that this metabolite was of similar acute toxicity than the parent substance.

IN-R3274 was tested in *S. typhimurium* and *E. coli* in the presence and absence of S9-mix and did not induce point mutations in any of bacterial tested strains (Ames test) (*RAR B.6.8.1.5. Study 2, 2009*). In order to predict the genotoxic potential of the metabolite, complete standard test battery should be used.

A final conclusion on the genotoxic potential of metabolite IN-R3274 cannot be drawn based on data provided. The available data on acute toxicity demonstrated that this metabolite was of similar acute toxicity than the parent substance. Since there is no data on repeated toxicity potency of IN-R3274 a conclusion if the metabolite is of lower, equal or higher toxicity than the parent cannot be reached.

Toxicological evaluation of metabolite IN-KQ960

Metabolite IN-KQ960 was detected in mouse, rat, rabbit, dog and human microsomes incubation samples (< 8% of the sample radioactivity) and in rat hepatocytes (< 5% of the sample radioactivity) in low amounts in comparative *in vitro* metabolism studies (*RAR B.6.1.3.1., 2018; RAR B.6.1.3.2., 2018*).

IN-KQ960 is a postulated metabolite in the crops (grapes, lettuce), soil and water sediment systems. The metabolite IN-KQ960 is included in the residue definition for surface water and ground water. Assessment of relevance of metabolite IN-KQ960 in groundwater according to SANCO/221/2000 guidance document is available (please see chapter 2.12).

A final conclusion on the genotoxic potential of IN-KQ960 cannot be drawn as only Ames test was provided. Furthermore, based on the study (*RAR B.6.8.1.6. Study 1, 2014*) submitted the *in vitro* mutagenic potential of IN-KQ960 was negative in *S. typhimurium* and in *E. coli* WP2 uvrA (pKM101) without metabolic activation, however, the result of the point mutation assay was equivocal in the strain *E. coli* WP2 uvrA (pKM101) with metabolic activation.

Metabolite IN-KQ960 was evaluated for mammalian toxicity (including chromosome damage, genotoxicity and other endpoints) by *in-silico* modelling tools using only one a knowledge base expert system for the prediction of toxicological hazard [Derek, 2008 and Derek Nexus, version 3.0.1, 2013). It was declared that this system produced no alerts for possible toxicities of IN-KQ960. However, this QSAR prediction submitted was considered unacceptable.

A final conclusion on the genotoxic potential cannot be drawn for the metabolite IN-KQ960 based on the information provided. Since there is no data on repeated toxicity potency of IN-KQ960 a conclusion if the metabolite is of lower, equal or higher toxicity than the parent cannot be reached.

Toxicological evaluation of metabolite IN-JX915 (tautomer of cymoxanil)

Metabolite IN-JX915 was seen in comparative *in vitro* metabolism study (*RAR B.6.1.3.1., 2018*). It was detected in mouse, rat, rabbit, dog and human microsomes incubate (with cofactor) and accounted less than 7% of the sample radioactivity.

Additionally, IN-JX915 is a postulated metabolite in the crops (grapes), soil, water (photolysis) and water sediment systems.

Metabolite IN-JX915 was evaluated for mammalian toxicity by *in-silico* modelling tools by using QSAR software including DEREK Nexus (v.6.01/Nexus v.2.2.1, Jan 2018), OECD Toolbox (v.4.1), US EPA T.E.S.T (version 4.2.1), ToxTree (v. 2.6.13) and VEGA (v.1.1.3). Due to limitations of the assessment and documentation of (Q)SAR data the study is considered as supportive only. Based on this evaluation, the toxicological profile of the metabolite IN-JX915 does not differ significantly from the active substance cymoxanil; however, some important aspects should be taken into account. It should be noted that based on the assessment the metabolite IN-JX915 can be regarded more toxic than the parent substance as its acute oral estimated toxicity was LD_{50} (rat) = 228.91 mg/kg bw. Since the results from QSAR skin sensitisation models revealed that this metabolite of cymoxanil shared structural similarities for the protein binding reactive domains with cymoxanil, skin sensitisation hazard classification should be assigned to it too. On the other hand fertility or overall reproductive performance is not suspected to be impaired by IN-JX915, similarly to cymoxanil based on the results of this assessment. However, (Q)SAR prediction regarding reproductive and development toxic potential of the metabolite is wrong according to the experimental results for the parent cymoxanil and its CLP classification (Repr. 2, H361fd). Therefore, reproductive toxicity hazard classification could be assigned to these metabolites too. The data provided do not allow final conclusion to be reached on the genotoxic potential of this metabolite.

A conclusion on the genotoxic potential cannot be drawn for the metabolite IN-JX915 based on the information provided. The available *in-silico* modelling data on acute toxicity demonstrated that metabolite IN-JX915 might be considered more toxic than the parent substance. However, since there is no data on repeated toxicity potency of IN-JX915 a conclusion if the metabolite is of lower, equal or higher toxicity than the parent cannot be reached.

Toxicological evaluation of metabolite IN-T4226

Metabolite IN-T4226 was seen in comparative *in vitro* metabolism study (*RAR B.6.1.3.1., 2018*). It was detected in mouse, rat, dog and human microsomes incubate (with cofactor) and accounted less than 7% of the sample radioactivity.

IN-T4226 is a postulated metabolite in the crops (grapes), soil and water sediment systems.

Metabolite IN-T4226 was evaluated for mammalian toxicity by *in-silico* modelling tools by using QSAR software including DEREK Nexus (v.6.01/Nexus v.2.2.1, Jan 2018), OECD Toolbox (v.4.1), US EPA T.E.S.T (version 4.2.1), ToxTree (v. 2.6.13) and VEGA (v.1.1.3). Due to limitations of the assessment and documentation of (Q)SAR data the study is considered as supportive only. Based on this evaluation, the toxicological profile of the metabolite IN-T4226 does not differ significantly from the active substance cymoxanil; however, some important aspects should be taken into account. According to this evaluation, the metabolite IN-T4226 has low acute oral toxicity based on the lowest estimated LC_{50} value (rat) of 1225.64 mg/kg bw and this result classifies the metabolite IN-T4226 in a comparable acute toxic hazard category to cymoxanil (Acute Tox. 4). Since the results from QSAR skin sensitisation models revealed that this metabolite of cymoxanil shared structural similarities for the protein binding reactive domains with cymoxanil, skin sensitisation hazard classification should be assigned to it too. On the other hand fertility or overall reproductive performance is not suspected to be impaired by IN-T4226, similarly to cymoxanil based on the results of this assessment. However, (Q)SAR prediction regarding reproductive and development toxic potential of the metabolite is wrong according to the experimental results for the parent cymoxanil and its CLP classification (Repr. 2, H361fd). Therefore, reproductive toxicity hazard classification could be assigned to these metabolites too. The data provided do not allow final conclusion to be reached on the genotoxic potential of this metabolite.

A conclusion on the genotoxic potential cannot be drawn for the metabolite IN-T4226 based on the information provided. The available *in-silico* modelling data on acute toxicity demonstrated that metabolite IN-T4226 and parent substance cymoxanil might be of comparable acute toxicity. However, since there is no data on repeated toxicity potency of IN-T4226 a conclusion if the metabolite is of lower, equal or higher toxicity than the parent cannot be reached.

Toxicological evaluation of metabolite IN-M2417

IN-M2417 was seen in comparative *in vitro* metabolism study only. It was chromatographically resolved from major metabolite IN-W3595 and both making up ≥30% of total radioactivity in liver microsomes in the presence of cofactor (*RAR B.6.1.3.1., 2018*).

The metabolite of cymoxanil IN-M2417 was evaluated for mammalian toxicity by *in-silico* modelling tools in three repots. The first QSAR software included Derek Nexus (v.6.01/Nexus v.2.2.1 (Jan 2018)), OECD Toolbox (v.4.1), VEGA (v.1.1.3) [first report] and TOPKAT (version 6.1), DEREK for Windows (version 12.0), HazardExpert module of Pallas 3.0, Toxtree v1.60 and CAESAR [second report]. The assessment in the first report relied mainly on CMR (carcinogenicity, mutagenicity and reproductive/developmental toxicity) and endocrine effects. Due to limitations of the documentation of (Q)SAR data the study is considered as supportive only. The assessment of this metabolite based on (Q)SAR data is considered acceptable. The toxicological profile of the metabolite IN-M2417 does not differ significantly from the active substance cymoxanil according to this assessment; however, some important aspects should be taken into account. Fertility or overall reproductive performance is not suspected to be impaired by the metabolite IN-M2417, similarly to cymoxanil based on the results of this assessment. However, (Q)SAR prediction regarding reproductive and development toxic potential of the metabolite is wrong according to the experimental results for the parent cymoxanil and its CLP classification (Repr. 2, H361fd). Therefore, reproductive toxicity hazard classification could be assigned to this metabolite too. The long-term toxicological profile of the metabolite IN-M2417 is very similar to cymoxanil technical grade, showing no carcinogenic potential. Based on (Q)SAR prediction provided, the metabolite IN-M2417 is unlikely to have genotoxic potential.

Based on the second report the assessment relied on (systemic (carcinogenicity, mutagenicity and developmental toxicity) and local toxicological effects (irritation and skin sensitization) of the compound IN-M2417. QSAR software included TOPKAT (version 6.1), DEREK for Windows (version 12.0), HazardExpert module of Pallas 3.0, Toxtree v1.60 and CAESAR. The toxicological profile predicted by computational methods for the active substance cymoxanil and the compound IN-M2417 was almost identical with the only exception of one out of the nine modules used to predict carcinogenicity that assigns a greater concern to the compound IN-M2417 compared to Cymoxanil. Based on the overall assessment of IN-M2417 no significant concern was identified for developmental toxicity and the only different from the active substance cymoxanil (positive) was identified by TOPKAT. Based on the overall (Q)SAR assessment developmental toxicity profile of the compound IN-M2417 is ambiguous. Since cymoxanil is harmonised classified as skin sensitiser (Skin Sens. 1, H317) and due to identical (Q)SAR predictions obtained for both substances, the same sensitization potential could be assigned to the compound IN-M2417 too. Based on (Q)SAR prediction provided, the compound IN-M2417 is unlikely to have genotoxic potential as identical predictions are obtained for cymoxanil.

The third QSAR analysis evaluated the metabolite IN-M2417 for potential genotoxicity by *in-silico* modelling tools using QSAR software including DEREK Nexus (v.6.01/Nexus v.2.2.1, Jan 2018), OECD QSAR Toolbox (v.4.3) and OASIS TIMES V2.29.1. Based on this QSAR analysis IN-M2417 would not be expected to be of genotoxic concern.

Due to limitations of the assessment and documentation of (Q)SAR data two out of three reports were considered as supportive only. A final conclusion on the genotoxicity and the general toxicity cannot be drawn for this metabolite as no other data were provided.

Toxicological evaluation of metabolite IN-R3273

Metabolite IN-R3273 was seen in comparative *in vitro* metabolism study (*RAR B.6.1.3.1., 2018*). It was detected in mouse, rat, dog and human microsomes incubate (with cofactor) and accounted less than 7% of the sample radioactivity.

IN-R3273 is a postulated metabolite in the crops (grapes) and water (photolysis).

The metabolite of cymoxanil IN-R3273 was evaluated for mammalian toxicity by *in-silico* modelling tools using QSAR software including Derek Nexus (v.6.01/Nexus v.2.2.1 (Jan 2018)), OECD Toolbox (v.4.1) and VEGA (v.1.1.3). The assessment relied mainly on CMR (carcinogenicity, mutagenicity and reproductive/developmental toxicity) and endocrine effects. Due to limitations of the assessment and documentation of (Q)SAR data the study is considered as supportive only. The toxicological profile of the metabolite does not differ significantly from the active substance cymoxanil according to this assessment; however, some important aspects should be taken into account. Fertility or overall reproductive performance is not suspected to be impaired by IN-R3273, similarly to cymoxanil based on the results of this assessment. However, (Q)SAR prediction regarding reproductive and development toxic potential of the metabolite is wrong according to the experimental results for the parent cymoxanil and its CLP classification (Repr. 2, H361fd). Therefore, reproductive toxicity hazard classification could be assigned to this metabolite too. The long-term toxicological profile of the IN-R3273 is very similar to cymoxanil technical grade, showing no carcinogenic potential. The data provided do not allow final conclusion to be reached on the genotoxic potential of the metabolite.

The second QSAR analysis evaluated the metabolite IN-R3273 for potential genotoxicity by *in-silico* modelling tools using QSAR software including DEREK Nexus (v.6.01/Nexus v.2.2.1, Jan 2018), OECD QSAR Toolbox

(v.4.3) and OASIS TIMES V2.29.1. Based on this QSAR analysis the structural alerts "*Primary unsaturated heterocyclic amines*" for DNA binding by OECD in QSAR Toolbox was present only in IN-R3273. Therefore, for the metabolite IN-R3273 final conclusion on the genotoxic potential cannot be reached reliably as positive predicted outcome was elicited unlike cymoxanil. This positive predicted outcome cannot be entirely disregarded.

A conclusion on the genotoxicity and the general toxicity cannot be drawn for this metabolite as no other data were provided. Since there is no data on repeated toxicity potency of IN-R3273 a conclusion if the metabolite is of lower, equal or higher toxicity than the parent cannot be reached.

Toxicological evaluation of Metabolite A

Metabolite A was seen in ADME study only: it was detected in rat urine (< 10% of the administered radioactive) but not in bile samples (*RAR B.6.1.1.2., 1995 and 1997 (supplement)*).

The metabolite of cymoxanil Metabolite A was evaluated for mammalian toxicity by *in-silico* modelling tools using QSAR software including Derek Nexus (v.6.01/Nexus v.2.2.1 (Jan 2018)), OECD Toolbox (v.4.1) and VEGA (v.1.1.3). The assessment relied mainly on CMR (carcinogenicity, mutagenicity and reproductive/developmental toxicity) and endocrine effects. Due to limitations of the assessment and documentation of (Q)SAR data the study is considered as supportive only. The toxicological profile of the Metabolite A does not differ significantly from the active substance cymoxanil according to this assessment; however, some important aspects should be taken into account. Fertility or overall reproductive performance is not suspected to be impaired by the Metabolite A, similarly to cymoxanil based on the results of this assessment. However, (Q)SAR prediction regarding reproductive and development toxic potential of the metabolite is wrong according to the experimental results for the parent cymoxanil and its CLP classification (Repr. 2, H361fd). Therefore, reproductive toxicity hazard classification could be assigned to this metabolite too. The long-term toxicological profile of the Metabolite A is very similar to cymoxanil technical grade, showing no carcinogenic potential. The data provided do not allow final conclusion to be reached on the genotoxic potential of the metabolite.

A conclusion on the genotoxicity and the general toxicity cannot be drawn for this metabolite as no other data were provided. Since there is no data on repeated toxicity potency of Metabolite A, a conclusion if the metabolite is of lower, equal or higher toxicity than the parent cannot be reached.

Toxicological evaluation of metabolite IN-W3595

As demonstrated in ADME studies in the rat, cymoxanil is subject to extensive degradation, generating a polar fraction containing mainly bound glycine. Major metabolite IN-W3595 and several further metabolites identified in low or very low amounts are intermediates leading to the formation of glycine used for incorporation and conjugation (*RAR B.6.1.1.1., 1995; RAR B.6.1.1.2., 1995 and 1997 (supplement); RAR B.6.1.1.5., 2001;RAR B.6.1.1.6., 2003*). Major metabolite IN-W3595 was detected in urine (10-45% of urine radioactivity recovered), faeces (<7%) and bile (<36%), irrespective of dose regimen or sex (*RAR B.6.1.1.1., 1995; RAR B.6.1.1.2., 1995 and 1997 (supplement); RAR B.6.1.1.6., 2003*). Additionally, the major metabolite IN-W3595 was the same in all investigated species (mouse, rat, rabbit, dog and human) in comparative *in vitro* metabolism study and making up ≥30% of total radioactivity in liver microsomes in the presence of cofactor (*RAR B.6.1.3.1., 2018*).

IN-W3595 is a postulated metabolite in the crops (grapes, lettuce), soil (aerobic) and water sediment systems.

No data regarding toxicity potency of metabolite IN-W3595 were submitted.

Since no specific studies on metabolite IN-W3595 is available, the following default assumption is made by the RMS: this metabolite is considered as studied in the toxicity studies conducted with the active substance cymoxanil because it contributes to more than 10% of the administered dose in total radioactive material recovered in the urine as detected in ADME studies. These results of ADME studies allow for a comparison with the general toxicity studies conducted with the parent compound. Therefore, in order to conduct a consumer risk assessment the reference values of the parent compound (cymoxanil) can be applied to metabolite IN-W3595.

2.6.8.2 Supplementary studies on the active substance

Immunotoxicity

The potential of cymoxanil to cause immunotoxicity has been investigated in rats and mice in two 28-day immunotoxicity studies. An overview of two immunotoxicity studies with cymoxanil is presented in the table below.

In the **first immunotoxicity study in rats** (*RAR B.6.8.2.1., 1999a*), based on decrease in body weight gain and food efficiency in females from the dose of 800 ppm (equal to 58.98 mg/kg bw/day for females), the overall systemic NOAEL was set at 400 ppm (equal to 31.3 mg/kg bw/day for females). The NOAEL for immunotoxicity was be established at > 1600 ppm (equal to 107.7 mg/kg bw/day in males) as no effects on immunotoxicity (thymus, spleen weight and the humoral immune response to SRBC) were observed in rats.

In the **second immunotoxicity study in mice** (*RAR B.6.8.2.2., 1999b*), based on decrease in body weight gain and food efficiency in female mice at the dose of 2400 ppm (equal to 552.4 mg/kg bw/day for females), the overall systemic NOAEL was set at 1200 ppm (equal to 268.5 mg/kg bw/day for females). The overall NOAEL for immunotoxicity was established at > 1200 ppm (equal to 218.39 mg/kg bw/day for males) as no effects on immunotoxicity (thymus, spleen weight and the humoral immune response to SRBC) were observed in mice.

Hence, in none of these two studies immunotoxic effects were evident up to high doses (107.7 mg/kg bw/day in rats and 218.4 mg/kg bw/day in mice). However, in should be noted that the target of orally administered cymoxanil was thymus, based on findings in two short-term oral toxicity studies (*90 day study in dogs, RAR B.6.3.2.3. Study 2, 1999*and *1 year dog study, RAR B.6.3.3.2., 2003).* The resulting classification is available in Commission Regulation (EU) No 605/2014 (6th adaptation to technical and scientific progress of Regulation (EC) No 1272/2008) and the classification for human health regarding this point is the following: STOT RE 2, H373 (blood, thymus). See discussions under the section *Summary of reproductive toxicity (point 2.6.3.1.1 Short summary and overall relevance of the provided information on specific target organ toxicity – repeated exposure).*

Table 39: Summary table of animal studies on immunotoxicity

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance(Batch No; purity), dose levels duration of exposure**	**Results:** **- NOAEL/LOAEL** **- target tissue/organ** **-critical effect at LOAEL**	**Reference**
28-Day immunotoxicity feeding study Immunotoxicity US EPA OPPTS 870.7800 (1998) GLP Rat Crl:CD®(SD)IGS BR 10/sex/group Acceptable	Cymoxanil, DPX-T3217-113, 97.8% 0, 200, 400, 800, 1600 ppm Equal to 0, 13.56, 26.97, 53.86, 107.71 mg/kg bw/day (males) Equal to 0, 15.62, 31.32, 58.98, 117.43 mg/kg bw/day (females) Positive control: cyclophosamide monohydrate	Immunotoxicity NOAEL: ≥107.7 mg/kg bw/day Immunotoxicity LOAEL: Not obtained. No effects on immunotoxicity (thymus, spleen weight and the humoral immune response to SRBC) up to highest dose Systemic NOAEL: 31.3 mg/kg bw/day Systemic LOAEL: 58.98 mg/kg bw /day based on ↓body weight gain (♀25.5%), ↓mean food efficiency (♀20.8%)	RAR B.6.8.2.1., 1999a
28-Day immunotoxicity feeding study Immunotoxicity US EPA OPPTS 870.7800 (1998) GLP Mice Crl:CD-1®(ICR)BR 10/sex/group Acceptable	Cymoxanil, DPX-T3217-113, 97.8% Males: 0, 30, 300, 600 or 1200 ppm equal to 0, 5.15, 55.96, 108.33 and 218.39 mg/kg bw/day Females: 0, 30, 300, 1200 or 2400 ppm equal to 0, 7.15, 71.01, 268.51 and 552.44 mg/kg bw/day Positive control: cyclophosamide monohydrate	Immunotoxicity NOAEL: ≥218.4 mg/kg bw/day Immunotoxicity LOAEL: Not obtained. No effects on immunotoxicity (thymus, spleen weight and the humoral immune response to SRBC) up to highest dose Systemic NOAEL: 268.5 mg/kg bw/day Systemic LOAEL: 552.4 mg/kg bw /day based on ↓body weight gain (♀78.6%), ↓mean food efficiency on day 14-21 (♀33.3%)	RAR B.6.8.2.2., 1999b

Assessment of IN-Q8761 (cymoxanil Z-isomer) based on (Q)SAR data

Cymoxanil Z-isomer (IN-Q8761) was evaluated for mammalian toxicity by *in-silico* modelling tools using QSAR software including Derek Nexus (v.6.01/Nexus v.2.2.1 (Jan 2018)), OECD Toolbox (v.4.1) and VEGA

(v.1.1.3). The assessment relied mainly on CMR (carcinogenicity, mutagenicity and reproductive/developmental toxicity) and endocrine-related effects. The toxicological profile of IN-Q8761 does not differ significantly from the active substance cymoxanil according to this assessment; however, some important aspects should be taken into account. Fertility or overall reproductive performance is not suspected to be impaired by IN-Q8761, similarly to cymoxanil based on the results of this assessment. However, (Q)SAR prediction regarding reproductive and development toxic potential of the metabolite is wrong according to the experimental results for the parent cymoxanil and its CLP classification (Repr. 2, H361fd). Therefore, reproductive toxicity hazard classification could be assigned to IN-Q8761 too. The long-term toxicological profile of IN-Q8761 is very similar to cymoxanil technical grade, showing no carcinogenic potential. Based on the fact that the parent compound, cymoxanil is unlikely to be genotoxic *in vivo* and that (Q)SAR predictions are identical for both compounds, IN-Q8761 is unlikely to have genotoxic potential.

2.6.9 Summary of medical data and information

Based on the four Reports submitted by the Applicants, medical surveillance of workers at cymoxanil or the formulations manufacturing plants (3 different sites) has revealed that cymoxanil has no effect on health according to regular medical examinations and blood tests. No cases of acute cymoxanil intoxication have been reported and no health interfering symptoms like skin anomalies or sensitization have been observed according to the Reports submitted.

The Applicant’s literature search revealed only one epidemiological study (please see RAR Volume 3CA B-6, point B.6.9.4). Exposure of the population arising from actual use of cymoxanil (its actual application to potato crops) was included in a large hospital-based case-control study of the association between residential exposure to pesticides and the risk of Parkinson’s disease in The Netherlands. Increased risk of Parkinson's disease was observed for exposure to (a cluster of) pesticides used on rotating crops (this cluster of compounds included cymoxanil). However, high correlations between these 21 compounds limited the ability to identify individual pesticides responsible for this association. This study provides some evidence for an association between environmental exposure to specific pesticides and the risk of Parkinson's disease, and generates new leads for further epidemiological and mechanistic research.

According to the applicants, there is no known antidote if this product is ingested. Effects following poisoning should be treated symptomatically by a physician.

2.6.10 Toxicological end points for risk assessment (reference values)

Table 40: Overview of relevant studies for derivation of reference values for risk assessment

Species	Study (method/type, length, route of exposure)	Test substance (Batch No; purity w/w)	Critical effect	NOAEL (mg/kg bw/day)	LOAEL (mg/kg bw/day)	Cross reference
Wistar rat (HsdCpb:WU)	28 days rat oral OECD 407 (1995)	Cymoxanil 0972, 98.8 %	↓body weight gain at week 1 (17.4%); ↓ food consumption throughout weeks 1 and 2 (>10%), ↑ relative liver (12.7%) and kidneys (11.8%) weight	74.4	143.5	RAR B.6.3.1.1., 1999a Section 2.6.3
Swiss albino mice: HsdOla:MF 1	28 days mice oral OECD 407 (1995)	Cymoxanil 0972, 98.8 %	↓body weight gain (41.8%); ↓ food consumption throughout all dosing period (>10%), ↓absolute kidneys (18%) weight	172.7	303.4	RAR B.6.3.1.2., 1999a Section 2.6.3
Sprague-Dawley rat (Crl:CD®BR)	90 days rat oral OECD 408 (1981)	Cymoxanil DPX-T3217-107, 96.8%	Histopathological findings in testes (bilateral elongate spermatid degeneration) and ↑ relative testes weight (10%)	6.54	47.6	RAR B.6.3.2.1. Study 1, 1993 Section 2.6.3 and 2.6.6
Wistar rat (HsdCpb:WU)	90 days rat oral OECD 408 (1981)	Cymoxanil 0972, 98.8%	↓body weight (11.3%), ↓ body weight gain (14.6%), ↓ food consumption (15.6%), ↑ creatinine (34%), ↑ total bilirubin (85.8%), ↑ relative kidney weight (17.3%), ↑ relative liver weight (15.7%)	85.1	174.3	RAR B.6.3.2.1. Study 2, 1999b Section 2.6.3 and 2.6.6
Swiss albino mice: Hsd0la:MF1	90 days mice oral OECD 408 (1981)	Cymoxanil 0972, 98.8%	↓body weight gain (21.4%), ↑ total bilirubin (114.8%)	84.4	256.6	RAR B.6.3.2.2., 1999b Section 2.6.3 and 2.6.6
Beagle dog	90 days dog oral OECD 409 (1981)	Cymoxanil DPX-T3217-113, 97.8%	Clinical signs (diarrhoea and decreased defecation), ↓ body weight gain (females), ↓ food consumption (females) and alteration of haematological parameters (↓RBC, ↓Hb, ↓Ht more severely in males)	3.0	5.13	*RAR B.6.3.2.3. Study 1, 1993* Section 2.6.3 and 2.6.6
Beagle dog	90 days dog oral OECD 409 (1981)	Cymoxanil 498VF973, 98.8%	Clinical signs ('weakness'), loss body weight gain, ↓ food consumption (g/animal/day) during 5 weeks (23.2 – 46.7%*); ↓ absolute (>55%) and relative (>45%) thymus weight; histological alterations in thymus with increasing severity	4.9	9.7	RAR B.6.3.2.3. Study 2, 1999 Section 2.6.3 and 2.6.6
Beagle dog	1 year dog oral OECD 452 (1981)	Cymoxanil DPX-T3217-113, 97.8%	Alterations of haematological parameters [↑MCV at termination, ↓ RBC at week 12/25, ↓ Hb at week 2/12/25]; alterations of clinical chemistry [↓	3.0	5.7	RAR B.6.3.3.1., 1994 Section 2.6.3 and 2.6.6

Species	Study (method/type, length, route of exposure)	Test substance (Batch No; purity w/w)	Critical effect	NOAEL (mg/kg bw/day)	LOAEL (mg/kg bw/day)	Cross reference
			potassium; bilateral cataract			
Beagle dog	1 year dog oral OECD 452 (1981)	Cymoxanil 89800028, 98.8% (first 4 weeks) 19800042, 99.2% (for remainder of study)	Histological changes in testes (minimal/slight bilateral atrophy) with an apparent trend in the incidence and severity	1.3	2.8	RAR B.6.3.3.2., 2003 Section 2.6.3 and 2.6.6
Sprague-Dawley rats, Crl:CD Br	Combined chronic toxicity /carcinogenicity study OECD 453 (1981)	Cymoxanil DPX-T3217-113 (Blend of lots 80317145 and 80321154); 97.8%	Clinical findings (↑hyperreactivity), ↓ body weight (15.3%), ↓ body weight gain (21.8%), histopathological findings (elongate spermatid degeneration, retinal atrophy) *Cymoxanil did not reveal any oncogenic potential*	4.08	30.3	RAR B.6.5.1.1., 1994a Section 2.6.3 and 2.6.5
Wistar rats, HsdCpb:WU strain, in-house bred	Combined chronic toxicity /carcinogenicity study OECD 453 (1981)	Cymoxanil 0972, 98.8% and 498 VF973, 99.3%	Histopathological findings (lymphoid hyperplasia in rectum)* * - *Due to deviations of the study the NOAEL (♂) cannot be set properly with respect to findings on male reproductive organs.* *Cymoxanil did not reveal any oncogenic potential*	4.7	23.5	*RAR B.6.5.1.2.*, 2003 Section 2.6.3 and 2.6.5
Mouse, Crl:CD-1®BR	Carcinogenicity study OECD 451 (1981)	Cymoxanil DPX-T3217-113, 97.8%	Histopathological findings in liver (apoptosis, pigment, granuloma, diffuse centrilobular hypertrophy) and epididymis (tubular dilatation, aggregate lymphoid and sperm cysts) *Cymoxanil did not reveal any oncogenic potential*	4.19	42.0	RAR B.6.5.2.1., 1994b Section 2.6.3 and 2.6.5
Carcinogenicity study OECD 451 (1981)	Carcinogenicity study OECD 451 (1981)	Cymoxanil 498VF973, 98.8%	Macroscopic and histopathological findings in mesenteric lymph nodes (dicolouration and haemorrhage) of males found dead and sacrificed moribund; histopathological findings in ovaries (follicular cysts) in females terminal sacrificed and combined fates *Cymoxanil did not reveal any oncogenic potential*	91.4	178.3	RAR B.6.5.2.2., 2002 Section 2.6.3 and 2.6.5
Rat, Hsd Cpb: WU	Two-generation reproduction toxicity study OECD 416	Cymoxanil 0972 and 498VF973, 98.8%	**Parental** ↓bw gain premating (F0 males: 17%, F1 males: 14%, F0 female: 10%), gestation (F1 female: 20%), lactation	31.6 (Parental)	94.0 (Parental)	RAR B.6.6.1.1., 2001 Section 2.6.3 and 2.6.6

Species	Study (method/type, length, route of exposure)	Test substance (Batch No; purity w/w)	Critical effect	NOAEL (mg/kg bw/day)	LOAEL (mg/kg bw/day)	Cross reference
	(1983)		(F0 female: 78%); ↓FC premating (F1 males: 10%, F0/F1 female: 9%), gestation (F0/F1 female: 11%/8%), lactation (F0/F1 females: 33%/26%) **Reproductive** F1 generation: ↓ mean number of corpora lutea, ↓mean number of implantations, ↑post-implantation loss (%), ↓mean litter size, ↓ live pups born (%), **Offspring** ↓bw (both sexes combined F1 pups Days 14 and 21: >10%; F2 pups Days 7, 14 and 21: >8%) *The average values of the intakes by F0 and F1 generations are not available, therefore, the lowest values of mean intakes were used (F0 generation, males)*	31.6 (Reproductive) 10.5 (Offspring)	94.0 (Reproductive) 31.6 (Offspring)	
Rat, Crl:CD®BR	Two-generation reproduction toxicity study OECD 416 (1983)	Cymoxanil, DPX-T3217-113, 97.8%	**Parental** clinical signs (end of tail missing, necrotic tip of tail, sore), ↓bw (>10%) premating (F0 males, F0 female), gestation (F0 female); ↓bw gain premating (F0 males: 18%, F1 males: 14%, F0 female: 23%, F1 female: 14%), gestation (F0 female: 12%); ↓FC (>10%) premating (F0/F1 males, F1 female), 1st gestation (F1 female) **Reproductive** Did not cause adverse effects at highest dose tested **Offspring** ↓viability index (%) of F1 pups (days 1-4) ↓bw (both sexes combined F2B pups, Days 4, 7, 14 and 21: 13-18%)	32.1 (Parental) 6.5 (Offspring)	97.9 (Parental) 32.1 (Offspring)	RAR B.6.6.1.2., 1993 Section 2.6.3 and 2.6.6
Rat, Hsd Cpb: WU	One generation reproduction toxicity study OECD 415 (1983)	Cymoxanil 0972, 98.8%	**Parental** ↓bw gain female (gestation 12%); ↓FC female: all premating (9%), all gestation (≥10%), all lactation (≥20%) periods **Reproductive** ↓female fertility index (%), ↓	68.4 (Parental) (combined) 127.7	127.7 (Parental) (combined) 235.2	RAR B.6.6.1.3., 1998 Section 2.6.3 and 2.6.6

Species	Study (method/type, length, route of exposure)	Test substance (Batch No; purity w/w)	Critical effect	NOAEL (mg/kg bw/day)	LOAEL (mg/kg bw/day)	Cross reference
			mean number of corpora lutea, (%), ↓mean number of implantations, ↑pre-implantation loss (%), ↑post-implantation loss (%), ↓mean litter size; gross necropsy: bilateral small & flaccid testes of 5 males **Offspring** ↓bw (both sexes combined pups Days 14 and 21: >10%)	(Reproductive) (combined) Not obtained (Offspring)	(Reproductive) (combined) 68.4 (Offspring) (combined)	
Rat, Crl:CD BR	Developmental toxicity (teratogenicity) study OECD 414 (1981)	Cymoxanil DPX-T3217-113, 97.8%	**Maternal:** ↓ body weight gain (45%) over 7-9 days of gestation ↓ food consumption (12%) over 7-9 days of gestation **Developmental:** ↑incidence of skeletal variations and delayed ossification Foetal toxicity at higher dose levels: ↑ incidence of skeletal malformations (hemi vertebra at ≥75 mg/kg/day; exencephalic head and fused ribs at 150 mg/kg/day); ↑incidence of skeletal variations (partially ossified sternebra, unossified sternebra, wavy ribs and partially ossified pelvis at 150 mg/kg/day)	10 (Maternal& Developmental)	25 (Maternal &Developmental)	RAR B.6.6.2.1., 1993 Section 2.6.3 and 2.6.6
Wistar rats	Developmental toxicity (teratogenicity) study OECD 414 (1981)	Cymoxanil 0972, 98.8 %	**Maternal:** ↓body weight gain (50%/20%) over 6-15/0-20 days of gestation; ↓food consumption (25%/13%) over 6-15/0-20 days of gestation **Developmental:** ↑incidence of skeletal minor anomalies (dumb-bell shaped thoracic vertebra 6/13); Foetal toxicity at higher dose level: ↑incidence of skeletal variations: delayed ossification (servical vertebra: 7/7 and supraoccipital) and minor anomalies (dumb-bell shaped thoracic vertebra 6/13, hypoplasia of sternum: sternebra no. 1/2 and rudimentary 14th rib) at ≥60 mg/kg bw/day	60 (Maternal) Not obtained (Developmental)	120 (Maternal) 30 (Developmental)	RAR B.6.6.2.2., 1998 Section 2.6.3 and 2.6.6

Species	Study (method/type, length, route of exposure)	Test substance (Batch No; purity w/w)	Critical effect	NOAEL (mg/kg bw/day)	LOAEL (mg/kg bw/day)	Cross reference
			↑incidence of skeletal variations: delayed ossification (sternum, vertebra, phalanges and supraoccipital) and minor anomalies (dumb-bell shaped thoracic vertebra 6/13, hypoplasia of sternum: sternebra no. 1/2, rudimentary 14th rib and vertebra) at ≥120 mg/kg bw/day			
New Zealand White rabbits	Developmental toxicity (teratogenicity) study OECD 414 (1981)	Cymoxanil, 7800-20-C, 94.2%	**Maternal:** clinical observations (anorexia/ reduced faecal output) **Developmental:** ↑incidences of skeletal malformations (vertebra and/or rib alterations linked with scoliosis)	8 (Maternal) 16 (Developmental)	16 (Maternal) 32 (Developmental)	RAR B.6.6.2.4., 1981 Section 2.6.3 and 2.6.6
New Zealand White rabbits	Developmental toxicity (teratogenicity) study OECD 414 (1981)	Cymoxanil, INT-3217-90, 95.8%	**Maternal:** No effects even at the highest dose tested **Developmental:** ↑incidences of visceral malformations (cleft palate and hydrocephaly)	≥ 32 (Maternal) 8 (Developmental)	Not obtained (Maternal) 32 (Developmental)	RAR B.6.6.2.5., 1982 Section 2.6.3 and 2.6.6
New Zealand White rabbits	Developmental toxicity (teratogenicity) study OECD 414 (1981)	Cymoxanil 0972, 98.8 %	**Maternal:** ↓body weight gain (160%) over 6-18 days of gestation; ↓food consumption (17%) over 6-19 days of gestation **Developmental:** ↑incidences of visceral malformations (dilation of heart ventricles), visceral variants (slight renal pelvis dilation), skeletal variants (incomplete/poor ossification of fore limb), skeletal minor anomalies (accessory floating rib no. 13)	15 (Maternal) 15 (Developmental)	25 (Maternal) 25 (Developmental)	RAR B.6.6.2.6., 1999 Section 2.6.3 and 2.6.6
Rat Crl:CD®(SD) IGS BR	28-Day immunotoxicity feeding study Immunotoxicity US EPA OPPTS 870.7800 (1998)	Cymoxanil, DPX-T3217-113, 97.8%	↓body weight gain (♀25.5%), ↓mean food efficiency (♀20.8%) *No effects on immunotoxicity (thymus, spleen weight and the humoral immune response to SRBC) up to highest dose*	31.3	58.98	RAR B.6.8.2.1., 1999a Section 2.6.3 and 2.6.8
Mice Crl:CD-1®(ICR)BR	28-Day immunotoxicity feeding study Immunotoxicit	Cymoxanil, DPX-T3217-113, 97.8%	↓body weight gain (♀78.6%), ↓mean food efficiency on day 14-21 (♀33.3%)	268.5	552.4	RAR B.6.8.2.2., 1999b Section 2.6.3 and 2.6.8

Species	Study (method/type, length, route of exposure)	Test substance (Batch No; purity w/w)	Critical effect	NOAEL (mg/kg bw/day)	LOAEL (mg/kg bw/day)	Cross reference
	y US EPA OPPTS 870.7800 (1998)		*No effects on immunotoxicity (thymus, spleen weight and the humoral immune response to SRBC) up to highest dose*			

2.6.10.1 Toxicological end point for assessment of risk following long-term dietary exposure – ADI (acceptable daily intake)

The estimation of the Acceptable Daily Intake (ADI) is based on the relevant lowest no-observed adverse effect level (NOAEL) observed in studies with respect to subchronic and chronic toxicity, neurotoxicity (if available and necessary), carcinogenicity and reproductive toxicity.

Cymoxanil is unlikely to be genotoxic *in vivo* and it does not reveal any oncogenic potential. The harmonised classification of cymoxanil for adverse effects on sexual function, fertility and development is Repr. 2, H361d based on Commission Regulation (EU) No 605/2014 (6th adaptation to technical and scientific progress of Regulation (EC) No 1272/2008).

The dog is the most sensitive species. Considering all results from relevant studies as summarised in Table above, the lowest NOAEL of 1.3 mg/kg bw/day was obtained in 1-year dog study (*RAR B.6.3.3.2., 2003*). This NOAEL is based on histological findings in the testes (minimal/slight bilateral atrophy) at 2.8 mg/kg bw/ day (100 ppm) with an apparent trend in the incidence and severity. A safety factor of 100 is considered appropriate to be applied to the NOAEL from the 1-year study in dog mentioned resulting in an **ADI** of **0.013 mg/kg bw/day**. With respect to the teratogenic effects seen at dose levels of 25 – 75 mg/kg bw/day, a margin of safety of at least 1900 is evident.

2.6.10.2 Toxicological end point for assessment of risk following acute dietary exposure - ARfD (acute reference dose)

For the determination of the ARfD, results from oral studies that used acute or short term exposure are considered to be the most relevant.

Cymoxanil is of moderate toxicity in the acute oral toxicity study in rats. In the short term (90 days) studies, no acute effects have been noted. Studies on subacute toxicity (28 day studies in rats and mice) are available, but range finding studies and therefore only a limited range of parameters has been investigated.

Based on the results of the studies regarding developmental toxicity, skeletal and visceral malformations were demonstrated in two developmental toxicity studies in rats (from 75 mg/kg bw/day) and in three out of four developmental toxicity studies in rabbits (from 25 mg/kg bw/day). The lowest developmental NOAEL of 8 mg/kg bw/day was obtained in developmental toxicity (teratogenicity) study in rabbits (*RAR B.6.6.2.5., 1982*), whereas the overall maternal toxicity was not established even at the highest dose tested. A safety factor of 100 is judged to be appropriate for setting the ARfD. In conclusion, the **ARfD** was calculated to be **0.08 mg/kg bw/day**. The margin of safety to the LOAEL for malformations is higher than 300.

2.6.10.3 Toxicological end point for assessment of occupational, bystander and residents risks – AOEL (acceptable operator exposure level)

The systemic AOEL is usually based on a suitable (mostly oral) short-term study. The lowest oral NOAEL and LOAEL obtained with cymoxanil were those from 1-year study in dog (*RAR B.6.3.3.2., 2003*) revealing a NOAEL of 1.3 mg/kg bw/day. This NOAEL is based on histological findings in the testes (minimal/slight bilateral atrophy) at 2.8 mg/kg bw/ day (100 ppm) with an apparent trend in the incidence and severity.

For setting the systemic AOEL, the usual uncertainty (safety) factor of 100 is applicable. In addition, in line with EU practice, correction for oral absorption should be made if this was below 80%. In case of cymoxanil, an oral absorption rate of 76% was calculated in the rat (*RAR B.6.1.1.2., 1995 and 1997 (supplement)*). In the absence of an ADME study in dogs, this data obtained in rats should be used for adjustment. Therefore an **AOEL** of **0.01 mg/kg bw/day** is proposed. The margin of safety to the developmental effects is higher than 2000.

2.6.10.4 Toxicological end point for assessment of occupational, bystander and residents risks – AAOEL (acute acceptable operator exposure level)

No harmonized guidance for the derivation of an AAOEL is available. The RMS proposes to establish the AAOEL using the same basis as for the ARfD, i.e. a NOAEL of 8 mg/kg bw/day from developmental toxicity (teratogenicity) study in rabbits (*RAR B.6.6.2.5., 1982*), an uncertainty (safety) factor of 100 and in addition a correction for limited oral absorption rate of 76%. Therefore, the **AAOEL** of **0.061 mg/kg bw/day** is proposed.

2.6.11 Summary of product exposure and risk assessment

There were presented three representative formulations for renewal of approval of cymoxanil.

2.6.11.1. Summary of Cymoxanil 45 WG exposure and risk assessment

The representative formulation Cymoxanil 45 WG is a water-dispersible granular formulation (WG) containing 450 g cymoxanil/kg. The toxicological studies (i.e., acute oral and dermal toxicity, skin and eye irritation, skin sensitisation studies) have been performed with the formulation Cymoxanil 45 WG. Cymoxanil 45 WG has low toxicity with respect to acute oral, acute dermal and is not irritating to the rabbit skin and inhalation routes of administration. It has been found that the product should be classified for irritancy to rabbit eye and classified for skin sensitization to the guinea pig according to Regulation (EC) 1272/2008.

Table 2.6.11.1-1: Summary of acute toxicity studies of Cymoxanil 45 WG

Endpoint	Value	Classification according to Reg. 1272/2008	Reference
Acute oral toxicity	LD_{50} >2000 mg/kg	None	RAR B.6.1.1., 2004a (Vol 3 CP)
Acute dermal toxicity	LD_{50} >2000 mg/kg	None	RAR B.6.1.2., 2004b (Vol 3 CP)
Acute Inhalation toxicity	LD_{50}> 5 mg/L (dust/mist)	None	Theoretical consideration
Skin irritation	Not irritating	None	RAR B.6.1.4., 2004c (Vol 3 CP)
Eye Irritation	Irritating	Yes	RAR B.6.1.5., 2004d (Vol 3 CP)
Skin sensitisation	Sensitising	Yes	Calculation method

According to the Regulation (EC) No. 1272/2008, due to the presence of 450 g/kg of the active substance cymoxanil and taking into account the results of toxicity studies, Cymoxanil 45 WG should be classified as follows:
Serious eye irritation, Cat. 2 - H319: Causes serious eye irritation,
Skin sensitisation, Cat. 1 - H317: May cause an allergic skin reaction,
Reproductive, Cat 2 - H361df: Suspected of damaging fertility or the unborn child,
Specific target organ toxicity after repeated exposure, Cat 2 – H373: May cause damage to organs (blood, thymus, eye).

Cymoxanil 45 WG is diluted with water and applied primarily using tractor-mounted field crop sprayers with air-assisted boom and nozzles directed downward for potatoes and upward for grape for field spraying and for greenhouse spraying by manual equipment. It is intended to be used as fungicide on potato, tomato and grape. It is to be applied maximum 0.15kg a.s/ha with spray volume 150-600-1500 l/ha. The critical GAP used for the exposure assessment of operators, workers, bystander and residents is summarised in Representative uses and GAP by Cymoxanil table in Volume Cymoxanil_dRAR_23-LoEP. The dermal absorption values for respectively the neat formulation and the spray dilution were considered 0.056% and 19%. The obtained predicted exposure was compared with the appropriate AOEL (0.01 mg/kg bw/day)/ AAOEL (0.061 mg/kg/bw per day) and expressed as % of AOEL/AAOEL.

The following exposure models were used for assessment of exposure of operators, workers, residents and bystanders:

EFSA Guidance: *Guidance on the assessment of exposure of operators, workers, residents and bystanders in risk assessment for plant protection products. EFSA Journal 2014;12(10):3874*
Dutch Greenhouse model.

Operators

Following calculations using the EFSA model it was demonstrated that there is no undue risk of exposure for operators to potato and tomato crops in fields when standard protected workwear (e.g., arms, body and legs covered) and gloves are worn The risk for the operator using of Cymoxanil 45 WG in grapes is acceptable when standard protected workwear and gloves is worn during loading/mixing and application. The risk to operators for indoor use of Cymoxanil 45 WG is considered acceptable when gloves, workwear and respiratory PPE are worn.

Bystander
According EFSA calculators there is no undue risk for bystander's adults and for children when Cymoxanil 45 WG is applied as intended use.

Resident
According to EFSA calculator, there is no unacceptable risk anticipated for an adult or a child resident incidentally exposed to Cymoxanil 45 WG in potatoes and tomatoes field.
There is no undue risk to resident after accidental short-term exposure to Cymoxanil 45 WG according to the GAP (150-1000L water) when spraying grapes if is considered buffer strip expanded to 10 m and drift reducing equipment using.

Worker
According to EFSA calculator, there is no unacceptable risk anticipated for a worker wearing working clothing when re-entering potatoes and tomatoes crops. There is no unacceptable risk anticipated for a worker wearing working clothing and gloves when re-entering grapevine after 4 days after last application. The risks are acceptable for workers re-entering the treated tomatoes indoor with workwear and gloves.

2.6.11.2. Summary of Dauphin 45 / FDJ03 exposure and risk assessment

The representative formulation Dauphin 45 / FDJ03 is a wettable granule (WG) containing 450 g cymoxanil/kg is use as fungicide. The toxicological studies (i.e., acute oral and dermal toxicity, skin and eye irritation, skin sensitisation studies) have been performed with the formulation Dauphin 45 / FDJ03. According to the data obtained with the active substance cymoxanil, Dauphin 45/FDJ03 containing 450 g/kg Cymoxanil has a moderate acute oral toxicity, and is neither dermally toxic nor is irritating to the rabbit skin or toxic by inhalation. Studies with the formulation Dauphin 45/FDJ03 revealed that it is non-irritating to the rabbit eye and it is not a skin sensitiser to the guinea pig.

Table 2.6.11.1-2: Summary of acute toxicity studies of Dauphin 45 / FDJ03

Endpoint	Value	Classification according to Reg. 1272/2008	Reference
Acute oral toxicity	LD_{50} =960 mg/kg	H302	RAR B.6.1.1., 1992 (Vol 3 CP)
Acute dermal toxicity	LD_{50} >2000 mg/kg	None	RAR B.1.2., 1994 (Vol 3 CP)
Acute Inhalation toxicity	LD_{50}> 5.06 mg/L air	None	RAR B.6.3., 1992 (Vol 3 CP)
Skin irritation	Not irritating	None	Calculation method
Eye Irritation	Not Irritating	None	RAR B.6.1.5., 2010 (Vol 3 CP)
Skin sensitisation	Not sensitising	None	RAR B.6.1.6., 2010 (Vol 3 CP)

Considering the toxicological classification of the active substance cymoxanil and the data herewith submitted, the product Dauphin 45/FDJ03 with respect to human health should be labelled as:

H302 Harmful if swallowed
H361fd Suspected of damaging fertility. Suspected of damaging the unborn child
H373 May cause damage to organs through prolonged or repeated exposure if swallowed

The Plant Protection Product Dauphin 45/FDJ03 containing 450 g/kg Cymoxanil is intended to be used as fungicide on potatoes, grapes and tomatoes outdoor. Dauphin 45/FDJ03 is diluted with water and applied primarily using tractor-mounted/trailed boom sprayer application downward for potatoes, tomatoes and tractor-mounted/trailed air assisted sprayer upward application for grape for field spraying and hand held equipment (knapsack) for high crops. It is to be applied maximum 0.12kg a.s/ha with spray volume 100-200-600-1000 l/ha. The critical GAP used for the exposure assessment of operators, workers, bystander and residents is summarised in Representative uses and GAP by Cymoxanil table in Volume Cymoxanil_dRAR_23-LoEP.
Two dermal absorption studies were provided by Applicant, which are conducted with different formulation than Dauphin 45/FDJ03, however dermal absorption studies were not accepted. According to the EFSA Guidance on

dermal absorption (2017), a default dermal absorption value of 10% is considered for the exposure estimations of the concentrate, and 50% is applied for the in-use dilution. The obtained predicted exposure was compared with the appropriate AOEL (0.01 mg/kg bw/day)/ AAOEL (0.061 mg/kg/bw per day) and expressed as % of AOEL/AAOEL.
The following exposure model was used for assessment of exposure of operators, workers, residents and bystanders:

EFSA Guidance: *Guidance on the assessment of exposure of operators, workers, residents and bystanders in risk assessment for plant protection products. EFSA Journal 2014;12(10):3874*

Operators
The risk for the operator using of Dauphin 45/FDJ03 in potatoes and tomatoes is acceptable when standard protected workwear and gloves is worn during loading/mixing and application.
Following calculations using the EFSA model, it was demonstrated that there is no unacceptable risk for operators during the grapes spraying by tractor mounted application if are worn work wear, gloves during mixing/loading and application, RPE during mixing/loading, and operator are working in close cabin.
Acceptable risk was demonstrated for operators spraying vineyard by hand held equipment when protected workwear and gloves and RPE during mixing/loading and gloves during application is worn.
According to the model estimations the risk for operator spraying tomatoes by manual application hand-held equipment is unacceptable even are worn PPE and using RPE.

Bystander
According to EFSA calculator, it can be concluded that there is no undue risk for an adult or a child bystander if exposed to Dauphin 45/FDJ03 when are using nozzle reduction equipment and buffer strip is extended to 10m.

Resident
Resident exposure is not acceptable for all intended uses neither even when the more realistic situation with the first tier DFR and DT_{50} is considered, nor using drift reduction and buffer strip expanded to 10 meter when spraying potato, tomato and grape.

Worker
According to EFSA calculator, there is no unacceptable risk anticipated for a worker wearing working clothing when re-entering potatoes treated with the Dauphin 45/FDJ03. There is no unacceptable risk anticipated for a worker wearing working clothing and gloves when re-entering tomatoes after 1 day after application and re-entering grape fields after 3 days.

2.6.11.3. Summary of Rival Duo exposure and risk assessment

The representative formulation Rival Duo is a suspension concentrate (SC) containing 50 g/l cymoxanil and 400 g/l propamocarb and is used as fungicide. The toxicological studies have not be performed, for product evaluation has been used a tiered approach with the utilisation of the calculation method. Cymoxanil 50 g/l + Propamocarb 400 g/l SC (Rival Duo) is of low acute toxicity by the oral and dermal routes, is also of low toxicity by the inhalation route, was found to be non-irritating to skin and the eyes. Rival Duo was predicted to be a potential skin sensitiser according the Calculation method, but an in vivo study, confirms that the formulation is not sensitising to skin in a guinea pig (Maximisation study). Based on the results of the calculation and the available studies, Rival Duo is not classified for acute toxicity, irritation or sensitisation according to Regulation (EC) No 1272/2008.

Table 2.6.11.1-3: Summary of acute toxicity studies of Rival Duo (Cymoxanil 50 g/l + Propamocarb 400 g/l)

Endpoint	Value	Classification according to Reg. 1272/2008	Reference
Acute oral toxicity	LD_{50} >2000 mg/kg	None	Calculation method of Reg. (EC) No. 1272/2008
Acute dermal toxicity	LD_{50} >2000 mg/kg	None	Calculation method of Reg. (EC) No. 1272/2008
Acute Inhalation toxicity	MLC_{50}> 5.184 mg/L	None	Calculation method of Reg. (EC) No. 1272/2008
Skin irritation	Not irritating	None	Calculation method of Reg. (EC) No. 1272/2008
Eye Irritation	Not Irritating	None	Calculation method of Reg.

			(EC) No. 1272/2008
Skin sensitisation (Maximisation Study)	Not sensitising	None	RAR B.6.1.6., 2012 (Vol 3 CP)

According to the Regulation (EC) No. 1272/2008, due to the presence of 50 g/kg of the active substance cymoxanil which classified According Regulation (EC) No 1272/2008, H361fd and taking into account the results of toxicity studies, Rival Duo should be classified as follows:
Repr.2; H361fd, Suspected of damaging fertility. Suspected of damaging the unborn child.
In the absence of product specific, the default values for an SC type product have been utilised in the risk assessment for both cymoxanil and propamocarb i.e. 10% for the concentrate and 50% for the dilution according to the Guidance on Dermal Absorption (EFSA Journal 2017; 15(6): 4873).
The Plant Protection Product Rival Duo containing 50 g/l cymoxanil and 400 g/l propamocarb is intended to be used as fungicide on potatoes outdoor. Rival Duo is diluted with water and applied primarily using tractor mounted boom sprayer downward for potatoes.
For exposure calculation as the worst-case is given the highest application rate (0.125 kg cymoxanil/ha and 1 kg propamocarb/ha) and lowest water volume 300 l/ha.
The obtained predicted exposure was compared with the appropriate AOEL (0.01 mg/kg bw/day)/ AAOEL (0.061 mg/kg/bw per day) and expressed as % of AOEL/AAOEL.

The following exposure model was used for assessment of exposure of operators, workers, residents and bystanders:

EFSA Guidance: *Guidance on the assessment of exposure of operators, workers, residents and bystanders in risk assessment for plant protection products. EFSA Journal 2014;12(10):3874*

Operator
According to the EFSA calculator, there is no unacceptable acute and longer term risk anticipated for operator wearing work wear (arms, body and legs covered) and gloves during mixing/loading and application is used.

Bystander
According to the model estimations, there is no undue risk for bystander's adults and for children when 'Cymoxanil 50 g/L + Propamocarb 400 g/L SC' (Rival Duo) is applied as intended use.

Resident
There is no undue risk to resident after accidental short-term exposure to cymoxanil when 'Cymoxanil 50 g/L + Propamocarb 400 g/L SC' (Rival Duo) according to the GAP and when spraying potato with drift reduction equipment and buffer strip expanded to 10 meter.

Worker
According to EFSA calculator, there is no unacceptable risk anticipated for a worker wearing working clothing when re-entering potatoes crops treated with 'Cymoxanil 50 g/L + Propamocarb 400 g/L SC' (Rival Duo) according to the GAP.

2.7 RESIDUE

2.7.1 Summary of storage stability of residues

Studies investigating stability of residues during storage of samples in lettuce and potatoes were reviewed during cymoxanil Annex I inclusion process. In addition, Cymoxanil Task Force submitted storage stability studies on tomatoes, potatoes and grapes, Agria submitted one study on grapes and lettuce, and SFP submitted storage stability studies on tomatoes, lettuce and grapes. A summary of all data is presented in the table below.

Residues of cymoxanil can be considered as stable for at least 12 months in lettuce, for at least 18 months in tomato RAC and 5 months in processed tomatoes, for at least 12.5 months in potato and for at least 24 months in grapes when stored at -18°C.

Table 41: Summary of storage stability of cymoxanil in plant matrices

Commodity categories	**Commodity**	**Fortification level**	**Storage period**	**Reference***	**Owner**

Commodity categories	Commodity	Fortification level	Storage period	Reference*	Owner
High water content	Lettuce	0.515 mg/kg	12 months (whole) 1 month (homogenised)	**Volume 3, Annex B, B.7.6.5 (DAR)** **CA 6.1/01 (CTF)** SIP 1379, Freschi G. 2004	n.a.
	Lettuce	0.2 mg/kg	12 months	**CA 6.1/08 (CTF)** **CA 6.1/01 (SFP)** B0210, Jonchere F. 2012	UPL, SFP
	Tomato	0.5 mg/kg	18 months (whole fruit) 1 month (homogenised fruit)	**CA 6.1/04 (CTF)** SIP 1381, Lucini L. 2006b	CTF
	Tomato	0.1 mg/kg	150 days (RAC, juice, canned tomatoes, puree, wet pomace)	**CA 6.1/07 (CTF)** B2124, Schlewitz P. 2014a	CTF
	Tomato	0.2 mg/kg	12 months	**CA 6.1/08 (CTF)** **CA 6.1/01 (SFP)** B0210, Jonchere F. 2012	UPL, SFP
	Tomato	0.1 mg/kg	6 months	**CA 6.1/01 (Agria)** WSD0042, Harper H. 2013	Agria
High starch content	Potato	0.25 mg/kg	12.5 months	**Volume 3, Annex B, B.7.6.5 (DAR)** **CA 6.1/02 (CTF)** AMR 3296-95, Nathan E.C. 1996	n.a.
	Potato	0.2 mg/kg	12 months	**CA 6.1/05 (CTF)** GAB-0704, Weber H. 2011	CTF
High acid content	Grapes	0.5 mg/kg	2 years (whole fruit) 1 month (homogenised fruit)	**CA 6.1/03 (CTF)** SIP 1380, Lucini L. 2006a	CTF
	Grapes	0.5 mg/kg	12 months	**CA 6.1/06 (CTF)** A7187, Perny A. 2010	CTF
	Grapes	0.2 mg/kg	130 days	**CA 6.1/09 (CTF)** **Ca 6.1/02 (SFP)** B4062, Schlewitz P. 2014b	UPL, SFP
	Grapes	0.1 mg/kg	6 months	**CA 6.1/01 (Agria)** WSD0042 Harper H. 2013	Agria

* - For renewal of approval of the a.s. cymoxanil, three dossiers from three different applicants were submitted to RMS LT therefore same reference numbers were allocated to different study reports in different dossiers. The applicant submitted the study is indicated to each reference, giving "CTF" for Cymoxanil Task Force, "Agria" to Agria SA and "SFP" to Société Financière de Pontarlier.

The data on the stability of residues in crops are sufficient to cover the length of storage of all representative uses for all three applicants.

No study was submitted for storage stability in animal matrices. As no livestock feeding studies have been conducted, it is therefore not required to conduct storage study stability on products of animal origin

2.7.2 Summary of metabolism, distribution and expression of residues in plants, poultry, lactating ruminants, pigs and fish

Metabolism in plants

The plant metabolism of cymoxanil was carried out in four crops, representing three crop groupings – fruit crop (grapes and tomatoes), leafy crop (lettuce) and root and tuber crop (potatoes). The application method was foliar for all these crops[7]. The representative use for cymoxanil in the EU is on potatoes (CTF, SFP and Agria), grapes and tomatoes (CTF and SFP).

Most of these studies have already been subject to a uniform principles evaluation and EU peer review, either for the first inclusion of cymoxanil into Annex I of Council Directive 91/414/EEC or during the subsequent review of cymoxanil MRLs under article 12(1) of Regulation (EC) No 396/2005. In these studies cymoxanil was observed to rapidly degrade in plants to glycine which then leads to natural incorporation into endogenous biomolecules such that significant residues of parent cymoxanil or structurally-related metabolites are not expected in plant commodities.

In the renewal dossier, an additional two crop metabolism studies are provided: CA 6.2.1/02, Rosenwald, J., 2011 in grapes (submitted by CTF and SFP) and CA 6.2.1/05, Tan, N.; Brands, Ir. C., 2009 (submitted by CTF) in lettuce. In these new studies, some of the known photolytic and hydrolytic degradates of cymoxanil were able to be detected, more so than in the earlier studies. These metabolites were almost exclusively found in the surface wash fraction rather than in the plant extracts, confirming their probable abiotic origin. This was exaggerated in the new grape study in which the grapevines were grown under protection between the final spray and harvest which is not normal agronomic practice for grapes, and which may have impeded normal photolysis and weathering processes. This may also explain why the TRRs in the grapes in this grape study were ~10 times higher than might have been expected when compared to the TRRs seen in the other plant metabolism studies considering the relative rate of [2-^{14}C]cymoxanil being applied, and thus why the metabolites were able to be detected in this study and at relatively significant amounts compared to the previous findings. However, these newer studies do not alter the overall conclusion that once absorbed into the plant tissues, cymoxanil is very rapidly degraded to glycine which then enters the metabolic pool and is naturally incorporated. Generally, little cymoxanil or structurally related metabolites were seen in the plant extracts in these new studies.

The overall relative abundance of cymoxanil and its metabolites observed in all of the available plant metabolism studies is summarised in the table below. Since the grape metabolism data reported in the literature paper at reference CA 6.2.1/10 (CTF) is simply a repetition of the regulatory study at reference CA 6.2.1/01 (CTF), only the data from CA 6.2.1/01 (CTF) are included in the summary table. Two articles from public literature (submitted by SFP) on metabolism in grapes, tomatoes and potatoes (Cohen, Y. and Gisi, U., 1993 and Roberts et al., 1999) were also not included in this table, as they lacked essential information to evaluate the reliability of this information.

[7] There is also literature information (supplementary) on metabolism in tomatoes (Cohen, Y. and Gisi, U., 1993), concluding that when [^{14}C]Cymoxanil was applied to the root it was taken up by the root system within 1 h and translocated to cotyledons, stem and leaves within 16 h. The compound was degraded, mostly to glycine, within 16-44 h, in the root and all parts of the shoot.

Table 42: Summary of the relative abundance (%TRR) of cymoxanil and metabolites identified in all of the plant metabolism studies.

Crop group	**Fruit crop**							**Leafy crop**				**Root and tuber vegetables**		
Commodity	**Grapes**				**Tomatoes**			**Lettuce**				**Potatoes**		
Study reference	CA 6.2.1/01 (CTF)		CA 6.2.1/02 (CTF) CA 6.2.1/03 (SFP)		CA 6.2.1/03 (CTF)	CA 6.2.1/04 (CTF)	CA 6.2.1/10 (CTF) CA 6.2.1/01 (SFP)	CA 6.2.1/05 (CTF)		CA 6.2.1/06 (CTF)	CA 6.2.1/07 (CTF)	CA 6.2.1/08 (CTF)	CA 6.2.1/09 (CTF)	CA 6.2.1/10 (CTF) CA 6.2.1/01 (SFP)
Author, Year	Han, J. C.-Y., Baude, F. J., (date not reported)		Rosenwald, J., 2011		Melkebeke, Ir. T. & van Noorloos, B., 2003a	Li, Y., 1997	Belasco, I. J., et al, 1980	Tan, N.; Brands, Ir. C., 2009		Fox, G. C., 1999	Melkebeke, Ir. T. & van Noorloos, B., 2003b	Li, Y., Hausmann, S. M., 1996	Melkebeke, Ir. T. & van Noorloos, B., 2003c	Belasco, I. J., et al, 1980
GAP	8 × 210 g a.s./ha		6 × 100 – 120 g a.s./ha		4 × 240 g a.s./ha	3 × 632 g a.s./ha	8 × 140 g a.s./ha	4 × 240 g a.s./ha		4 × 840 g a.s./ha	3 × 240 g a.s./ha	3 × 400 g a.s./ha	8 × 240 g a.s./ha	4 – 5 × 210 g a.s./ha
DALA	10 (5th spray)	10 (final spray)	7	28	13	3	7	3	10	3	11	3	10	10 – 11
TRR [mg/kg] =	1.6	2.4	11.89 – 16.14*	11.30 – 14.06*	0.125	1.10	7.9	3.83	1.04	10.78	1.07	0.69	1.05	1.45 – 2.41
Component	**% TRR (mg/kg)**													
Cymoxanil	**5.0**	**4.0**	**5.4-38.3**	**5.3-12.1**	ND	1.1	0.8	**50.1**	3.6	2.1	1.4	ND	ND	<0.1
Glycine	**23.0**	**23.0**	**35.4-51.5†**	**56.5-69.5†**	ND	**65.2**	**30.0**	**14.0**	**29.7**	**30.6**	**13.0**	**78.5**	**27.0**	**44.7**
Starch/sugars	**14.2**	**14.4**			ND	**9.6**	**7.0**	NA	NA	**21.1**	NA	NA	NA	**14.0**
IN-W3595	NA	NA	**1.5-2.9**	**2.7-7.1**	ND	ND	NA	**4.9**	**10.3**	ND	**18.1**	ND	ND	NA
IN-KP533	NA	NA	**6.0-16.6**	**1.1-2.4**	NA	<1	NA	**5.7****	**8.2****	**2.8**	NA	ND	NA	NA
IN-T4226	NA	NA	**5.1-5.9**	<0.1-0.1	ND	<1	NA	ND	ND	ND	ND	ND	ND	NA
IN-KQ960	NA	NA	<0.1-0.4	<0.1	NA	ND	NA	**1.8†****	0.3†**	**7.4**	NA	ND	NA	NA
IN-U3204	1.2	0.6	**4.3-16.7**	**1.2-21.1**	NA	ND	NA			ND	NA	ND	NA	<0.1
IN-R3273	<0.1	<0.1	**<0.1-2.3**	**0.6-3.2**	NA	ND	NA	ND	ND	ND	NA	ND	NA	<0.1
IN-JX915	NA	NA	<0.1-**4.4**	**0.4-3.9**	NA	ND	NA	ND	ND	ND	NA	ND	NA	NA
IN-18474	NA	NA	NA	NA	NA	ND	NA	NA	NA	ND	NA	ND	NA	NA
IN-R3274	NA	NA	NA	NA	ND	ND	NA	ND	ND	ND	ND	ND	ND	NA
IN-Q8761	NA	NA	NA	NA	NA	ND	NA	0.2**	0.3**	ND	NA	ND	NA	NA

Crop group	**Fruit crop**							**Leafy crop**				**Root and tuber vegetables**		
Commodity	**Grapes**				**Tomatoes**			**Lettuce**				**Potatoes**		
Study reference	CA 6.2.1/01 (CTF)		CA 6.2.1/02 (CTF) CA 6.2.1/03 (SFP)		CA 6.2.1/03 (CTF)	CA 6.2.1/04 (CTF)	CA 6.2.1/10 (CTF) CA 6.2.1/01 (SFP)	CA 6.2.1/05 (CTF)		CA 6.2.1/06 (CTF)	CA 6.2.1/07 (CTF)	CA 6.2.1/08 (CTF)	CA 6.2.1/09 (CTF)	CA 6.2.1/10 (CTF) CA 6.2.1/01 (SFP)
Author, Year	Han, J. C.-Y., Baude, F. J., (date not reported)		Rosenwald, J., 2011		Melkebeke, Ir. T. & van Noorloos, B., 2003a	Li, Y., 1997	Belasco, I. J., et al, 1980	Tan, N.; Brands, Ir. C., 2009		Fox, G. C., 1999	Melkebeke, Ir. T. & van Noorloos, B., 2003b	Li, Y., Hausmann, S. M., 1996	Melkebeke, Ir. T. & van Noorloos, B., 2003c	Belasco, I. J., et al, 1980
GAP	8 × 210 g a.s./ha		6 × 100 – 120 g a.s./ha		4 × 240 g a.s./ha	3 × 632 g a.s./ha	8 × 140 g a.s./ha	4 × 240 g a.s./ha		4 × 840 g a.s./ha	3 × 240 g a.s./ha	3 × 400 g a.s./ha	8 × 240 g a.s./ha	4 – 5 × 210 g a.s./ha
DALA	10 (5th spray)	10 (final spray)	7	28	13	3	7	3	10	3	11	3	10	10 – 11
TRR [mg/kg] =	1.6	2.4	11.89 – 16.14*	11.30 – 14.06*	0.125	1.10	7.9	3.83	1.04	10.78	1.07	0.69	1.05	1.45 – 2.41
Component	**% TRR (mg/kg)**													
CAU	NA	NA	NA	NA	ND	NA	NA	NA	NA	NA	ND	NA	ND	NA
INT-3204	NA	NA	NA	NA	ND	NA	NA	NA	NA	NA	ND	NA	ND	NA
AS 999/glycine-related	NA	NA	NA	NA	NA	NA	NA	1.0**	**11.4****	NA	NA	NA	NA	NA
Total identified compounds	69.5	69.3	89.6-93.6	91.2-95.2	0	75.9	44.6	77.7	65.6	64.0	32.5	-	27.0	69.4
Total characterised**			0.6-3.6	0.7-0.9	73.0	<2		10.6	11.2	8.0	57.6	86.6	38.5	
Total extracted			91.8-97.2	91.9-96.1	80.9	92.2		92.4	77.0	72.0	91.3	98.3	68.3	
Post extraction solids (PES)			2.9-8.1	3.9-8.1	13.2	7.1		7.2	15.6	27.9	4.6	1.7	36.4	
Accountability	69.5	69.3	99.9-100.1	99.9-100	94.1	100	44.6	95.5	92.4	99.9	94.7	88.3	101.9	69.4

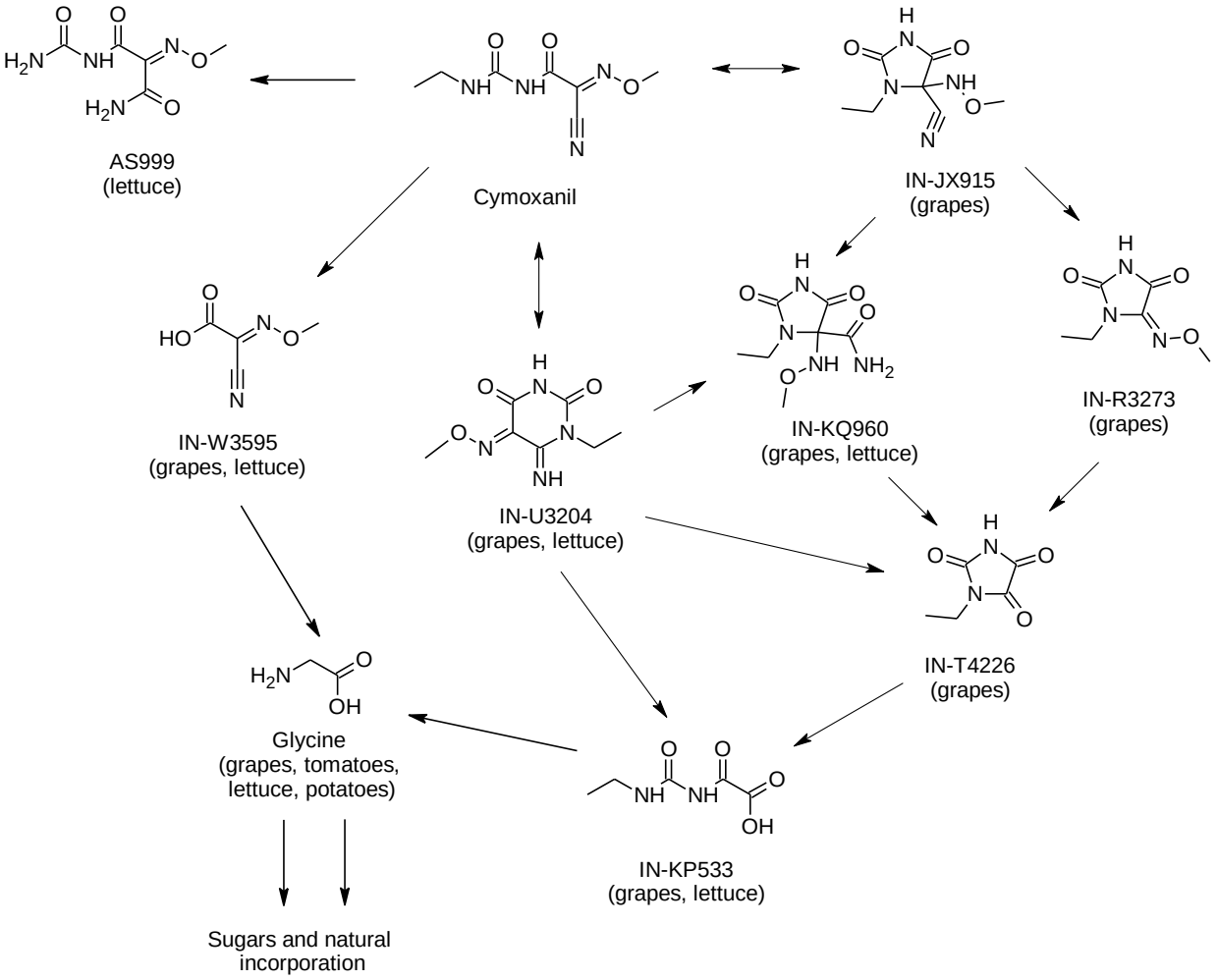
NA = Not analysed;
ND = Not detected;
†These metabolites are grouped together as they were not differentiated in the study,
* Range of three plants.
**These metabolites were only very tentatively identified.
Metabolites that were identified at ≥10% of the TRR in a commodity or ≤10% of the TRR but ≥0.05 mg a.s. equiv./kg are highlighted in bold typeface.

In the above summary table of the relative abundance (%TRR) of cymoxanil and metabolites identified in all of the plant metabolism studies, major metabolites that were identified at ≥10% of the TRR in a commodity or ≤10% of the TRR but ≥0.05 mg a.s. equiv./kg are highlighted in bold typeface. All of these major metabolites are known hydrolytic or photolytic degradates of cymoxanil or arise as the result of natural incorporation. The results are fairly consistent across the various studies and commodities in showing that glycine and starch/sugars are the most predominant components, accounting together for between 30 and 75% of the residue. Other natural incorporation accounted for additional radioactivity when specifically determined or accounted for the frequent observation of an unidentified polar fraction in the metabolism studies.

The most significant of the identified metabolites which were present at close to or above 10% of the TRR are IN-W3595, IN-KP533, IN-KQ960, AS 999 and IN-U3204. IN-W3595 was present at 5 – 18% of the TRR in lettuce although this metabolite was not detected in tomatoes and potatoes and was a more minor component of grapes (1.5-7.1% TRR). IN-KP533 was present at 17% of the TRR in grapes, tentatively identified at a moderate level in lettuce and not detected in tomatoes and potatoes. IN-KQ960 was present at 7.4% of the TRR in one of the lettuce studies, was a less significant component in one of the other lettuce studies and was not detected in the other crops apart from a trace levels in one of the grape studies. AS 999 was tentatively identified only in one of the lettuce studies. IN-U3204 was identified as a significant metabolite in grapes only, at 21% of the TRR, and was tentatively as a more minor residue in one of the lettuce studies. Metabolites IN-T4226, IN-R3273 and IN-JX915 were found only in one grape study at levels <10% TRR but >0.05 mg a.s. equiv./kg.

From the identified metabolites, a summary of the metabolic pathway of cymoxanil in plants is presented below.

Proposed metabolic pathway of [2-^{14}C]cymoxanil in crops following foliar spray application



Metabolism in animals

On the basis of the representative uses of cymoxanil on grapes, tomatoes and potatoes supported at renewal, the findings of the confined rotational crop metabolism study showing that residues in rotated feed crops are not expected and the low log P_{OW} meaning that cymoxanil has a low propensity for bioaccumulation, residues of cymoxanil in commodities of animal origin (including fish) are not expected and the data requirement for livestock metabolism studies is not triggered for all three applicants. A livestock dietary burden calculation is presented in section 2.7.5 of this Volume. No metabolism studies on poultry, ruminants, pigs or fish are required. However, a lactating ruminant metabolism study performed in the goat was submitted for the first inclusion of cymoxanil into Annex I of Council Directive 91/414/EEC and reviewed under uniform principles.

The metabolism of [2-^{14}C]cymoxanil was investigated in a lactating goat after administration of a daily dose of 10 mg/kg/day in the feed for 3 consecutive days. This corresponded to an intake of 0.35 mg/kg body weight per day and approximately 175 N-rate compared to the maximum exposure level expected in the ruminant diet from the representative uses of cymoxanil at renewal. In total, 68.8% of the dose was recovered (24% in urine, 18% in faeces, 0.5% in cage washes, 2.2% in expired air, 3% in milk and 22% in tissues, intestinal tract and the reaming carcass). The unaccounted for radioactivity was attributed to [^{14}C]methane, produced in the rumen.

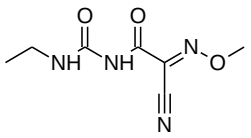
Although the animal was dosed for 3 days instead of the required 5 days for a ruminant metabolism study, the total radioactive residue had already reached a plateau in milk by the second day and so this is considered to be acceptable. The steady state TRR in milk was about 0.28 mg a.s. equiv./l. The TRRs in the tissues amounted to

2.133 mg a.s. equiv./kg in liver, 0.457 mg a.s. equiv./kg in kidney, 0.084 mg a.s. equiv./kg in muscle, 0.073 mg a.s. equiv./kg in fat, 0.197 mg a.s. equiv./kg in the intestinal contents, 0.255 mg a.s. equiv./kg in the intestinal tract and 0.492 mg a.s. equiv./kg in whole blood.

No cymoxanil or structurally-related metabolites were identified in any of the milk, liver, kidney, muscle, fat or urine samples and instead the radiolabel was determined to have been incorporated into natural products. In milk, a mixture of lipids, carbohydrates and fatty acids were identified with lactose as the largest component amounting to 45.8% of the TRR (0.13 mg a.s. equiv./l). The metabolic profiles of liver and kidney were similar, and the residue was identified as mostly formic or acetic acids, the sum amounting to 78.1% of the TRR (1.57 mg a.s. equiv./kg) in liver and 75.1% of the TRR (0.34 mg a.s. equiv./kg) in kidney. 55% of the TRR in muscle and 28.0% of the TRR in fat were extracted but given the low concentration of radioactivity, these extracts were not identified, and no exhaustive extraction was attempted. The urine was found to contain radiolabelled amino acids with glycine the only conclusively identified amino acid. Formic acid was also identified in urine.

Cymoxanil was found to be very labile in the rumen and is completely metabolised by the microorganisms present within about 7.5 min. No structurally-related metabolites or known hydrolytic/photolytic products of cymoxanil could be detected at this time point. Within four hours after administration, volatile [^{14}C]fatty acids (formic acid, acetic acid, valeric acid) were detected which were further incorporated into lipids, sugars, proteins, and other natural products. This would explain why cymoxanil and structurally-related metabolites could not be detected in the goat milk and tissues – cymoxanil had already degraded in the rumen and the radiolabel incorporated into, and absorbed from, the general metabolic pool in the gut.

The following metabolic pathway for cymoxanil in the goat is proposed.



Cymoxanil

↓

Rumen

↓

Rumen volatile fatty acids
(formic, acetic and valeric acids)

↓ Absorption into the goat

Natural incorporation into fatty acids, glycerol, amino acids, acetylated compounds and formyl methionine and other formylated compounds

2.7.3 Definition of the residue

Plants

Results in metabolism studies are fairly consistent across the various studies and commodities (grapes and tomatoes – fruit crops, lettuce – leafy crop and potatoes- root and tuber vegetables, representing 3 different metabolism groups) in showing that glycine and starch/sugars are the most predominant components, accounting together for between 30 and 75% of the residue. Residue pattern is considered similar in 3 different metabolism groups, therefore a general residue definition could be proposed. The most significant of the identified metabolites which were present at close to or above 10% of the TRR or >0.05 mg a.s. equiv./kg are IN-W3595, IN-KP533, IN-KQ960, AS 999, IN-T4226, IN-U3204, IN-R3273 and IN-JX915.

IN-W3595 was present at 5 – 18% of the TRR in lettuce although this metabolite was not detected in tomatoes and potatoes and was a more minor component of grapes 1.5-7.1% TRR. Due to general toxicity, it is considered to share the same toxicological reference values as parent compound.

IN-KP533 was present at 17% of the TRR in grapes, tentatively identified at a moderate level in lettuce and not detected in tomatoes and potatoes. The available data on general toxicity (acute toxicity) demonstrated that the compound might be considered less toxic than the parent substance. A final conclusion on the genotoxic potential cannot be drawn for the metabolite IN-KP533 based on data provided. The available data on acute toxicity demonstrated that metabolite IN-KP533 might be considered less toxic than the parent substance. However, since there is no data on repeated toxicity potency of IN-KP533 a conclusion if the metabolite is of lower, equal or higher toxicity than the parent cannot be reached.

IN-KQ960 was present at 7.4% of the TRR in one of the lettuce studies, was a less significant component in one of the other lettuce studies and was not detected in the other crops apart from a trace levels in one of the grape studies. A final conclusion on the genotoxic potential cannot be drawn for the metabolite IN-KQ960 based on the information provided. Since there is no data on repeated toxicity potency of IN-KQ960 a conclusion if the metabolite is of lower, equal or higher toxicity than the parent cannot be reached.

AS 999 was tentatively identified only in one of the lettuce studies. The available data on general toxicity (acute toxicity) demonstrated that the compound might be considered less toxic than the parent substance. It is expected that the toxicological profile will not differ significantly from the active substance cymoxanil regarding potential for genotoxicity, carcinogenic potential, apparent risks of causing organ-target mediated effects in vertebrates, fertility or overall reproductive performance. The data provided do not allow final conclusion to be reached on the genotoxic potential of this metabolite. Since there is no data on repeated toxicity potency of AS 999 a conclusion if the metabolite is of lower, equal or higher toxicity than the parent cannot be reached.

IN-U3204 was identified as a significant metabolite in grapes only, at 21% of the TRR, and was tentatively identified as a more minor residue in one of the lettuce studies. It is expected that the toxicological profile of the metabolite IN-U3204 will not differ significantly from the active substance cymoxanil. The long-term toxicological profile of the IN-U3204 is very similar to cymoxanil technical grade, showing no carcinogenic potential. However, due to limitations of the assessment and documentation the data provided do not allow final conclusion to be reached on the genotoxic potential of the metabolite.

Metabolites IN-T4226, IN-R3273 and IN-JX915 were found only in one grape study at levels <10% TRR but >0.05 mg a.s. equiv./kg. However, residue levels in this study are rather high compared to other studies, and growing them in greenhouse might have caused this difference. It is expected that the toxicological profile of these metabolites will not differ significantly from the active substance cymoxanil. The data provided do not allow final conclusion to be reached on the genotoxic potential of these metabolites. The available in-silico modelling data on acute toxicity demonstrated that metabolite IN-JX915 might be considered more toxic than the parent substance. However, since there is no data on repeated toxicity potency of metabolites IN-T4226, IN-R3273 and IN-JX915 a conclusion if the metabolite is of lower, equal or higher toxicity than the parent cannot be reached.

For all metabolites (except IN-W3595) a final conclusion on the genotoxic potential cannot be drawn, and a conclusion if the metabolite is of lower, equal or higher toxicity than the parent cannot be reached. Detail assessment on the toxicity of the metabolites could be found in section 2.6.8 of this document. All of these metabolites are known photolytic/hydrolytic degradates.

The proposed residue definitions for plant commodities are detailed below:

- Enforcement: cymoxanil;

- Risk assessment: cymoxanil.

Livestock

Based on available livestock metabolism studies and as livestock exposure was below the trigger value, no proposal has been done for livestock residue definitions (both enforcement and risk assessment).

2.7.4 Summary of residue trials in plants and identification of critical GAP

The representative crops in the original EU review of cymoxanil were potato and lettuce. For renewal the Cymoxanil Task Force and SFP are seeking to support uses on potatoes, tomatoes and grapes, while the Agria SA is seeking to support the use on potatoes only. Residue trials evaluated under Directive 91/414/EEC and new trials provided for renewal are presented for each applicant separately.

Grapes

Crop	EU zone	Number of applications	Interval between applications (days)	Application rate per treatment (kg a.s./ha)	Growth stage at last application	PHI (days)	Applicant
Grapes	CEU, SEU	5	7	0.12	BBCH 89	28	Cymoxanil Task Force
Grapes	SEU	4	7-10	0.12	BBCH 85	28	SFP

Grapes were not a representative use of Annex I inclusion. Therefore, new trials have been submitted by the applicants Cymoxanil Task Force (CTF) and Société Financière de Pontarlier (SFP) to support their proposed GAPs. Residue levels of trials complying with these GAPs are reported in the overview table.

Cymoxanil Task Force submitted 14 NEU (conducted in 1996, 2000, 2001 and 2010) and 12 SEU trials (conducted in 1996, 1997, 2000 and 2010) out of which 13 NEU and 12 SEU trials were accepted. Two trials in NEU were conducted at the locations for maximum of 4 km distance from each other with applications made on the same day, these two trials are not considered independent. CTF GAP is supported for NEU and SEU.

Although there is no GAP in NEU for Société Financière de Pontarlier (SFP), 4 NEU trials (conducted on 2009) and 4 SEU trials (conducted in 2009) were submitted. These trials were conducted at a more critical GAP that proposed (8 x 120 g/ha instead of 4 x 120 g/ha). Only 3 NEU and 3 SEU trials were accepted as two trials in NEU and two trials in SEU were conducted in the same or neighbouring towns sharing the same ZIP code these two trials are not considered independent.

As all residue levels are <LOQ and trials conducted at a more critical gap, 3 trials NEU and 3 trials SEU are considered sufficient to support the SFP GAP in SEU.

As intended GAPs on grapes are quite the same between applicants (the only difference is the number of applications - 4 vs 5), pooling all data on grapes for MRL calculation might be considered. Pooled data set gives an MRL of 0.2 mg/kg. If both applicants data set are not merged the calculated MRLs based on each applicant data are: 0.2 mg/kg (CTF) and 0.02 mg/kg (SFP)

Tomatoes

Crop	EU zone	Number of applications	Interval between applications (days)	Application rate per treatment (kg a.s./ha)	Growth stage at last application	PHI (days)	Applicant
Tomatoes (protected)	EU all zones	5	7	0.15	BBCH 89	3	Cymoxanil Task Force
Tomatoes (field)	SEU	5	7	0.15	BBCH 89	3	Cymoxanil Task Force
Tomatoes (field)	SEU	5	7	0.12	BBCH 87	3 (fresh consumption)	SFP

Tomatoes were not a representative use of Annex I inclusion. Therefore, new trials have been submitted by the applicants Cymoxanil Task Force (CTF) and Société Financière de Pontarlier (SFP) to support their proposed GAPs. Residue levels of trials complying with these GAPs are reported in the overview table.

Cymoxanil Task Force submitted 18 residue trials on protected tomatoes (4 trials in NEU and 14 in SEU) conducted in 2001, 2012 and 2013 out of which all were accepted. CTF GAP is supported for indoor use on tomatoes.

For field use on tomatoes Cymoxanil Task Force submitted 24 residue trials in SEU (conducted in 2006, 2007, 2010, 2012 and 2013) out of which 23 trials were accepted (one trial was conducted at a >25% less application rate with residues being <LOQ and not possible to upscale using proportionality principle). CTF GAP is supported for outdoor use on tomatoes in SEU.

Société Financière de Pontarlier (SFP) submitted 4 trials in NEU and 4 trials in SEU (SEU trials are the same as submitted by CTF) conducted in 2010. Even though there is no representative use on field tomato in NEU for renewal, the study submitted was evaluated to have a broader view of cymoxanil residues in tomatoes. 4 trials in NEU and 4 trials in SEU (all below LOQ) are considered sufficient to support SFP outdoor use on tomatoes in SEU and derive MRL value.

Potatoes

Crop	EU zone	Number of applications	Interval between applications (days)	Application rate per treatment (kg a.s./ha)	Growth stage at last application	PHI (days)	Applicant
Original inclusion into Annex I (DAR 2007)							
Potatoes	NEU	4	7-10	0.120	BBCH 95	7	Oxon
Potatoes	SEU	5	7	0.120	BBCH 95	7	Oxon
Potatoes	EU all zones	6-8	7-10	0.175	BBCH 95	14	DuPont
Renewal of approval							
Potatoes	EU all zones	5	5	0.15	BBCH 95	7	Cymoxanil Task Force
Potatoes	CEU	8	7-10	0.100	BBCH 95	7	SFP
Potatoes	SEU	8	7-10	0.120	BBCH 95	7	SFP
Potatoes	NEU, SEU	6	7	0.125	BBCH 95	14	Agria

Data on residues of cymoxanil in potato was submitted for the first inclusion of cymoxanil into Annex I of Council Directive 91/414/EEC and was reviewed under uniform principles.

As several parameters deviate from the GAP intended for renewal for CTF and SFP, the proportionality approach cannot be applied. Therefore these trials cannot support the GAP intended for the renewal for CTF and SFP. However, some of these "old" trials are compliant to representative GAP on potatoes for Agria, as the worst case scenario. However, as the LOQ in these studies (0.05 mg/kg) is not compatible with the current MRL for potatoes (0.01 mg/kg) and LOD is not stated, these study results could not be used in the risk assessment.

New residue trials were provided by all three applicants. Residue levels of trials complying with these GAPs are reported in the overview table.

Cymoxanil Task Force submitted 14 NEU (conducted in 2006, 2007 and 2010) and 6 SEU (conducted in 2007, 2008 and 2010) trials out of which 13 NEU and 6 SEU trials were accepted. Two trials in NEU were conducted at the locations less than 4 km distance from each other with applications made 15 days apart, these two trials are not considered independent. As all residue values are below LOQ, CTF GAP is considered supported for outdoor use on potatoes in both NEU and SEU.

Société Financière de Pontarlier submitted 9 NEU (conducted in 2009, 2010 and 2018) and 5 SEU trials (conducted in 2009, 2010 and 2018) out of which 2 NEU and 2 SEU trials were accepted. 7 trials in NEU and 3 trials in SEU were conducted at less critical GAP (PHI of 14 days instead of 7 days) and could not be used in the assessment. As SFP submitted only 2 acceptable trials in each zone, additional trials in the Northern and Southern zone of Europe are required.

Agria SA submitted 8 NEU (conducted in 2017) and 7 SEU trials (conducted in 2017 and 2018) out of which 8 NEU and 6 SEU trials were accepted. Two trials in SEU were conducted at the locations less than 1 km distance from each other with applications made on the same day, these two trials are not considered independent. As all residue values are below LOQ, Agria GAP is considered supported for outdoor use on potatoes in both NEU and SEU.

As intended GAPs on potatoes are quite different between applicants, it is not relevant to pool all data on potatoes, therefore an MRL is calculated separately for each data set.

Table 43: Overview of the available residues trials data and MRL calculation

Commodity	**Region/ Indoor** (a)	**Residue levels observed in the supervised residue trials relevant to the supported GAPs** (mg/kg)	**Comments/Source**	**Calculated MRL** (mg/kg)	**HR** (b) (mg/kg)	**STMR** (c) (mg/kg)
Residue definition for monitoring (Mo): Cymoxanil **Residue definition for risk assessment (RA): Cymoxanil**						
Grapes (CTF)	NEU (13)	2xND (<0.007), 7xND (<0.02), 3xND (<0.05), <0.05 (0.024)	MRL calculation is based on combined NEU+SEU datasets as they are similar (Mann-Whitney U-test) and GAP in NEU and SEU are the same. **Data set sufficient to derive an MRL.**	**0.2§**	<0.05	0.02
	SEU (12)	2xND (<0.007), 2xND (<0.01), ND (<0.02), 4xND (<0.03), 2x<0.05, 0.19			0.19	0.03
Grapes (SFP)	NEU (3)	3x<LOQ (<0.02)	Residue trials on grapes conducted at a more critical GAP than proposed for SFP use on grapes. MRL calculation is based on combined NEU+SEU datasets as they are similar (Mann-Whitney U-test) and GAP in NEU and SEU are the same. **Data set (reduced number of trials) is considered sufficient to derive an MRL.** (3 NEU and 3 SEU all <LOQ)	**0.02§**	<0.02	<0.02
	SEU (3)	3x<LOQ (<0.02)			<0.02	<0.02
Tomatoes (CTF)	Indoor (18)	4x<0.01, 3x<0.04 (0.01), 2x0.01, 3x<0.04 (0.02), 0.02, 0.024, 2x0.04, 0.05, 0.23	**Data set sufficient to derive an MRL.**	**0.3**	0.23	0.015
Tomatoes (CTF)	SEU (23)	4xND (<0.001), ND (<0.002), 3xND (<0.003), ND (<0.003†), 2x<0.01 (0.005†), <0.01 (0.008†), <0.01 (0.009†), 3x<0.01, 2x<0.02, 0.02, 4x<0.05	Residue trials on tomatoes compliant with or more critical† than CTF GAP. **Data set sufficient to derive an MRL.**	**0.09**	<0.05	0.009
Tomatoes (SFP)	NEU (4)	2xND(<0.002), 2x<0.02	No GAP on tomatoes in NEU was supported by SFP. However the submitted residue trials are	-	-	-

Commodity	**Region/ Indoor** (a)	**Residue levels observed in the supervised residue trials relevant to the supported GAPs** (mg/kg)	**Comments/Source**	**Calculated MRL** (mg/kg)	**HR** (b) (mg/kg)	**STMR** (c) (mg/kg)
	SEU (4)	ND(<0.002), 2x<0.02, 0.02	used for MRL calculation (same GAP in both zones, similar datasets). **Data set not sufficient to derive an MRL.**		-	-
Potatoes (CTF)	NEU (13)	11xND (<0.003), 2x<0.01	MRL calculation is based on combined NEU+SEU datasets as they are similar (Mann-Whitney U-test) and GAP in NEU and SEU are the same. **Data set sufficient to derive an MRL.**	**0.01***	<0.01	<0.01
	SEU (6)	4xND (<0.003), 2x<0.01			<0.01	<0.01
Potatoes (SFP)	NEU (2)	2xND (<0.002)	**Data set not sufficient to derive an MRL.**	-	-	-
	SEU (2)	2xND (<0.002)			-	-
Potatoes (Agria)	NEU (8)	8xND (<0.003)	MRL calculation is based on combined NEU+SEU datasets as they are similar (Mann-Whitney U-test) and GAP in NEU and SEU are the same. **Data set sufficient to derive an MRL.**	**0.01***	<0.01	<0.01
	SEU (6)	6xND (<0.003)			<0.01	<0.01

* Indicates that the MRL is proposed at the limit of quantification.

§ The current MRL for grapes is 0.3 mg/kg. In the Evaluation of confirmatory data following the Article 12 MRL review for cymoxanil (EFSA Journal 2019;17(10):5823) it is proposed that MRL for grapes should be lowered to 0.05 mg/kg. CTF submitted the MRL application form to maintain the current MRL of 0.3 mg/kg for grapes.

Mo: residue levels expressed according to the monitoring residue definition; RA: residue levels expressed according to risk assessment residue definition.

(a): NEU: Outdoor trials conducted in northern Europe, SEU: Outdoor trials conducted in southern Europe, Indoor: indoor EU trials or Country code: if non-EU trials.

(b): Highest residue. The highest residue for risk assessment (RA) refers to the whole commodity and not to the edible portion.

(c): Supervised trials median residue. The median residue for risk assessment (RA) refers to the whole commodity and not to the edible portion.

(d): Conversion factor to recalculate residues according to the residue definition for monitoring to the residue definition for risk assessment.

2.7.5 Summary of feeding studies in poultry, ruminants, pigs and fish

The representative uses of cymoxanil at renewal are on grapes, tomatoes and potatoes (CTF and SFP) or potatoes alone (Agria). Of these, only potatoes may form part of the livestock diet. The confined rotational crop study indicates that significant residues of cymoxanil in following crops are not likely to occur and thus a consideration of potential feed items in addition to potatoes is not required. A livestock dietary burden calculation was performed using the EFSA "pesticides_mrl_guidelines_animal_model_2017 (version 1)" calculator which implements "OECD Guidance Document, Series on testing and assessment No. 64 and Series on pesticides No. 32" and "OECD Guidance Document on Residues in livestock, Series on Pesticides No. 37". The input parameters and the results of the calculations are reported in the following tables. Dietary burden was calculated using STMR and HR values from residue trials submitted by CTF and Agria, residue trials submitted by SFP were not sufficient to derive MRL, STMR or HR values. Residue values (all <LOQ of 0.05 mg/kg) in studies from the DAR 2007 were not taken into account.

Table 44: Input values for the dietary burden calculation

Commodity	Median dietary burden		Maximum dietary burden	
	Input value (mg/kg)	Comment	Input value (mg/kg)	Comment
Residue definition for risk assessment in plants: cymoxanil				
Potato culls	0.01*	STMR from the residues trials at renewal	0.01*	HR from the residues trials at renewal
Potato process waste	0.01*	STMR-P (the default processing factor of 20 is modified to 1 as it is an LOQ residues situation)	0.01*	HR-P (the default processing factor of 20 is modified to 1 as it is an LOQ residues situation)
Potato dried pulp	0.01*	STMR-P (the default processing factor of 38 is modified to 1 as it is an LOQ residues situation)	0.01*	HR-P (the default processing factor of 38 is modified to 1 as it is an LOQ residues situation)

*- Residues are <LOQ (<0.01 mg/kg) and even <LOD (0.003 mg/kg) for the majority of the residues samples.

Table 45: Results of the initial dietary burden calculation

	Intake (%)	Maximum dietary burden (mg/kg bw/d)	Median dietary burden (mg/kg bw/d)	Highest contributing commodity	Max dietary burden (mg/kg DM)	Dietary burden triggered?
Residue definition for risk assessment in plants: cymoxanil						
Cattle (beef)						
Potato process waste	40	0.001	0.0012	Potato process waste	0.048	No
Potato culls	30					
Cattle (dairy)						
Potato process waste	30	0.002	0.0015	Potato process waste	0.04	No
Potato culls	30					
Sheep (ram/ewe)						

	Intake (%)	Maximum dietary burden (mg/kg bw/d)	Median dietary burden (mg/kg bw/d)	Highest contributing commodity	Max dietary burden (mg/kg DM)	Dietary burden triggered?
Potato process waste	40	0.002	0.0016	Potato process waste	0.048	No
Potato culls	30					
Sheep (lamb)						
Potato process waste	20	0.001	0.0011	Potato process waste	0.027	No
Potato culls	20					
Swine (breeding)						
Potato process waste	20	0.001	0.001	Potato process waste	0.042	No
Potato culls	50					
Swine (finishing)						
Potato culls	50	0.001	0.001	Potato culls	0.027	No
Potato dried pulp	20					
Poultry (broiler)						
Potato culls	10	0.001	0.001	Potato culls	0.01	No
Potato dried pulp	20					
Poultry (layer)						
Potato culls	10	<0.001	<0.001	Potato culls	0.01	No
Potato dried pulp	15					
Poultry (turkey)						
Potato culls	20	0.001	0.001	Potato culls	0.01	No
Fish (carp)						
Potato protein concentrate	3			Potato protein concentrate		

The calculated median and maximum dietary burdens do not exceed the trigger of 0.004 mg/kg bw/d for all livestock animals. In addition, the log P_{OW} for cymoxanil (pH 5 – 7, cymoxanil is too unstable in alkaline conditions for a log P_{OW} to be determined at alkaline pH) is 0.59 – 0.67. Therefore, cymoxanil has a low propensity for bioaccumulation. Taken together, residues of cymoxanil in commodities of animal origin are not expected and the data requirements for livestock feeding studies are not triggered.

A working document on the nature of pesticide residues in fish is elaborated (SANCO/11187/2013, Appendix J), however it has not been formally noted as a guidance document since several points need to be addressed (Standing Committees of 25-26 February 2013 and 22-23 April 2013).

Additionally, the Summary Report of the Standing Committee meeting on Plants, Animal, Food and Feed (Section Phytopharmaceuticals - Residues), 24-25 November 2014 states: 'It clarified that the Commission working document on the nature of pesticide residues in fish was discussed in 2013 and it was concluded that it is not yet finalised and ready to be noted as a guidance document. The Commission emphasised that for the time being there are no agreed test guidelines and that hence the pertinent data requirements can be waived. This was also clarified in general at the meeting of the Committee's section on Plant Protection Products - Legislation on 09/10 October

2014, and laid down in document SANCO/10181/2013 Rev 2.1. Such test guidelines must be published in the form of an update of the respective Commission Communications.'

Based on the working document on the Nature of Pesticide Residues in Fish (Annex 2 Feedstuffs Table; SANCO/11187/2013 31 January 2013 rev 3), potato protein represents a minor component of aquaculture diets (3 and 0% of carp and trout diet, respectively). Considering the proposed cGAP, there is a no-residue situation for cymoxanil in potato. Taking also into account that log P_{OW} is <3 for cymoxanil, transfer of residues into fish tissues is considered negligible. Consequently, no fish dietary burden has been calculated.

2.7.6 Summary of effects of processing

The representative use of cymoxanil on potato, grapes or tomato is not expected to result in residues of cymoxanil at or above 0.01 mg/kg for SFP and Agria GAPs. Therefore a study to investigate the effect of processing on the nature of the residue is not required for these applicants.

For Cymoxanil Task Force cymoxanil residue levels above 0.1 mg/kg were observed in two occasions in grapes and protected tomatoes (one residue at 0.19 mg/kg in grape and one residue at 0.23 mg/kg in protected tomato - commodities that may be subject to hydrolysis), therefore high temperature hydrolysis study is in principle required.

As the calculated chronic exposure is less than 10% of the ADI (only 0.9% ADI) the requirement for a high temperature hydrolysis study (OECD507) could be waived. In addition, cymoxanil degradation seems to be rather due to physical factors and not to biotic factors. In the hydrolysis studies (evaluated in Fate and behaviour section, Doc M-CA7, chapter 7.2.1.1) has been demonstrated that cymoxanil is extensively hydrolysed at pH 7-9 at 20°C and at pH 5 at 50°C. The observed metabolites were such as IN-U3204 (max 60.8% AR), IN-JX915 (max 11.0 % AR), IN-W3595 (max 41.5% AR), IN-KP533 (max 57.4 % AR), IN-R3273 (10.2% AR) and IN-KQ960 (14.1% AR). Therefore, higher temperatures are not expected to affect the nature of the hydrolysis products but only the kinetic of the reactions. Cymoxanil is expected to be hydrolysed under the conditions set in the OECD 507 (pH 4 at 90°C or pH 5 at 100°C or pH 6 at 120°C). Other metabolites, different than the ones reported above, are not expected under standard OECD 507 hydrolytic conditions. These are the same metabolites that were identified in the primary crop metabolism studies. Metabolites IN-W3595 and IN-KP533 were identified as the final products of the depicted metabolic pathway while the other identified compounds were only intermediates. Therefore, it can be assumed that metabolites IN-W3595 and IN-KP533 will form the majority of the hydrolysis residues (found at max 41.5 and 57.4 % AR in hydrolysis studies). These metabolites are considered to be incorporated into natural products such as glycine therefore cymoxanil would be the only relevant residue in processed commodities

An evaluation of the distribution of residues between inedible peel/pulp is not relevant for the intended crops, grape, tomato and potato, which is not separated in this way. For potato, which can be peeled, as no residues of cymoxanil is expected in the whole treated potato crops (lower than the LOQ (<0.01 mg/kg)), there is no need to investigate the distribution of residues between peel/pulp.

Even not triggered, magnitude of residues studies in processed grape ant tomato commodities were submitted by Cymoxanil Task Force. Residue levels are in most cases below LOD for raw and processed commodities therefore specific processing factors for processed commodities could not be calculated and proposed.

2.7.7 Summary of residues in rotational crops

The metabolism of [2-^{14}C]cymoxanil in rotational crops was investigated following one pre-plant application at 1212 g a.s./ha (1.6 N rate in the representative GAP for renewal (CTF and Agria uses) and 1.3 N rate in the representative GAP for renewal for SFP uses) on bare soil and subsequent sowing of sugar beet, lettuce and wheat 30, and 120 days later. Due to the short soil DT_{50} values (less than 10 days for parent and metabolites), consideration of a background steady state concentration on top of the seasonal rate is not required, and 270-365 days interval is not considered necessary. Immature green wheat foliage was collected 28 – 34 days after sowing. Wheat straw and grain, beet tops and roots, and lettuce heads were collected at harvest 46 – 102 days after sowing. Samples of soil were also taken prior to crop sowing and at each harvest.

The total radioactive residue (TRR) in the soil immediately after application was 1.04 mg a.s. equiv./kg mg and declined to 0.31 and 0.20 mg a.s. equiv./kg after aging for 30 and 120 days, respectively. The lettuce samples contained no significant residues (<0.01 mg a.s. equiv./kg) after either rotation. Low levels of TRR (≤0.07 mg a.s. equiv./kg) were detected in the wheat and sugar beet matrices from the 30 and 120 day plant back intervals. None of the individual components in chromatograms of extracts of the sugar beet and wheat matrices exceeded 0.02 mg a.s. equiv./kg and all components were characterised as polar molecules based upon poor retention on a reversed-phase HPLC column. No cymoxanil or known plant/photolytic/hydrolytic metabolites could be detected in any of the rotated plant commodities. The results confirm that residues of cymoxanil are not expected in succeeding crops. No metabolic pathway can be proposed.

2.7.8 Summary of other studies

Effect on the residue level in pollen and bee products

In 14 September 2018, a new guideline SANTE/11956/2016 was published. However, this guideline will not be implemented until 1 January 2020. Considering that the time (and season period) of the release of this new publication, it was not possible to consider it for this dossier.

No study was conducted specifically to measure the Cymoxanil levels in honey after application of this fungicide to crops. However, tomatoes and potatoes are considered as having no melliferous capacity and therefore, no concern is expected from the use of cymoxanil on these crops.

Concerning the uses of cymoxanil on grapes, according the supported critical gap, cymoxanil is applied as a foliar spray on grapes, BBCH 11 – 89.

Considering the following points:

- a rapid degradation of cymoxanil in environmental compound (soil and surface water) and in plant (crop and leaves);

- cymoxanil in plants and in animal matrices is rapidly degraded to glycine followed by natural incorporation over the course of a few days;

- few ecotoxicological studies on bees which have been conducted at an exaggerated dose, are showing also a rapid degradation;

the risk of having cymoxanil residue in bee product and therefore the risk of consumer exposure for the bees is considered extremely low.

This low risk of having cymoxanil residue in bee product has also been confirmed, 13 articles/publications relating to cymoxanil residues in bee products (wax, honey, pollen and bee bread) were found in literature search and submitted by applicants CTF and SFP.

None of the article was considered fully reliable. However some information on cymoxanil monitoring data in bee products could be used as supportive information. In these articles monitoring results from 12 EU MS in 2012-2013, Belgium in 2012-2013, Colombia, Brazil, Kenya in 2013-2015, Poland in 2009-2012 and 2014-2017 and USA in 2009-2012 were analysed. No cymoxanil residues were found in bee products, except several samples from Kenya and USA with residue levels not exceeding the 0.01 mg/kg in honey and pollen. No adverse data was recorded.

2.7.9 Estimation of the potential and actual exposure through diet and other sources

The ADI and ARfD for cymoxanil are detailed in toxicological section and are summarised in the table below.

The definition of residue in plant commodities is defined as the parent compound for consumer risk assessment and monitoring.

Table 46: Endpoints used for the risk assessment

Endpoint	Value (mg/kg bw/d)	Study	Safety factor
Cymoxanil			
ADI	0.013	Dog, 1-year study	100
ARfD	0.08	Rabbit, developmental	100

The consumer risk assessment was performed using revision 3.1 of the EFSA PRIMo (Pesticide Residue Intake Model). For the chronic and acute intake assessment the proposed MRL, STMR and HR derived from residue trials were considered for plant commodities.

Table 47: Input values for chronic and acute risk assessments

Commodity	Chronic risk assessment		Acute risk assessment	
	Input value (mg/kg)	Comment	Input value (mg/kg)	Comment
Residue definition for risk assessment: Cymoxanil				
EU representative uses				
Grapes	0.2	Calculated MRL	0.19	HR
Tomatoes	0.3	Calculated MRL	0.23	HR
Potatoes	0.01*	Calculated MRL	<0.01	HR

Table 48: TMDI calculation linked to EU representative uses

European Food Safety Authority

EFSA PRIMo revision 3.1; 2019/03/19

Cymoxanil

LOQs (mg/kg) range from:	0,01	to:	0,01
Toxicological reference values			
ADI (mg/kg bw/day):	0,013	ARfD (mg/kg bw):	0,08
Source of ADI:	RAR	Source of ARfD:	RAR
Year of evaluation:	2020	Year of evaluation:	2020

Input values

Details - chronic risk assessment | Supplementary results - chronic risk assessment

Details - acute risk assessment/children | Details - acute risk assessment/adults

Comments:

Normal mode

Chronic risk assessment: JMPR methodology (IEDI/TMDI)

No of diets exceeding the ADI : ---

	Calculated exposure (% of ADI)	MS Diet	Exposure (µg/kg bw per day)	Highest contributor to MS diet (in % of ADI)	Commodity / group of commodities	2nd contributor to MS diet (in % of ADI)	Commodity / group of commodities	3rd contributor to MS diet (in % of ADI)	Commodity / group of commodities	Exposure resulting from MRLs set at the LOQ (in % of ADI)	Exposure resulting from commodities not under assessment (in % of ADI)
TMDI/NEDI/IEDI calculation (based on average food consumption)	10%	GEMS/Food G06	1,32	8%	Tomatoes	2%	Table grapes	0,2%	Potatoes	0,2%	10%
	8%	RO general	0,99	4%	Tomatoes	3%	Wine grapes	0,3%	Potatoes	0,3%	8%
	7%	PT general	0,88	4%	Wine grapes	2%	Tomatoes	0,4%	Table grapes	0,4%	7%
	6%	GEMS/Food G07	0,72	2%	Tomatoes	2%	Wine grapes	0,5%	Table grapes	0,3%	6%
	5%	GEMS/Food G15	0,66	3%	Tomatoes	2%	Wine grapes	0,5%	Table grapes	0,3%	5%
	5%	GEMS/Food G08	0,66	3%	Tomatoes	2%	Wine grapes	0,5%	Table grapes	0,3%	5%
	5%	NL toddler	0,65	2%	Table grapes	2%	Tomatoes	0,3%	Potatoes	0,3%	5%
	5%	FR adult	0,63	4%	Wine grapes	1%	Tomatoes	0,2%	Table grapes	0,1%	5%
	5%	GEMS/Food G11	0,60	2%	Tomatoes	2%	Wine grapes	0,6%	Table grapes	0,3%	5%
	5%	DE child	0,60	2%	Tomatoes	2%	Table grapes	0,2%	Potatoes	0,2%	5%
	4%	GEMS/Food G10	0,58	3%	Tomatoes	0,6%	Wine grapes	0,5%	Table grapes	0,2%	4%
	4%	DE women 14-50 yr	0,46	2%	Tomatoes	1%	Wine grapes	0,5%	Table grapes	0,1%	4%
	4%	IT toddler	0,46	3%	Tomatoes	0,2%	Table grapes	0,1%	Potatoes	0,1%	4%
	3%	IE adult	0,45	2%	Wine grapes	0,9%	Tomatoes	0,4%	Table grapes	0,2%	3%
	3%	DE general	0,43	2%	Tomatoes	1%	Wine grapes	0,4%	Table grapes	0,1%	3%
	3%	NL child	0,41	2%	Table grapes	1%	Tomatoes	0,3%	Potatoes	0,3%	3%
	3%	FR child 3 15 yr	0,41	2%	Tomatoes	0,6%	Wine grapes	0,5%	Table grapes	0,1%	3%
	3%	DK adult	0,39	1%	Wine grapes	1%	Tomatoes	0,3%	Table grapes	0,1%	3%
	3%	IT adult	0,38	3%	Tomatoes	0,2%	Table grapes	0,0%	Potatoes	0,0%	3%
	3%	UK vegetarian	0,38	1%	Tomatoes	1%	Wine grapes	0,1%	Table grapes	0,1%	3%
	3%	UK adult	0,37	2%	Wine grapes	1%	Tomatoes	0,1%	Potatoes	0,1%	3%
	3%	PL general	0,36	2%	Tomatoes	0,5%	Table grapes	0,3%	Potatoes	0,3%	3%
	3%	ES adult	0,34	2%	Tomatoes	0,6%	Wine grapes	0,1%	Potatoes	0,1%	3%
	2%	ES child	0,32	2%	Tomatoes	0,1%	Potatoes	0,1%	Table grapes	0,1%	2%
	2%	NL general	0,32	1,0%	Tomatoes	0,9%	Wine grapes	0,4%	Table grapes	0,2%	2%
	2%	SE general	0,27	2%	Tomatoes	0,3%	Potatoes			0,3%	2%
	2%	UK toddler	0,27	1%	Tomatoes	0,4%	Table grapes	0,3%	Potatoes	0,3%	2%
	2%	FI 3 yr	0,26	1%	Tomatoes	0,4%	Potatoes	0,4%	Table grapes	0,4%	2%
	2%	FI adult	0,26	1%	Tomatoes	0,5%	Wine grapes	0,1%	Table grapes	0,1%	2%
	2%	LT adult	0,22	1%	Tomatoes	0,2%	Potatoes	0,0%	Table grapes	0,2%	2%
	2%	DK child	0,22	1%	Tomatoes	0,3%	Table grapes	0,2%	Potatoes	0,2%	2%
	2%	FR toddler 2 3 yr	0,21	1%	Tomatoes	0,4%	Wine grapes	0,1%	Potatoes	0,1%	2%
	2%	FI 6 yr	0,21	1%	Tomatoes	0,3%	Potatoes	0,3%	Table grapes	0,3%	2%
	1%	UK infant	0,15	0,8%	Tomatoes	0,3%	Potatoes	0,0%	Table grapes	0,3%	1%
	0,4%	FR infant	0,05	0,2%	Tomatoes	0,1%	Potatoes	0,1%	Wine grapes	0,1%	0,4%
	0,3%	IE child	0,03	0,1%	Tomatoes	0,1%	Table grapes	0,0%	Potatoes	0,0%	0,3%

Table 49: IESTI calculation linked to EU representative uses

Acute risk assessment /children	Acute risk assessment / adults / general population	Acute risk assessment /children	Acute risk assessment / adults / general population
Details - acute risk assessment /children	Details - acute risk assessment/adults	Hide IESTI new calculations	Show IESTI new calculations

The acute risk assessment is based on the ARfD.
The calculation is based on the large portion of the most critical consumer group.

IESTI new calculations:
The calculation is performed with the MRL and the peeling/processing factor (PF), taking into account the residue in the edible portion and/or the conversion factor for the residue definition (CF). For case 2a, 2b and 3 calculations a variability factor of 3 is used. Since this methodology is not based on internationally agreed principles, the results are considered as indicative only.
Since this methodology is not based on internationally agreed principles, the results are considered as indicative only.

Show results of IESTI calculation for all crops

Unprocessed commodities

Results for children
No. of commodities for which ARfD/ADI is exceeded (IESTI): ---

IESTI

Highest % of ARfD/ADI	Commodities	MRL / input for RA (mg/kg)	Exposure (µg/kg bw)
17%	Table grapes	0,2 / 0,19	14
17%	Tomatoes	0,3 / 0,23	13
2%	Wine grapes	0,2 / 0,19	1,8
2%	Potatoes	0,01 / 0,01	1,5

Expand/collapse list

Total number of commodities exceeding the ARfD/ADI in children and adult diets (IESTI calculation)

Results for adults
No. of commodities for which ARfD/ADI is exceeded (IESTI): ---

IESTI

Highest % of ARfD/ADI	Commodities	MRL / input for RA (mg/kg)	Exposure (µg/kg bw)
8%	Table grapes	0,2 / 0,19	6,4
6%	Wine grapes	0,2 / 0,19	4,5
5%	Tomatoes	0,3 / 0,23	3,6
0,4%	Potatoes	0,01 / 0,01	0,30

IESTI new
Results for children
No. of commodities for which ARfD/ADI is exceeded (IESTI new): ---

IESTI new

Highest % of ARfD/ADI	Commodities	MRL / input for RA (mg/kg)	Exposure (µg/kg bw)
11%	Tomatoes	0,3 / 0,3	9,1
11%	Table grapes	0,2 / 0,2	8,8
2%	Wine grapes	0,2 / 0,2	1,9
0,8%	Potatoes	0,01 / 0,01	0,66

Total number of commodities found exceeding the ARfD/ADI in children and adult diets (IESTI new calculation)

IESTI new
Results for adults
No. of commodities for which ARfD/ADI is exceeded (IESTI new): ---

IESTI new

Highest % of ARfD/ADI	Commodities	MRL / input for RA (mg/kg)	Exposure (µg/kg bw)
7%	Tomatoes	0,3 / 0,3	5,8
6%	Wine grapes	0,2 / 0,2	4,7
5%	Table grapes	0,2 / 0,2	4,1
0,4%	Potatoes	0,01 / 0,01	0,31

Processed commodities

Results for children
No of processed commodities for which ARfD/ADI is exceeded (IESTI): ---

IESTI

Highest % of ARfD/ADI	Processed commodities	MRL / input for RA (mg/kg)	Exposure (µg/kg bw)
11%	Wine grapes / juice	0,2 / 0,2	8,7
7%	Tomatoes / juice	0,3 / 0,3	5,7
4%	Tomatoes / sauce/puree	0,3 / 0,3	2,9
1%	Potatoes / fried	0,01 / 0,01	0,93
0,7%	Potatoes / dried (flakes)	0,01 / 0,05	0,59
#NUM!	#NUM!	#NUM!	#NUM!

Results for adults
No of processed commodities for which ARfD/ADI is exceeded (IESTI): ---

IESTI

Highest % of ARfD/ADI	Processed commodities	MRL / input for RA (mg/kg)	Exposure (µg/kg bw)
5%	Wine grapes / juice	0,2 / 0,2	4,2
3%	Tomatoes / sauce/puree	0,3 / 0,3	2,5
2%	Wine grapes / wine	0,2 / 0,19	1,8
1%	Table grapes / raisins	0,2 / 0,89	1,1
0,1%	Potatoes / chips	0,01 / 0,01	0,08
0,07%	Potatoes / dried (flakes)	0,01 / 0,05	0,06

Results for children
No of processed commodities for which ARfD/ADI is exceeded (IESTI new): ---

IESTI new

Highest % of ARfD/ADI	Processed commodities	MRL / input for RA (mg/kg)	Exposure (µg/kg bw)
11%	Wine grapes / juice	0,2 / 0,2	8,7
7%	Tomatoes / juice	0,3 / 0,3	5,7
4%	Tomatoes / sauce/puree	0,3 / 0,3	2,9
0,7%	Potatoes / dried (flakes)	0,01 / 0,05	0,59
0,5%	Potatoes / fried	0,01 / 0,01	0,44
#NUM!	#NUM!	#NUM!	#NUM!

Results for adults
No of processed commodities for which ARfD/ADI is exceeded (IESTI new): ---

IESTI new

Highest % of ARfD/ADI	Processed commodities	MRL / input for RA (mg/kg)	Exposure (µg/kg bw)
5%	Wine grapes / juice	0,2 / 0,2	4,2
3%	Tomatoes / sauce/puree	0,3 / 0,3	2,5
2%	Wine grapes / wine	0,2 / 0,2	1,9
1%	Table grapes / raisins	0,2 / 0,94	1,2
0,1%	Potatoes / chips	0,01 / 0,01	0,08
0,07%	Potatoes / dried (flakes)	0,01 / 0,05	0,06

2.7.10 Proposed MRLs and compliance with existing MRLs

Considering available residue trials, MRLs related to EU intended uses can be proposed and are summarized below.

Table 50: Proposed MRL for EU representative uses

Commodity code	Commodity	Proposed EU MRL (mg/kg)	Existing EU MRL (mg/kg)	Comment
0151000	Grapes	0.2	0.3	The submitted data do not provide evidence that the existing MRL has to be modified. However, in the Evaluation of confirmatory data following the Article 12 MRL review for cymoxanil (EFSA Journal 2019;17(10):5823) it is proposed that MRL for grapes should be lowered to 0.05 mg/kg. CTF submitted the MRL application form to maintain the current MRL of 0.3 mg/kg for grapes.
0231010	Tomatoes	0.3	0.4	The submitted data do not provide evidence that the existing MRL has to be modified.
0211000	Potatoes	0.01*	0.01*	The submitted data do not provide evidence that the existing MRL has to be modified

2.7.11 Proposed import tolerances and compliance with existing import tolerances

Not relevant.

2.8 FATE AND BEHAVIOUR IN THE ENVIRONMENT

The environmental fate and behaviour of cymoxanil was reviewed in the EU evaluation as laid down in the 2008 peer review (EFSA Scientific Report (20008) 167). Since then new environmental studies were conducted to support registrations at EU Member State level. The dossier supporting the approval renewal of cymoxanil will include some of these additional studies. In addition new studies (a limited scope soil photolysis study with cymoxanil, aerobic soil degradation and adsorption desorption batch equilibrium studies with metabolites IN-U3204, IN-W3595, IN-T4226, IN-JX915, IN-R3273, IN-KQ960 and kinetic modelling assessment of laboratory aerobic soils are provided by the current EU review along the existing studies (aerobic soil and adsorption/desorption studies on parent and metabolites).

In aerobic soil cymoxanil degrades rapidly to form a number of very transient degradates which ultimately results in significant, mineralisation to carbon dioxide with up to 66% being produced.

During the laboratory studies with cymoxanil four metabolites were observed in amounts >10% of applied: IN-KQ960, IN-U3204, IN-W3595 and IN-T4226. Three additional metabolites IN-R3273, IN-JX195 and IN-18474 (oxamic acid) were observed as a minor metabolite in aerobic soils studies, exceeding 5% at one or more time points. Laboratory degradation studies have been conducted for six of these metabolites: IN-KQ960, IN-U3204, IN-W3595, IN-JX195, IN-T4226 and IN-R3273. Two additional minor metabolites IN-KP533 and oxalic acid were detected in laboratory soil studies but did not exceed 5% AR at more than one time point.

2.8.1 Summary of fate and behaviour in soil

The route of degradation of cymoxanil in soil under aerobic conditions in the laboratory was evaluated during the previous Annex I inclusion in the DAR (2008). All studies were accepted by the European Commission during previous EU reviews and are now out of date. Four main studies, Aikens (1998), Major (1993), Boucher (1993) and Anderson (2001) were reported and reviewed at the time of submission. These studies did not showed significant technical deficiencies and were included in the new submission. To fulfil data requirements two new aerobic soil degradation studies by Tan (2009) and Clark (2015) on cymoxanil were carried out by TF.

The original soil photolysis study Willems (1998) was considered to have some deficiencies, the key factor was used TLC method which is not valid to separate several metabolites from parent. The study of Willems (1998) was considered not to be acceptable in the DAR. A new soil photolysis studies by Copper (2017) and Oddy (2016) are presented by TF.

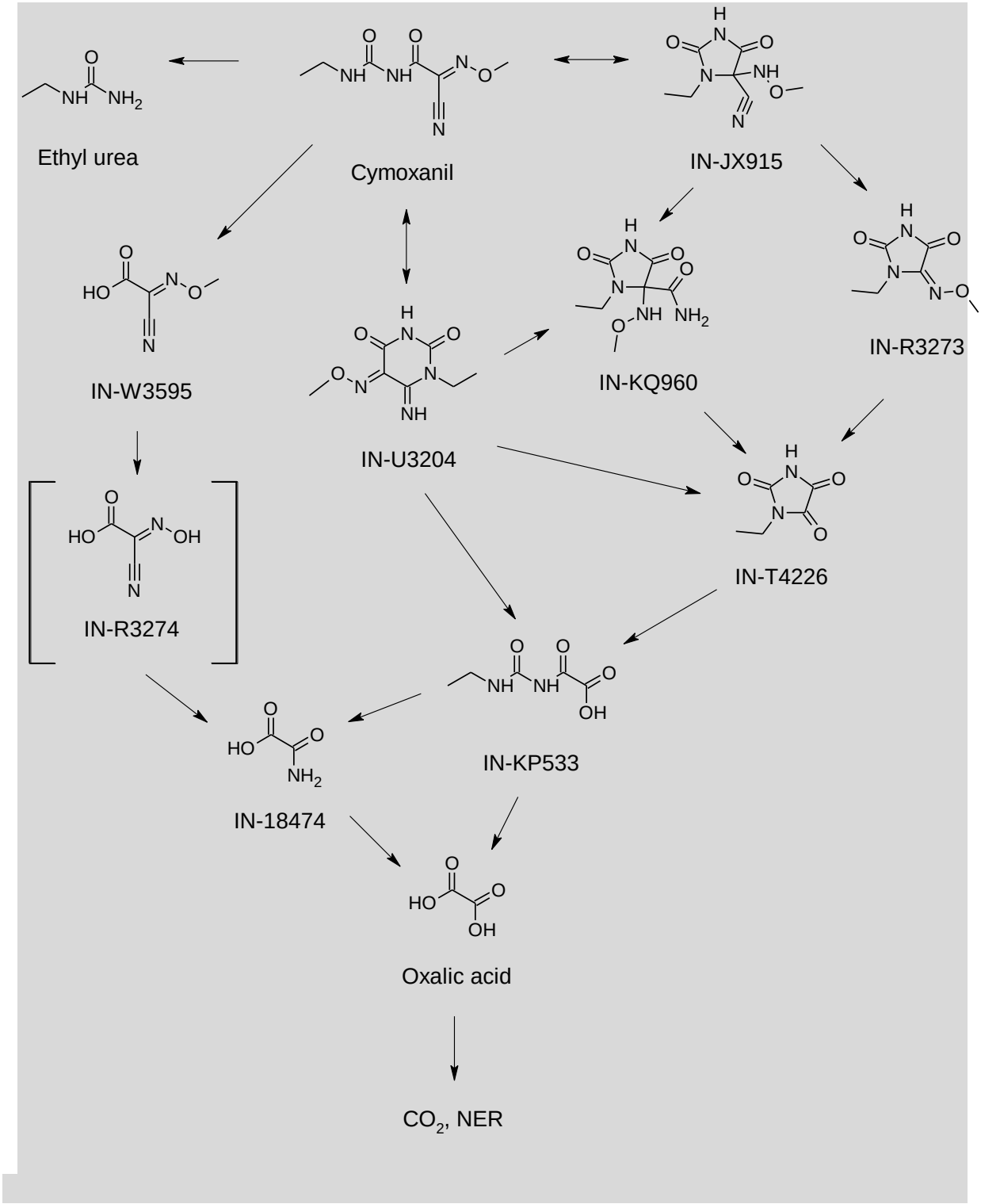
Route of degradation in soil

Aerobic degradation of cymoxanil in soil under aerobic dark conditions at 20ºC and 25ºC was investigated with [^{14}C] labelled compound in 6 different soils in 6 studies: two European soils (20ºC, 40-50% MWHC), three US soils (20-25ºC, 75% of 1/3 bar FC, 0.1 bar) and a Japanese Black Andosol (25ºC, 50% MWHC). The six soils covered a range of pH (5.7 - 7.7), clay content (8.8 – 38.0%) and organic carbon content (0.5 – 3.7%). The aerobic degradation of cymoxanil resulted in formation of four metabolites in amounts > 10% of applied: IN-KQ960, IN-U3204, IN-W3595 and IN-T4226. Three additional metabolites IN-R3273, IN-JX195 and IN-18474 (oxamic acid) which were observed as a minor metabolite, exceeding 5% at one or more timepoints. Laboratory degradation studies have been conducted for six of these metabolites; IN-KQ960, IN-U3204, IN-W3595, IN-JX195, IN-T4226 and IN-R3273. Minor metabolites IN-KP533 and oxalic acid were detected in laboratory soil studies but did not exceed 5% AR. Degradation pathway on cymoxanil and metabolites in aerobic soil is presented below.

The potential for photolytic breakdown of labelled cymoxanil at the soil surface was carried out is moist and dry loam soil under artificial light. Under dry soil conditions metabolite IN-JX915 in the irradiated soil reached 10.9 %. In dark control samples metabolite IN-Q8761 was measured at concentration for 6.9 %.

Studies investigating the anaerobic degradation of cymoxanil were not submitted for the approval since cymoxanil extensively degrades under aerobic conditions. It is unlikely to persist long enough to move into the saturated zone even under local or partial anaerobic conditions.

Proposed degradation pathway of cymoxanil in aerobic soil



Rate of degradation in soil, laboratory studies

Aerobic degradation of cymoxanil was investigated in four studies at 20ºC and two studies at 10 ºC. Two new aerobic degradation studies of cymoxanil were carried out by TF.

In a degradation rate study of cymoxanil in one soil (silty clay loam) at 10 ºC the non - extracted residue were a significant sink (maximum 55.2 % of AR at day 5), while the mineralisation accounted for 38.6 % of AR at the end of the incubation (on the day 7 after the study initiation). In another degradation rate study in one soil (silty clay loam) at 10 ºC the non - extracted residue were a significant sink (maximum 66.9 % of AR at day 9), while the mineralisation accounted for 56.9 % of AR at the end of the incubation (on the day 58 after the study initiation).

Under aerobic conditions the persistence endpints were in the range of 0.094 – 7.29 days at 20ºC and 0.197 – 2.95 days at 25ºC. After normalisation to FOCUS reference conditions (20ºC and pF2) the geometric mean of the DT_{50} values calculated for cymoxanil is 0.822 days.

Unextracted bound residues accounted for between 17.2 to 53.0% of applied cymoxanil by the end of the studies of bound residues. The majority of the bound radioactivity was recovered in the humin and fulvic acid fraction. Mineralization accounted for up to 66% AR at the end of the study. Two further degradation studies under lower temperatures (10ºC) were conducted with the same soil as used in aerobic degradation study at 20ºC.

In aerobic soil cymoxanil degrades rapidly to form a number of very transient degradates which ultimately results in significant, mineralisation to carbon dioxide with up to 66% being produced. Unextracted bound residues accounted for between 17.2 to 53.0% of applied cymoxanil by the end of the studies. Two additional minor metabolites IN-KP533 and oxalic acid were detected in laboratory soil studies but did not exceed 5% AR at more than one timepoint and were not considered further see Table 2.8.1-1.

Table 51: Summary of the maximum values of soil metabolites formed from cymoxanil

Metabolite	**% Applied radioactivity**	
	Aerobic	**Soil Photolysis**
IN-KQ960	12.0	ND
IN-W3595	18.1	2.3
IN-R3273	7.6[2]	ND
IN-T4226	11.7	1.2
IN-JX915	8.2	10.9
IN-U3204	24.7	8.3
IN-R3274	7.2 seen on a single occasion in one study	ND
IN-18474 Oxamic acid[1]	7.6	4.4
IN-KP533	2.7	ND
Oxalic acid[1]	<2.6	ND
N-ethyl urea[1]	69.8 seen in the metabolite study of IN-T4226	ND

Table 52: K_{OC} and 1/n (Freundlich exponent) adsorption values of IN-KQ960 in different soils

Soil	**Texture**	**OC [%]**	**K_f**	**K_{OC}**	**1/n**
Gross-Umstadt	loam	1.1	0.0357	3.23	0.827
Drummer	clay loam	3.1	0.1747	5.56	0.840
Lleida	clay loam	1.2	0.1067	8.99	0.960
Nambsheim	sandy loam	1.6	0.0459	2.82	0.850
Sassafras	sandy loam	0.8	0.0178	2.36	1.066
Arithmetic mean				**4.59**	**0.909**
Geometric mean				**4.04**	

Table 53: K_{OC} and 1/n (Freundlich exponent) adsorption values of IN-W3595 in different soils

Soil	**Texture**	**OC [%]**	**K_f**	**K_{OC}**	**1/n**
RefeSol 02-A	silt loam	1.06	0.03	2.3	0.859
Speyer 2.2	loamy sand	1.42	0.05	3.5	0.885
Speyer 2.4	loam	2.11	0.03	1.5	0.917

Arithmetic mean		**2.4**	**0.887**
Geometric mean		**2.3**	

Table 54: K_{OC} and 1/n (Freundlich exponent) adsorption values of IN-R3273 in different soils

Soil	Texture	OC [%]	K_f	K_{OC}	1/n
RefeSol 02-A	silt loam	1.06	0.23	21.8	0.940
Speyer 2.2	loam*y* sand	1.42	0.21	14.6	0.908
Speyer 2.4	loam	2.11	0.17	8.1	0.673
Arithmetic mean				**14.8**	**0.840**
Geometric mean				**13.7**	

Table 55: K_{OC} and 1/n (Freundlich exponent) adsorption values of IN-T4226 in different soils

Soil	Texture	OC [%]	K_f	K_{OC}	1/n
RefeSol 02-A	silt loam	0.92	0.001	0.1	0.62
Speyer 2.2	sandy loam	1.61	0.011	0.7	0.91
Speyer 2.4	loam	1.99	0.059	3.0	1.30
Arithmetic mean				**1.2**	**0.94**
Geometric mean				**0.59**	

Table 56: K_{OC} and 1/n (Freundlich exponent) adsorption values of IN-U3204 in different soils

Soil	Texture	OC [%]	K_f	K_{OC}	1/n
RefeSol 02-A	silt loam	0.92	0.127	14	1.12
Speyer 2.2	sandy loam	1.61	0.021	1.3	0.92
Speyer 2.4	loam	1.99	0.016	0.8	0.90
Arithmetic mean				**5.4**	**0.98**
Geometric mean				**2.4**	

Table 57: K_{OC} and 1/n (Freundlich exponent) adsorption values of IN-JX915 in different soils

Soil	Texture	OC [%]	K_f	K_{OC}	1/n
RefeSol 02-A	silt loam	0.92	0.023	2.47	0.986
LUFA 2.2	sandy loam	1.61	0.040	2.46	1.033
LUFA 2.4	loam	1.99	0.026	1.29	0.872
Arithmetic mean				**2.08**	**0.964**
Geometric mean				**1.99**	

Rate of degradation in soil, field studies

As cymoxanil does not trigger the need of field data, no new studies have been performed. DT50 of cymoxanil is 0.822 days at 20 ºC and 25 ºC and is very much shorter than 60 days under laboratory conditions.

Assessment in relation to the P-criteria

The criteria for persistence in soil as stated in Annex II to Regulation (EC) 1107/2009 are DT50 120 days (PBT) and 180 days (POP and vPvB). All results for cymoxanil are considerably below these criteria.

Adsorption to soil

New laboratory batch adsorption studies have been performed. Studies evaluated in original DAR were used and were considered appropriate. The adsorption of cymoxanil and its major metabolites IN-U3204, IN-KQ960, IN-T4226, INW3595 and IN-KP533 were determined in four European soils. The worst case K_{FOC} values were chosen to be used in the risk assessment in accordance with the conclusions laid out in the Draft Assessment Report.

Resulting K_{FOC} values of cymoxanil were in a range of 15.1 to 87.1 mL/g with the geometric mean of 19.6 L kg^{-1}. Arithmetic mean 1/n value was 0.873. No impact of soil pH on the adsorption was found.

The mobility of metabolite IN-KQ960 was investigated in two American and three European soils. Sorption of IN-KQ960 is pH independent. Metabolite IN-KQ960 may be considered to be mobile in soil with K_{FOC} range from 2.36 to 8.99 mL/g.

The mobility of metabolite IN-R3273 was investigated in three European soils. Sorption of IN-R3273 is pH independent. IN-R3273 may be considered to be mobile in soil with K_{FOC} range from 8.1 to 21.8 mL/g.

The mobility of metabolite IN-T4226 was investigated in three European soils. Sorption of IN-T4226 is pH independent. Metabolite IN-T4226 may be considered as very mobile in soil with K_{FOC} range from 0.1 to 3.0 mL/g.

The mobility of metabolite IN-U3204 was investigated in three European soils. Sorption of metabolite IN-U3204 is pH independent. Metabolite IN-U3204 may be considered as very mobile in soil.

The mobility of metabolite IN-JX915 was investigated in three European soils. Sorption of metabolite IN-JX915 is pH independent. IN-JX915 may be considered to be very mobile in soil.

The mobility of metabolite of IN-W3595 was investigated in in three European soils. Sorption of IN-W3595 is pH independent. IN-W3595 may be to be mobile with the K_{FOC} range from 1.5 to 3.5 mL/g.

Mobility in soil

The adsorption/desorption characteristics of cymoxanil was determined in standard batch equilibrium experiments. No correlation with soil pH was observed.

Table 58: K_{OC} and 1/n (Freundlich exponent) adsorption values of cymoxanil in different soils

Soil	Texture	OC [%]	K_f	K_{OC}	1/n
Speyer 2.2	sand	0.59	0.090	15.1	0.882
Midwest 1	sandy loam	1.0	0.910	87.1	0.871
Cranfield 115	clay loam	1.6	0.462	28.9	0.806
Cranfield 164	silt loam	2.0	0.856	43.4	0.867
Cranfield 164	silty clay loam	3.70	0.63	16.97	0.85
Speyer 2.2	loamy sand	2.36	0.50	23.0	0.78
Speyer 2.3	sandy loam	1.02	0.33	33.66	0.77
Speyer 6S	clay	1.89	0.77	43.96	0.91
ACE08-270A	sandy loam	0.5	0.0135	2.70	0.924
ACE08-270B	sandy loam	1.9	0.1020	5.37	0.957
QBR231008A	sandy clay loam	1.7	0.2392	14.07	0.924
QBR101108A	clay	4.1	0.6668	16.26	0.930
Arithmetic mean				**27.57**	**0.873**
Geometric mean				**19.59**	

Table 59: K_{OC} and 1/n (Freundlich exponent) adsorption values of IN-KQ960 in different soils

Soil	Texture	OC [%]	K_f	K_{OC}	1/n
Gross-Umstadt	loam	1.1	0.0357	3.23	0.827
Drummer	clay loam	3.1	0.1747	5.56	0.840
Lleida	clay loam	1.2	0.1067	8.99	0.960

Nambsheim	sandy loam	1.6	0.0459	2.82	0.850
Sassafras	sandy loam	0.8	0.0178	2.36	1.066
		Arithmetic mean		**4.59**	**0.909**
		Geometric mean		**4.04**	

Table 60: K_{OC} and 1/n (Freundlich exponent) adsorption values of IN-W3595 in different soils

Soil	**Texture**	**OC [%]**	**K_f**	**K_{OC}**	**1/n**
RefeSol 02-A	silt loam	1.06	0.03	2.3	0.859
Speyer 2.2	loam*y* sand	1.42	0.05	3.5	0.885
Speyer 2.4	loam	2.11	0.03	1.5	0.917
		Arithmetic mean		**2.4**	**0.887**
		Geometric mean		**2.3**	

Table 61: K_{OC} and 1/n (Freundlich exponent) adsorption values of IN-R3273 in different soils

Soil	**Texture**	**OC [%]**	**K_f**	**K_{OC}**	**1/n**
RefeSol 02-A	silt loam	1.06	0.23	21.8	0.940
Speyer 2.2	loam*y* sand	1.42	0.21	14.6	0.908
Speyer 2.4	loam	2.11	0.17	8.1	0.673
		Arithmetic mean		**14.8**	**0.840**
		Geometric mean		**13.7**	

Table 62: K_{OC} and 1/n (Freundlich exponent) adsorption values of IN-T4226 in different soils

Soil	**Texture**	**OC [%]**	**K_f**	**K_{OC}**	**1/n**
RefeSol 02-A	silt loam	0.92	0.001	0.1	0.62
Speyer 2.2	sandy loam	1.61	0.011	0.7	0.91
Speyer 2.4	loam	1.99	0.059	3.0	1.30
		Arithmetic mean		**1.2**	**0.94**
		Geometric mean		**0.59**	

Table 63: K_{OC} and 1/n (Freundlich exponent) adsorption values of IN-U3204 in different soils

Soil	**Texture**	**OC [%]**	**K_f**	**K_{OC}**	**1/n**
RefeSol 02-A	silt loam	0.92	0.127	14	1.12
Speyer 2.2	sandy loam	1.61	0.021	1.3	0.92
Speyer 2.4	loam	1.99	0.016	0.8	0.90
		Arithmetic mean		**5.4**	**0.98**
		Geometric mean		**2.4**	

Table 64: K_{OC} and 1/n (Freundlich exponent) adsorption values of IN-JX915 in different soils

Soil	Texture	OC [%]	K_f	K_{OC}	1/n
RefeSol 02-A	silt loam	0.92	0.023	2.47	0.986
LUFA 2.2	sandy loam	1.61	0.040	2.46	1.033
LUFA 2.4	loam	1.99	0.026	1.29	0.872
Arithmetic mean				**2.08**	**0.964**
Geometric mean				**1.99**	

Lysimeter studies

One field leaching study was evaluated in the original DAR. No additional studies were submitted for the renewal. Results are considered as appropriate for the risk assessment. The study was carried out in Germany, using lysimeter with tubes at 0-10 cm and 10-20 cm. The cymoxanil was applied in June 1993, 6 July 1993 and 14 July 1993 at 320 g a.s./ha (960 g a.s./ha in total) to potatoes growing at loamy sand soil (pH 5.4, OC 1.29) at Germany. Cymoxanil was not detected in any of the leachate sample (LOQ 0.01 µg/L. Metabolites IN-U3204, IN-T4226, IN-R3237, IN-18474 and oxalic acid were never seen above the detection limits of 0.05 µg/L.

2.8.2 Summary of fate and behaviour in water and sediment [equivalent to section 11.1 of the CLH report template]

Photolysis

The photolytic degradation of cymoxanil in water has been investigated under sterile conditions in acetate buffer solutions at pH 5 for up to 30 days. Cymoxanil degraded rapidly under photolytic conditions at pH 5. Two major degradates were seen at >10% IN-JX915 and IN-R3273, plus a further two metabolites IN-KP533 and IN-T4226 were seen at 7.9 and 6.7 % AR respectively, several additional minor components were also seen. The DT_{50} for cymoxanil under continuous irradiation was 1.8 days.

Under the impact of irradiation, degradation of cymoxanil owing to photolysis is strongly driven by formation of the cyclisation metabolite IN-JX915 (five-member ring system, maximum occurrence 52.6 % of AR), which rapidly further degrades to IN-R3273 (maximum occurrence 35.4 % of AR). No other major metabolites were observed. This pathway is clearly the major degradation route of cymoxanil in acidic solutions exposed to irradiation. The alternative hydrolysis processes (cyclisation to IN-U3204 and cleavage of the parent to form IN-W3595) were almost negligible at the investigated pH value. In the dark control samples almost no degradation of cymoxanil was observed.

Net photolysis half-life time of cymoxanil in sterile buffer solution at pH 5 was calculated to be 1.7 and 3.0 days (DuPont and Oxon study, respectively). The experimental net photolysis of cymoxanil ranged between 4.3 and 12.1 days under environmental conditions (midsummer day, approx. 40 °N). As demonstrated in one additional experiment, conducted in non-sterile pond water at pH 7.0, the impact of irradiation on the overall dissipation of cymoxanil in aquatic ecosystems loses its significance at neutral and alkaline conditions owing to the extensive abiotic hydrolysis of cymoxanil at higher pH values. Quantum yield (Φ) of cymoxanil was calculated in two studies to range between 0.0052 and 0.00058.

The DT_{50} of IN-JX915, owing to the influence of photolysis and hydrolysis, was calculated to be approx. 6.6 days at the investigated pH of 5.0. However, owing to the highly transient character of IN-JX915 during hydrolysis under neutral and alkaline conditions (hydrolysis DT_{50} < 2 days) it is expected that levels of photolytically formed IN-JX915 will be significantly lower in aquatic systems under environmental conditions (without considering biotic degradation).

Degradation half-life of IN-R3273 at pH 5.0, owing to the influence of photolysis and hydrolysis, was calculated to be 32.7 days, no reliable half-life time could be calculated for IN-R3273. Further minor photolysis products (< 10 % of AR) were IN-T4226 and IN-KP533 which derive from the degradation of IN-JX915 and IN-R3273.

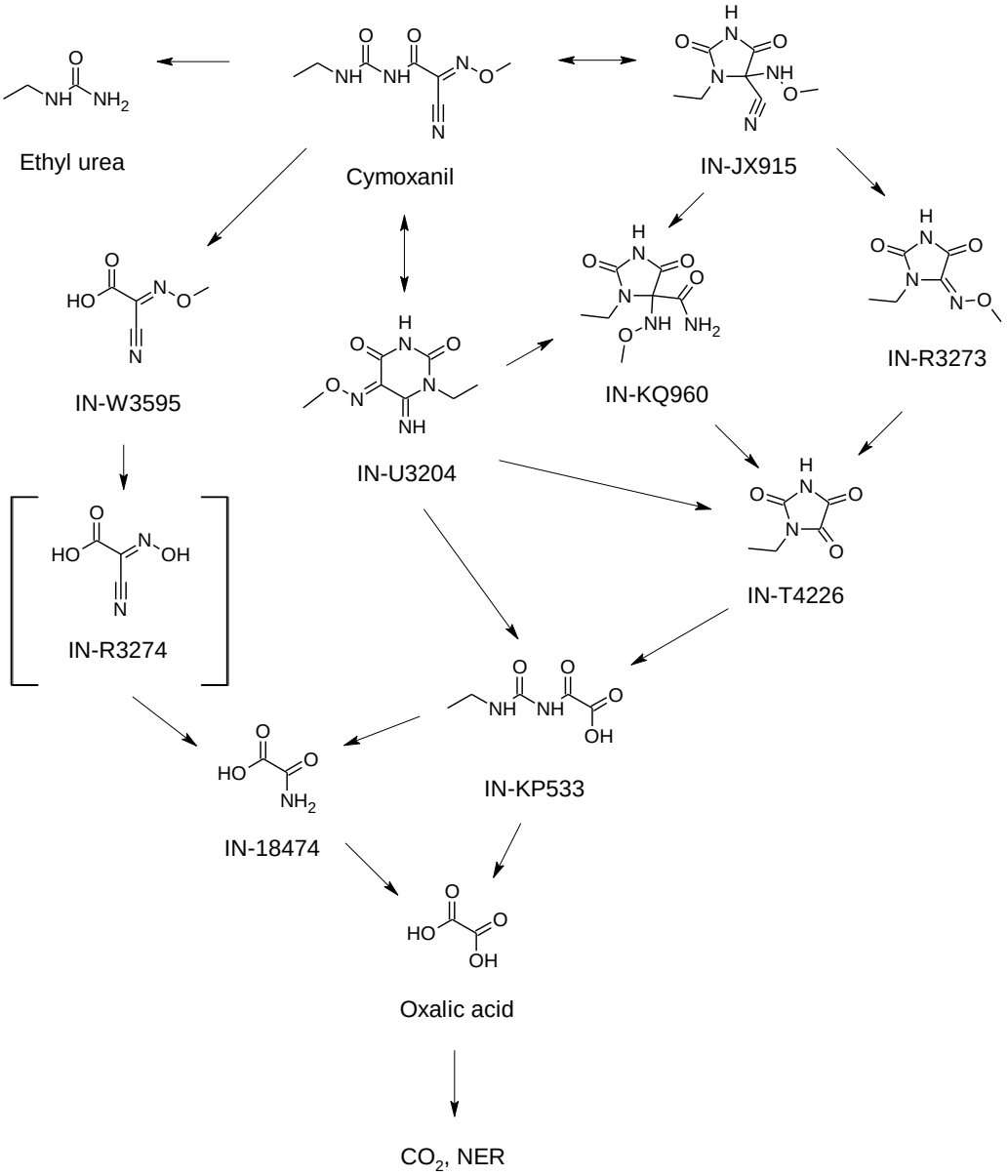
Biological degradation

In an aerobic mineralisation study (Irmer, 2019) the fate of cymoxanil was investigated in natural water at pH 8.2. The mineralisation of cymoxanil to CO_2 was observed in amounts up to 24.1 % AR and 29.0 % AR for the low and high test concentration, respectively. Degradation was very rapid. The DT_{50} values for cymoxanil were 2.52 hours (9.4 µg/L) and 2.42 hours (96.0 µg/L). The DT_{90} values were 10.8 hours (9.4 µg/L) and 99.1 hours (96.0 µg/L). Six major metabolites were detected with maximum values of 25.8 % AR (IN-KP533), 30.3 % AR (IN-W3595), 34.3 % AR (IN-U3204), 15.0 % AR (IN-R3274), 21.5 % AR (IN-KQ960) and 7.3 % AR (IN-R3273), respectively. Two major metabolites (M125 and M103) could not be identified. The metabolite M125 was very transient and its instability did not allow identification. M103 was sufficiently stable but it is very high polarity indicated a very small molecular structure. Due to this, it was not possible to identify M103 but it is assumed that it consists of oxalic acid.

In four aerobic water / sediment systems (pH of water phase 6.7 to 8.9) cymoxanil dissipated from the water phase to the sediment very rapidly. Once deposited in the sediment, parent continued to degrade very rapidly ultimately resulting in the evolution of significant quantities of radioactivity, evolved as $^{14}CO_2$. The maximum level of cymoxanil in the sediment phase never reached above 5% in any the studies. Seven transformation products (≥ 5% AR) were observed in the water phase, IN-W3595, IN-KP533, IN-U3204, IN-KQ960, AS999 (M5), IN-T4226 and IN-JX915. Only very minor transformation products in the sediment were detected which reached maximum concentrations of < 5.0% AR.

The proposed degradation pathway of cymoxanil in aquatic systems is shown below.

Proposed degradation pathway of cymoxanil in aquatic systems



Maximal aquatic metabolite levels selected for PECsw evaluations are summarised in the following Table 65.

Table 65: Maximum aquatic metabolite levels

Metabolite	**Maximum % AR**					Comment
	Hydrolysis [pH7]	**Photolysis**	**Water sediment total system**	**Aerobic mineralisation**	**Maximum % for PECsw**	
IN-U3204	52.7	0.56	25.4	34.3	25.4	Water phase of water sediment
IN-W3595	16.2	7.92	24.6	30.3	24.6	Water phase of water sediment
IN-JX915	5.0	52.6	10.1	-	52.6	Aqueous photolysis
IN-KQ960	9.0	-	14.3	21.5	14.3	Water phase of water sediment
IN-T4226	5.4	6.7	12	3.7	12	Water phase of water sediment

IN-R3273	10.2	35.4	5.8	5.9	35.4	Aqueous photolysis
IN-R3274	-	-	-	15	15	Aerobic mineralisation
IN-KP533	57.0	7.92	21.6	25.8	21.6	Water phase of water sediment
AS999 (M5)	-	-	29.2	-	29.2	Water phase of water sediment

Aerobic mineralisation in surface water

A surface water mineralisation study of Irmer (2019) has been conducted to meet the new data requirement laid out in Commission Regulation (EU) No 284/2013. Two concentrations (nominal rates of 9.4 and 96 µg/L) were tested in a natural water system. Both systems were incubated under aerobic conditions at 20°C for up to 61 days. Degradation was very rapid; The DT_{50} values for [acetyl-2-^{14}C]cymoxanil were 2.52 hours (9.4 µg/L) and 2.42 hours (96.0 µg/L). The DT_{90} values were 10.8 hours (9.4 µg/L) and 99.1 hours (96.0 µg/L). Cymoxanil mineralisation to CO_2 was high (exceeded 29.0 % of AR in the end of the study), no other volatiles were detected 0 % of AR. Calculated DT_{50} for cymoxanil in surface water were 0.09-0.15 days in non steril system.

Water/sediment studies

Aerobic water/sediment studies were conducted in five aerobic/water systems (pH of water phase 6.7 to 8.9) cymoxanil dissipated from the water phase to the sediment very rapidly.

Seven metabolites (>5 % AR) were observed in water phase: IN-W3595, IN-KP533, IN-U3204, IN-KQ960, AS999 (M5), IN-JX915 and IN-T4226. The maximum level of cymoxanil in the sediment phase never reached above 5 % in any studies. No metabolite was observed < 5 % AR in the sediment phases of all test systems investigated.

Degradation of cymoxanil in the whole system was fast with DT50 values in a range of 0.056-1.6 days following SFO kinetics with a geometric mean of 0.268. Since transfer of cymoxanil into sediment layer was negligible, dissipation in the water layer is almost consistent to degradation in the entire system.

Based on the entire system, the following metabolites are considered major (> 10 % of AR): IN-U3204 (maximum occurrence 25.4 % of AR by 1 DAT), IN-W3595 (24.6 % of AR by 4 DAT), IN-KQ960 (14.25 % of AR by 10 DAT), IN-JX915 (11.5 % of AR by 2 DAT), IN-KP533 (21.6 % of AR by 8 DAT), IN-T4226 (12.0 % of AR by 3 DAT), M5/ASS999 (29.2 % of AR by 3 DAT).

None of the observed metabolites in the water/sediment studies was persistent. Whole system half-lives for use as modelling endpoints are summarised in Table 8.2.2-2 for cymoxanil and its metabolites along with water dissipation half-lives for cymoxanil.

The proposed EU endpoints are shown in Table 66.

Table 66: Proposed EU endpoints relevant to the Predicted Environmental Concentration (PEC) in surface water of cymoxanil and its metabolites at Step 1, 2, 3 and 4

End-point	**Cymoxanil**	**IN-KQ960**	**IN-U3204**	**IN-W3595**	**IN-T4226**	**IN-JX915**	**IN-R3273**	**IN-R3274**	**IN-KP533**	**ASS99 (M5)**
DT_{50} water [days]	0.268[a]	160[b]	0.794[c]	5.48[d]	3.03[e]	2.45[f]	7.62[g]	10.3[h]	6.52[i]	63.6[j]
DT_{50} sediment [days]	0.268[a]	160[b]	0.794[c]	5.48[d]	3.03[e]	2.45[f]	7.62[g]	10.3[h]	6.52[i]	63.6[j]
DT_{50} whole system [days]	0.268[a]	160[b]	0.794[c]	5.48[d]	3.03[e]	2.45[f]	7.62[g]	10.3[h]	6.52[i]	63.6[j]

[a)] studies in the dark (worst-case) total system geometric mean DT_{50} (n=8); for details see **Document M-CA Section 7 Supplement, CA 7.2.2**

b) studies in the dark (worst-case) total system geometric mean DT_{50} (n=4); for details see **Document M-CA Section 7 Supplement, CA 7.2.2**
c) studies in the dark (worst-case) total system geometric mean DT_{50} (n=5); for details see **Document M-CA Section 7 Supplement, CA 7.2.2**
d) studies in the dark (worst-case) total system geometric mean DT_{50} (n=5); for details see **Document M-CA Section 7 Supplement, CA 7.2.2**
e) studies in the dark (worst-case) total system geometric mean DT_{50} (n=6); for details see **Document M-CA Section 7 Supplement, CA 7.2.2**
f) studies in the dark (worst-case) total system geometric mean DT_{50} (n=6); for details see **Document M-CA Section 7 Supplement, CA 7.2.2**
g) studies in the dark (worst-case) total system geometric mean DT_{50} (n=6); for details see **Document M-CA Section 7 Supplement, CA 7.2.2**
h) studies in the dark (worst-case) total system geometric mean DT_{50} (n=2); for details see **Document M-CA Section 7 Supplement, CA 7.2.2**
i) studies in the dark (worst-case) total system geometric mean DT_{50} (n=6); for details see **Document M-CA Section 7 Supplement, CA 7.2.2**
j) studies in the dark (worst-case) total system geometric mean DT_{50} (n=4); for details see **Document M-CA Section 7 Supplement, CA 7.2.2**

Table 67: Summary of modelling endpoints

Compartment	**System Name**	**Kinetic level**	**Kinetic Model**	**Calculated $DegT_{50}$ (days)**
Water column	Brandywine Creek	P-I: system $DissT_{50}$	SFO	0.5
	Lums Pond		SFO	1.5
	Oostvaardersplasssen (OVP)		SFO	0.056
	Schoonrewoerdseweil (SW) Slangen & Willems (2000)		SFO	0.148
	Kolkven (KV)		SFO	0.4
	Schoonrewoerdseweil (SW) Tan & Brands (2009)		SFO	0.2
	Goose River		SFO	0.104
	Chula		SFO	0.52
			Geometric mean	**0.268**
Sediment	Brandywine Creek	P-I: system $DegT_{50}$	SFO	0.5
	Lums Pond		SFO	1.5
	Oostvaardersplasssen (OVP)		SFO	0.056
	Schoonrewoerdseweil (SW) Slangen & Willems (2000)		SFO	0.148
	Kolkven (KV)		SFO	0.4
	Schoonrewoerdseweil (SW) Tan & Brands (2009)		SFO	0.2
	Goose River		SFO	0.104
	Chula		SFO	0.5
			Geometric mean	**0.268**
Total system	Brandywine Creek	P-I: system $DegT_{50}$	SFO	0.5
	Lums Pond		SFO	1.5
	Oostvaardersplasssen (OVP)		SFO	0.056
	Schoonrewoerdseweil (SW) Slangen & Willems (2000)		SFO	0.148
	Kolkven (KV)		SFO	0.4
	Schoonrewoerdseweil (SW)		SFO	0.2

	Tan & Brands (2009)			
	Goose River		SFO	0.104
	Chula		SFO	0.52
			Geometric mean	**0.268**

Ready biodegradability

The following study was evaluated in the original DAR of cymoxanil in 2008 (Luit, R., J. 2000). Study was performed to determine the biodegradability of cymoxanil (purity 98.8 %) in a carbon dioxide evolution test and in an activated sludge test. Biodegradation of the test substance was < 20 % after 28 days.

Two new biodegradability studies were presented (Desmares-Koopmans, M. J.E., 2008 and Feil, N. 2009). In Desmares-Koopmans (2008) study biodegradation of the test substance was < 20 % after 28 days. In Feil (2009) study the percentage biodegradation of the test substance after 28 days in consideration of nitrification was 23% ($ThOD_{NO3}$). In respect to studies results, cymoxanil is classified as not readily biodegradable.

Assessment in relation to the P-criteria

Following criteria for persistence in water and sediment are stated in Annex II to Regulation (EC) 1107/2009:

- DT_{50} in water: POP – 60 days, PBT – 40 days (fresh) and 60 days (marine), vPvB – 60 days (all water)
- DT_{50} in sediment: POP – 180 days, PBT – 120 days (fresh) and 180 days (marine), vPvB – 180 days (all sediment)
-

Data on fate and behaviour of cymoxanil in marine water is not available.

2.8.2.1 Rapid degradability of organic substances

Table 68: Summary of relevant information on rapid degradability

Method	Results*	Key or Supportive study[1]	Remarks	Reference

** data on full mineralization should be reported*

2.8.2.1.1 Ready biodegradability

Three acceptable studies on ready biodegradability were submitted (Luit, 2001; Desmares-Koopmans, 2000; Feil, 2009)

Luit, R. J., 2001

The ready biodegradability of cymoxanil was determined in Luit 2001 by observing the carbon dioxide demand (OECD 301B) using modified Sturm test over 10 days at 21.0 – 23.5 °C.

The ready biodegradability of cymoxanil was determined with the modified Sturm test at 10 mg TOC/L. The relative degradation values calculated from the measurements performed during the test period revealed no significant degradation of cymoxanil.

Sodium acetate was used as reference substance. The test solutions (pH approx. 7.6, Temp. 21.0 – 23.5 °C) were continuously stirred during the test duration of 10 days. Carbon dioxide produced in each test bottle was reacted with barium hydroxide contained in a gas scrubbing bottle and was precipitated as barium carbonate. The relative degradation values calculated from the measurements performed during the test revealed no significant degradation (< 10 %) of cymoxanil technical. In the toxicity control, more than 25 % degradation occurred within 14 days (based on theoretical CO_2).

Under the conditions of the modified Sturm test, cymoxanil is not considered to be readily biodegradable.

Desmares-Koopmans., M.J.E., (2008)

The ready biodegradability of cymoxanil was determined by observing the carbon dioxide demand (OECD 301B) with the modified Sturm test at 12 mg TOC/L in Desmares-Koopmans., M.J.E., (2008)

The relative degradation values calculated from the measurements performed during the test period revealed no significant degradation of cymoxanil. Cymoxanil was not readily biodegradable under the conditions of the modified Sturm test. The samples were incubated for 28 days at 21.3 to 22.6 °C in darkness. The percentage degradation in the sodium acetate positive control calculated by biochemical oxygen demand (BOD) reached 66% after 16 days. The percentage biodegradation of the test substance after 28 days was <20%.

Feil., N., (2009)

The ready biodegradability of cymoxanil was determined in Feil 2009 by observing the BOD (biochemical oxygen demand, OECD 301F) using manometric methods over 28 days at 21-22°C in the dark.

The ready biodegradability of cymoxanil was determined under a Manometric respirometry test over a period of 28 days. The relative degradation values calculated from the measurements performed during the test period revealed no significant degradation of cymoxanil. Cymoxanil was not readily biodegradable under the conditions of the test. The samples were incubated for 28 days at 21 to 22°C in darkness. The toxicity control contained both test material and the reference item sodium benzoate.

The percentage degradation in the sodium acetate positive control calculated by biochemical oxygen demand (BOD) reached 98% after 14 days. The percentage biodegradation of the test substance after 28 days in consideration of nitrification was 23% ($ThOD_{NO3}$).

Biodegradation of Cymoxanil technical

The mean biodegradation after 28 days of Cymoxanil technical in consideration of nitrification was 23% $ThOD_{NO3,}$ thus the 10 day window criterion was not passed. The biodegradation occurred late in the incubation period, since the sludge bacteria needed a certain time to adopt. Therefore, Cymoxanil technical is considered not to be readily biodegradable, but an inherent biodegradation potential can be assumed.

Biodegradation of Sodium Benzoate

The reference item sodium benzoate was sufficiently degraded to 98 % after 14 days and to 103% after 28 days of incubation.

Biodegradation in the toxicity control

In the toxicity control containing both the test item and the reference 36 % biodegradation was noted within 14 days and 48 % biodegradation was determined after 28 days of incubation based on $ThOD_{NH3.}$

Conclusion: Because the three studies meet CLP criteria on the ready biodegradability, the conclusion is that cymoxanil is not readily biodegradable.

2.8.2.1.2 BOD5/COD

No data provided.

2.8.2.2 Other convincing scientific evidence

No data provided.

2.8.2.2.1 Aquatic simulation tests

No data provided.

2.8.2.2.2 Field investigations and monitoring data (if relevant for C&L)

No data provided.

2.8.2.2.3 Inherent and enhanced ready biodegradability tests

Cymoxanil is not readily biodegradable.

2.8.2.2.4 Soil and sediment degradation data

Aerobic degradation of cymoxanil in soil under aerobic dark conditions at 20ºC and 25ºC was investigated with [^{14}C] labelled compound in 6 different soils in 6 studies. The aerobic degradation of cymoxanil resulted in formation of four metabolites in amounts > 10% of applied: IN-KQ960, IN-U3204, IN-W3595 and IN-T4226.
In four aerobic water / sediment systems (pH of water phase 6.7 to 8.9) cymoxanil dissipated from the water phase to the sediment very rapidly. Once deposited in the sediment, parent continued to degrade very rapidly ultimately resulting in the evolution of significant quantities of radioactivity, evolved as $^{14}CO_2$. The maximum level of cymoxanil in the sediment phase never reached above 5% in any the studies. Seven transformation products (≥ 5% AR) were observed in the water phase, IN-W3595, IN-KP533, IN-U3204, IN-KQ960, AS999 (M5), IN-T4226 and IN-JX915.

2.8.2.2.5 Hydrolysis

Hydrolysis of cymoxanil was investigated in sterile buffer solutions at pH4, 5, 7 and 9 in two independent studies, which gave consistent results. Once in contact with (sterile) buffer solutions, cymoxanil undergoes extensive hydrolysis strongly depending on the pH of the solution, leading to the formation of numerious metabolites. Cymoxanil is considered stable at a pH of 4, half-life times at pH 5, 7 and 9 were 144, 1.1 and 0.02 days at 25ºC. At 20ºC half-life times at pH 7 and 9 were determined to be 2.1 and 0.04 days. At pH 5 only minor metabolites were observed, no major metabolites were found. The major metabolites formed under sterile conditions at pH 7 and 9 were: IN-U3204 (60.8 % AR), IN-JX915 (11.0 % AR), IN-W3595 (41.5 % AR), IN-KP533 (57.4 % AR), IN-R3273 (10.2 % AR), IN-KQ960 (14.1 % AR). The metabolites IN-W3595, IN-KQ960, IN-R3273, IN-KP533 were considered stable under the conditions of sterile hydrolysis at both pHs. Metabolite IN-U3204 is highly unstable in aqueous solutions, rapidly degrading into IN-KP533, IN-T4226 and IN-KQ960. IN-T4226 is a further transient hydrolysis metabolite rapidly degrading into IN-KP533 by ring cleavage. Metabolites IN-KQ960 and IN-KP533 have to be considered stable under the conditions of sterile hydrolysis. Metabolite IN-JX915 rapidly degrades into IN-R3273, which in turn slowly degrades into IN-T4226.
The parent cleavage product IN-W3595 is considered rather stable under the conditions of hydrolysis in sterile buffer solutions. Ethyl urea, which is likely to be formed together with IN-W3595, was never quantified in environmental fate studies, since the labelling of the parent (cyanoacetamide position) does not allow to follow the fate of this cleavage product. Nevertheless, ethyl urea has to be considered a major degradation product of the hydrolysis of cymoxanil in sterile buffer solutions at neutral and alkaline pH, too. According to SANCO/221/2000, rev. 10, (2003) guidance document on the relevance of metabolites in groundwater, ethyl urea and its degradation products are considered compounds of no concern and therefore not further considered in the environmental risk assessment.

Hydrolysis half-life of the transient metabolites IN-U3204, IN-JX915 and IN-T4226 at pH 7 and pH 9 were estimated to be 2.5 and 0.5 days, 0.7 and 1.7 days, and 7.2 and 2.0 days, respectively. The metabolites IN-W3595, IN-KQ960, IN-R3273 and IN-KP533 have to be considered rather stable under the conditions of sterile hydrolysis at each pH, their amounts remained almost stable once the hydrolysis process has finished (which occurred by approx. 15 DAT at pH 7 and by 7 DAT at pH 9).

In the Oxon study (Slangen and Willams, 2003) several degradation products, not exceeding 10 % of AR individually, remained unidentified.

New hydrolysis study (Anand, 2007) on cymoxanil has been submitted to the existing hydrolysis studies (Lawler 1996, Willems 2003, Willems 2000, Brands 2006, Goodyear 2006) by TF. The active substance is stable at pH 5 and very quickly hydrolyses to under basic conditions pH 7 with half-life values of 0.02 days and pH 9 with half-life values of 1.4 days. Under acidic conditions, cymoxanil slowly hydrolysis with half- life value of 148 days at pH 4 at 25°C. At pH 7 and 25°C, degradation of IN-3204 was observed up to 52.7 % of AR after 2 days, IN-W3595 up to 16.2 of AR after 15 days, IN-KP533 up to 57.0 % of AR after 30 days. At pH 9 and 25°C, degradation of IN-W3595 was observed up to 39.0 % of AR after 3 days, IN-KP5333 up to 31.6 % of AR up to 10 days, IN-U3204 up to 60.8 % of AR after 0.17 days and IN-KQ960 up to 13.5 % of AR after 7 days.

2.8.2.2.6 Photochemical degradation

Cymoxanil degraded very rapidly under neutral and alkaline conditions but was considerably slower under acidic conditions with calculated half-lives of 31 minutes, 34 hours and 148 days respectively. Five degradates occurring at >10% were identified IN-U3204, IN-W3595, IN-KP533 and IN-KQ960. In five aerobic water / sediment systems (pH of water phase 6.7 to 8.9) cymoxanil dissipated from the water phase to the sediment very rapidly. Once deposited in the sediment, parent continued to degrade very rapidly ultimately resulting in the evolution of significant quantities of radioactivity, evolved as $14CO_2$. The maximum level of cymoxanil in the

sediment phase never reached above 5% in any the studies.
The aqueous photolysis of cymoxanil had been studied in sterile aqueous buffer at pH 5 at a temperature of 25 °Cin four studies.

2.8.2.2.7 Other / Weight of evidence

2.8.3 Summary of fate and behaviour in air

Cymoxanil has a low volatility of 3.8 x 10^{-5} Pa at 25ºC and is shown that has little potential for volatilisation in the environment. The atmospheric reaction between photochemically produced hydroxyl radicals and cymoxanil was estimated by the computer programme AOPWIN Ver 1.83. The bimolecular rate constant k_{OH} was estimated as 6.021 x 10^{-12} cm^3 $molecule^{-1}$ sec^{-1} at 25ºC an atmospheric DT_{50} for cymoxanil of 21.3 hours this is 1.776 days when a 12 hour day is considered.

2.8.3.1 Hazardous to the ozone layer

Table 69: Summary table of studies on hazards to the ozone layer

Method	Results	Remarks	Reference

2.8.3.1.1 Short summary and overall relevance of the provided information on hazards to the ozone layer

No data provided.

2.8.3.1.2 Comparison with the CLP criteria

No data provided.

2.8.3.1.3 Conclusion on classification and labelling for hazardous to the ozone layer

Not relevant.

2.8.4 Summary of monitoring data concerning fate and behaviour of the active substance, metabolites, degradation and reaction products

Laboratory, lysimeter and field data demonstrated the degradability of cymoxanil and its residues in the various compartments of the environment, with no indications for persistence or accumulation. Under recommended use conditions, no unacceptable leaching of parent compound or of any relevant metabolites to groundwater is to be detected.
During the literature review process some publications were found reporting on monitoring data. Summaries of these studies were provided by the applicants. The monitoring studies from France (surface water) showed that mean concentration of cymoxanil in run-off samples was 0.05-0.08 µg/L. The monitoring studies in Spain (surface water and groundwater) showed that the majority of water samples (>80 %) cymoxanil residues were not detected (LOD 0.022 µg/L. In Rioja Alta and Rioja Baja cymoxanil residues exceeded 0.1 µg/L in five and eight samples respectively out of a total of 136 and 164 samples.

2.8.5 Definition of the residues in the environment requiring further assessment

Compartment	Residue definition	Major metabolite in
Soil	Cymoxanil	Parent substance
	IN-U3204	Aerobic soil

	IN-W3595	Aerobic soil
	IN-T4226	Aerobic soil
	IN-JX 915	Aerobic soil
	IN-KQ960	Aerobic soil
Groundwater	Cymoxanil	Parent substance
	IN-U3204	Aerobic soil
	IN-W3595	Aerobic soil
	IN-KQ960	Aerobic soil
	IN-T4226	Aerobic soil
	IN-JX915	Aerobic soil
Surface water	Cymoxanil	Parent substance
	IN-U3204	Aerobic water/sediment
	IN-KQ960	Aerobic soil
	IN-W3595	Aerobic soil, Aerobic water/sediment
	IN-R3273	Aerobic soil, Aerobic water/sediment
	IN-T4226	Aerobic water/sediment
	Metabolite fraction M5 (AS999)	Aerobic water
	IN-JX915	Aerobic soil, Aerobic water/sediment
	IN-KP533	Aerobic water
Air	Cymoxanil	Parent substance

Definition of Relevant Air Residues

Based on the short chemical lifetime, accumulation of cymoxanil in the air is not to be expected. In case of any spray drift after application, cymoxanil is degraded very quickly by hydroxyl radicals and ozone. Further more the Henry's law constant of cymoxanil is less than 3.8×10^{-5} Pa m^3 mol^{-1}, suggesting little potential for volatilisation in the environment.

Conclusion: Cymoxanil volatilisation is negligible.

Definition of Relevant Soil Residues

As demonstrated in laboratory experiments, cymoxanil is very rapidly degraded by soil micro-organisms under aerobic conditions, the half-life in soils being 0.822 days. The following metabolites IN-KQ960, IN-W3595, IN-R3273, IN-T4226, IN-JX915 and IN-U3204 are formed at >5% (12.0%, 18.1%, 7.6%, 11.7%, 10.9% and 24.7% of applied respectively). These are further degraded with an average half-lives of 1.52, 0.986, 8.69, 0.210, 0.187 and 0.160 days to produce polar components associated with fulvic acid 15% of AR), humic acid (4% of AR) and the humin fraction (27% of AR). Further compounds are always significantly below 10%. Carbon dioxide is the final end product of microbial degradation yielding up to 60% after 120 days.

These results provide clear evidence that IN-KQ960, IN-W3595, IN-R3273, IN-T4226, IN-JX915 and IN-U3204 are the only major soil metabolites. IN-R3274 was observed as a minor metabolite in aerobic soils studies,

exceeding 5% at one timepoint only. IN-18474 oxamic acid was seen at 7.6 % but was considered to be of no concern at the last EU review [b]ased on SANCO/221/2000 –rev. 10. final, organic compounds of aliphatic structure with a chain length of 4 or less, which consist only of C, H, N or O atoms and which has no "alerting structures" such as epoxide, nitrosamine, nitrile or other functional groups of known toxicological concern, are considered substances of no concern.

Conclusion: Cymoxanil, IN-KQ960, IN-W3595, IN-T4226, IN-JX915 and IN-U3204 are the relevant soil residue.

Definition of Relevant Water Residues

Cymoxanil is not applied directly to aquatic systems. However, it might reach water bodies indirectly by spray drift and by surface or drainage run-off. Under biotic conditions, cymoxanil degrades very rapidly with a half-life of 0.325 days from the whole system and dissipates from the aqueous phase to sediment with a half-life of 0.325 days.

Several major transformation products (≥ 10% AR) were observed in the water layer IN-KQ960, IN-W3595, IN-R3273, IN-T4226, IN-JX915, IN-U3204, IN-KP533, M5 (AS999). Only very minor transformation products were detected in the sediment phase (not exceeding 5.0% AR on one or more occasions).

The only major photodegradation products exceeding 10% and not found at similar levels in the dark controls were IN-JX915 (52.6%) and IN-R3273 (35.4%), but only at pH 5.

Conclusion: Cymoxanil, IN-KQ960, IN-W3595, IN-R3273, IN-T4226, IN-JX915, IN-U3204, IN-KP53, IN-R3274 and M5 (AS999) are the relevant residue in water.

Definition of the residue for monitoring

Soil: Cymoxanil
Groundwater: Cymoxanil
Surface water: Cymoxanil
Sediment: Cymoxanil
Air: Cymoxanil

2.8.6 Summary of exposure calculations and product assessment

Exposure via soil

PECsoil was calculated for cymoxanil and metabolites IN-U3204, IN-JX915, IN-W3595, IN-T4226 and IN-KQ960. For all applications, initial PECsoil were presented.

Acceptable PECsoil were presented for the representative uses in potatoes, tomatoes and grapes. The scenario for potatoes with 750 g a.s/ha at BBCH 10 assuming 15 % crop interception results in the highest PECsoil. The scenario for tomatoes with 750 g a.s/ha at BBCH 50 % crop interception results in the highest PECsoil. The scenario for grapes with 500 g a.s/ha at BBCH 50 % crop interception results in the highest PECsoil.

PECsoil were calculated for single multiple applications using standard equation from FOCUS (1997) and default soil depth of 5 cm and bulk density of 1.5 g/cm. The PECplateau was calculated for 5 cm soil depth.

The complete PECsoil calculations are presented in Volume 3 B8 (PPP) for the three representative formulated products CYMOXANIL 45WG, RIVAL DUO, DAUPHIN/FDJ03. It was sufficient to estimate the risk for the areas of use resulting in highest loading of soil, covering other areas of use. No refinements of the first tier exposure estimates were necessary. PEC values in soil were calculated for cymoxanil, its major soil metabolites IN-U3204, IN-JX915, IN-W3595, IN-T4226, IN-KQ960.

Exposure via groundwater

The estimation of PECgw for cymoxanil and its soil metabolites has been carried out according to current guidance requirements. The models FOCUS-PEARL 4.4.4, FOCUS-PELMO 5.5.3 and MACRO 5.5.4 were used to simulate the leaching behaviour of cymoxanil and its metabolites. PECgw calculations were performed for representative uses considering all FOCUS groundwater scenarios that are parameterized for potatoes, tomatoes and grapes. Crop interception values of 15 % were considered for potatoes and tomatoes, 50 % for grapes.

From the modelling proposed by the Applicants, the following conclusions can be drawn:

- For the formulated product CYMOXANIL 45WG

When the formulated product is applied every year on tomatoes at the application rate of 5 x 150 g/ha, on potatoes at the application rate of 5 x 150 g/ha, on grapes at the application rate of 5 x 120 g/ha, the results of the simulations indicated that PECgw for cymoxanil and metabolites IN-U3204, IN-JX915, IN-W3595, IN-T4226, IN-KQ960, IN-R3273 are below to the trigger value of 0.1 µg/L in all FOCUS scenarios (Maximum PECgw values were <0.001 µg/L).

- For the formulated product RIVAL DUO

When the formulated product is applied every year on potatoes at the application rate 5 x 150 g/ha, the results of the simulations indicated that PECgw for cymoxanil and metabolites IN-U3204, IN-JX915, IN-W3595, IN-T4226, IN-R3273 are below to the trigger value of 0.1 µg/L in all FOCUS scenarios (Maximum PECgw values were <0.001 µg/L). Metabolite IN-KQ960 exceeded 0.1 µg/l in Northern zone in Hamburg and Jokioinen scenarios, but no use in Northern zone is intended.

- For the formulated product DAUPHIN/FDJ03

When the formulated product is applied every year on potatoes at the applications rates 8 x 120 g/ha, on tomatoes at the application rate 5 x 120 g/ha, on grapes at the application rate 4 x 120 g/ha, the results of the simulations indicated that PECgw for cymoxanil and metabolites IN-U3204, IN-JX915, IN-T4226, IN-R3273 are below to the trigger value of 0.1 µg/L in all FOCUS scenarios (Maximum PECgw values were <0.001 µg/L). Metabolite IN-KQ960 in Northern zone in Jokioinen for the potatoes late scenario and in Hamburg for the vines late scenario, but no use in Northern zone is intended.

The complete PECgw calculations are presented in Volume 3 B.8 (PPP) for the three representative formulated products CYMOXANIL 45WG, RIVAL DUO, DAUPHIN/FDJ03.

Exposure via surface water and sediments

The calculations for PEC in surface water and sediment were performed according to the recommendations of the FOCUS working group on surface water scenarios in a stepwise approach considering the pathways spray drift, drainage and runoff. The calculations were made for cymoxanil as well as for its metabolites IN-U3204, IN-KQ960, IN-W3595, IN-JX915, IN-T4226, IN-R3273, IN-R3274, IN-KP533, ASS999 (soil and water/sediment system). For all calculations, the following model versions were used STEPS1-3 (version 3.1) for Step 1, Step 2 and Step 3.

Use of 960 g a.s./ha in potatoes and tomatoes and 480 g a.s/ha in grapes were simulated. The crop interception was set to “minimal canopy” for cymoxanil and metabolites. Calculations were performed for all available Step 2 scenarios i.e. North and South Europe.

The complete PECsw calculations are presented in Volume 3 B.8 (PPP) for the three representative formulated products CYMOXANIL 45WG, RIVAL DUO, DAUPHIN/FDJ03.

2.9 EFFECTS ON NON-TARGET SPECIES

2.9.1 Summary of effects on birds and other terrestrial vertebrates

Data on toxicity of cymoxanil to birds and mammals were previously submitted and evaluated during the first EU of cymoxanil. Data were considered acceptable and resulting endpoints were cited in the EFSA Scientific Report (September 2008) 167, 1-116.

Birds

Acute toxicity

Three studies previously submitted in the EU and additionally two new studies have been submitted. One study (RAR B.9.1.1.2, 1999) was not considered valid for vomiting and regurgitation reasons. The endpoints are summarized in Table 70 below.

For the acute toxicity endpoint, in line with EFSA guidance, the geometric mean of all the available endpoints was proposed to be used for the risk assessment. It was considered acceptable to use the geometric mean approach as the most sensitive endpoint was not more than a factor of 10 below the geometric mean of all the tested species, and despite the available studies being performed according to different test guidelines (OECD and EPA), performance of the studies was equivalent in terms of dosage, number of animals and duration of exposure. As all the acute values were unbound, the unbound value was taken as a worst case estimation of the LC_{50} in the calculation of the geometric mean.

Table 70: LD50 values from avian acute toxicity studies and the calculation of the geometric mean value

Species	**Study Reference**	**LD_{50} (mg a.s./kg bw)**	**LD_{50} used in calculation of geometric mean**
Bobwhite quail	CA 8.1.1.1-01 RAR B.9.1.1.1., 1996	>2000	2000
Mallard duck	CA 8.1.1.1-02* RAR B.9.1.1.2., 1999	>2000*	486
	CA 8.1.1.1-03 RAR B.9.1.1.3., 1992	>486	
Japanese quail	CA 8.1.1.1-05 RAR B.9.1.1.5., 1995	>625	625
Zebra Finch	CA 8.1.1.1-04 RAR B.9.1.1.4., 2013	>2000	2000
Overall geometric mean used in risk assessment			**>1050****

*Study not acceptable, endpoint not used in the risk assessment
** Not used in the risk assessment, see information below

Short-term toxicity

Overall, four short-term dietary toxicity tests conducted with cymoxanil technical are available. Short–term studies on dietary toxicity to birds are no longer required under Reg (EU) 1107/2009 unless there is evidence that the short-term dietary toxicity is higher than the oral toxicity.
In two short term toxicity studies treatment-related mortality and clinical signs occur for concentrations tested, indicating higher toxicity compared to acute oral studies. Two studies previously submitted in the EU and additionally two new studies have been submitted.

Overall, four short-term dietary toxicity tests conducted with cymoxanil technical are available. Short–term studies on dietary toxicity to birds are no longer required under Reg (EU) 1107/2009 unless there is evidence that the short-term dietary toxicity is higher than the oral toxicity.
In two short term toxicity studies treatment-related mortality and clinical signs occur for concentrations tested, indicating higher toxicity compared to acute oral studies. Two studies previously submitted in the EU and additionally two new studies have been submitted.

Four short term dietary studies are available for cymoxanil: one on bobwhite quail and three on mallard duck.
In the study with bobwhite quail (RAR B.9.1.2.1**,** 1997), since there were no treatment-related mortalities (a single mortality occurred on Day 5 among the birds of the 2600 mg/kg diet group but was unrelated to treatment) in the dietary study there is no indication of increased toxicity due to dietary exposure.
However, in the study with mallard duck (RAR B.9.1.2.2., 2009) 1 bird died in group 7 (2500 ppm) and 5 birds died in group 8 (5000 ppm) (the highest rate tested) as treatment related mortalities. Also treatment related clinical signs (subdued behaviour and unsteadiness) were observed in all birds in group 6 (1250 ppm), 7 (2500 ppm) and 8 (5000 ppm) from study Day 4 to day 7. In the second study with mallard duck (RAR B.9.1.2.3**.**

1999) 20%, 30% and 80% treatment-related mortality occurred at 1250, 2500 and 5000 ppm, respectively. Hunched posture and uncoordinated movements were observed at all three higher concentrations, combined with lethargy at 2500 and 5000 ppm. In the third study with mallard duck (RAR B.9.1.2.4., 2009) two mortalities occurred in the highest (5000 mg a.s./kg feed) treatment level. No other fatalities or abnormal observations were noted among any other treatment levels.

In order to set an endpoint for short-term toxicity studies, the RMS proposed to use the geomean value of the LC_{50} set in four dietary toxicity studies. The geomean dietary LC_{50} was found to be > 548 mg/kg bw/day. As the endpoint for the most sensitive species (LC50 mallard duck is > 157 mg/kg bw/day) is not less than a factor of 10 below the geometric mean of all tested species, the geomean value can be used for acute risk assessment.

As geomean dietary **LD50 >548 mg/kg bw/day** is lower than geomean acute >1050 mg/kg bw/day, the dietary LD_{50} should be used in the acute risk assessment.

Table 71: D_{50} values from avian short-term toxicity studies and the calculation of the geometric mean value

Species	**Study Reference**	**LC_{50} (mg a.s./kg bw)**	**LC_{50} used in calculation of geometric mean**
Bobwhite quail	CA 8.1.1.2-01 RAR B.9.1.2.1., 1997	>999	999
Mallard duck	CA 8.1.1.2-02 RAR B.9.1.2.2., 2009	>157	301
Mallard duck	CA 8.1.1.2-03 RAR B.9.1.2.3., 1999	>260	
Mallard duck	CA 8.1.1.2-04 RAR B.9.1.2.4., 2009	>666	
Overall geometric mean used in risk assessment			**>548**

Reproductive toxicity

Overall, two reproductive toxicity tests conducted with cymoxanil technical are available. A **NOAEL of 14.9 mg a.s./kg bw/day** derived from the study for mallard duck (RAR B.9.1.3.2,1996b) was used in the long-term risk assessment as the endpoint from the most sensitive tested species.

Table 72: Summary of the toxicity values of Cymoxanil for birds

Test species	**Test material**	**Endpoint**	**Data point Author, year**
Acute			
Bobwhite quail (*Colinus virginianus*)	Cymoxanil	LD_{50} > 2000 mg a.s./kg bw NOEL <521 mg a.s./kg bw	CA 8.1.1.1-01 RAR B.9.1.1.1., 1996
Mallard duck (*Anas platyrhynchus*)	Cymoxanil	LD_{50} > 486 mg a.s./kg bw NOEL < 292 mg a.s./kg bw	CA 8.1.1.1-03 RAR B.9.1.1.3., 1992
Japanese quail (*Coturnix coturnix japonica*)	Cymoxanil	LD_{50} >625 mg a.s./kg bw NOEL=6 mg a.s./kg bw	CA 8.1.1.1-05 RAR B.9.1.1.5., 1995
Zebra Finch (*Taeniopygia guttata*)	Cymoxanil	LD_{50} > 2000 mg a.s./kg bw NOEL = 2000 mg a.s./kg bw	CA 8.1.1.1-04 RAR B.9.1.1.4., 2013
Overall geometric mean	Cymoxanil	LD_{50} > 1050 mg a.s./kg bw***	
Dietary toxicity (short-term)			
Mallard duck (*Anas*	Cymoxanil	LC_{50} = 4950 mg a.s./kg feed	CA 8.1.1.2-02

Test species	**Test material**	**Endpoint**	**Data point Author, year**
platyrhynchus)		(>157 mg a.s./kg bw/day)* NOEC = 156 mg a.s./kg feed (42 mg a.s./kg bw/day)	RAR B.9.1.2.2., 2009
	Cymoxanil	LC_{50} > 5000 mg a.s./kg feed (>666 mg a.s./kg bw/day) NOEC (mortality) = 2500 mg a.s./kg feed (= 537 mg a.s./kg bw/day)	CA 8.1.1.2-04 RAR B.9.1.2.4., 2009
	Cymoxanil	LC_{50} = 2944.5 mg a.s./kg diet (>260 mg a.s./kg bw/day)** NOEC = 313 mg a.s./kg diet (119 mg a.s./kg bw/day)	CA 8.1.1.2-03 RAR B.9.1.2.3., 1999
Bobwhite quail (*Colinus virginianus*)	Cymoxanil	LC_{50} > 5200 ppm (> 999 mg a.s./kg bw/day) NOEC = 1300 ppm (249 mg a.s./kg bw/day)	CA 8.1.1.2-01 RAR B.9.1.2.1., 1997
Overall geometric mean	Cymoxanil	**LC_{50} > 548 mg a.s./kg diet**	
Reproductive toxicity (long-term)			
Bobwhite quail (*Colinus virginianus*)	Cymoxanil	NOAEC = 300 ppm a.s. (equivalent to 27.9 mg a.s./kg bw/day)	CA 8.1.1.3-01 RAR B.9.1.3.1., 1996a
Mallard duck (*Anas platyrhynchus*)	Cymoxanil	NOAEL = 100 ppm a.s. (**equivalent to 14.9 mg a.s/kg bw/day**)	CA 8.1.1.3-02 RAR B.9.1.3.2., 1996b

* Since food consumption was reduced at dietary concentrations above and below the LC_{50} value, it is not possible to convert the LC_{50} to a reliable daily dose estimate. The highest dietary concentration that caused no significant impact on food consumption was 625 ppm, corresponding to 157 mg a.s./kg bw/day. Therefore, following a conservative approach, the LC_{50} can be assumed to be higher than 157 mg a.s./kg bw/day.

** Since food consumption was reduced at dietary concentrations above and below the LC_{50} value, it is not possible to convert the LC_{50} to a reliable daily dose estimate. The highest dietary concentration that caused no significant impact on food consumption was 625 ppm, corresponding to 260 mg a.s./kg bw/day. Therefore, following a conservative approach, the LC_{50} can be assumed to be higher than 260 mg a.s./kg bw/day.

***Not used in the risk assessment, see information in Volume 3 (PPP), section B.9.1.1

Mammals

Acute toxicity

Overall, two acute oral toxicity tests conducted with cymoxanil are available. One new study has been submitted. The lowest acute LD_{50} value for mammals (i.e. 485 mg a.s./kg bw) from new study (RAR B.6.2.1.2., 1992) is proposed to be used in the acute risk assessments for mammals and as new endpoint. The endpoints are summarized in Table 73 below.

Table 73: Summary of endpoints for mammals

Organism	**Test substance**	**Timescale/ Test type**	**Endpoint**	**Toxicity value**	**Reference**
Acute					
Rat	Cymoxanil	Acute oral	LD_{50}	760 mg/kg (♂) 1200 mg/kg (♀) 960 mg/kg (combined)	CA 5.2.1-01 RAR B.6.2.1.1., 1992 (EU agreed endpoint) DAR 2007
Rat	Cymoxanil	Acute oral	LD_{50}	538 mg/kg (♂) 356 mg/kg (♀)	CA 5.2.1-02 RAR B.6.2.1.2.,

				485 mg/kg (combined)	1992a (SFP)
Rat	Cymoxanil 45 WG	Single dose/ Acute oral	LD_{50}	> 2000 mg/kg bw	CP 7.1.1-01 RAR B.6.1.1., 2004a (Vol 3 CP)
Chronic					
Rat	Cymoxanil	Long-term (Reproductive)	NOAEL	10.5 mg/kg w/day	CA 5.6.1-01 RAR B.6.6.1.1., 2001 (EU agreed endpoint) DAR 2007
Rat	Cymoxanil	Rat developmental toxicity study	NOAEL	**10 mg/kg bw/day**	CA 5.6.2-01 RAR B.6.6.2.1., 1993 DAR 2007

Reproductive toxicity

Table 74: Data from the mammalian toxicology section, relevant to identify the ecotoxicologically relevant reproductive endpoint for mammals

Studies	Reports	Summary
28-day rat oral toxicity study (OECD 407) 0, 750, 1500, 3000, 5000 ppm equal to 0, 74.4, 143.5, 260.0, 400.3 mg/kg bw/d (males) 0, 79.8, 154.3, 287.8, 415.9 mg/kg bw/d (females) (the highest dose was increased from 1000 mg/kg/day to 2000 mg/kg/day at day 10 as no signs of toxicity were evident	RAR B.6.3.1.1., 1999a	**NOAEL = 74.4 (♂)** LOAEL = 143.5 (♂) based on ↓ body weight gain at I week (17.4%); ↓ food consumption throughout weeks 1 and 2 (>10%), ↑ relative liver (12.7%) and kidneys (11.8%) weight NOAEL = 154.3 (♀) LOAEL = 287.8 (♀) based on ↓ body weight at I week (12.1%) ↓ body weight gain at week 1 and 2 (>20%); ↓ food consumption throughout weeks 1 and 2 (>15%)
28-day mice oral toxicity study (OECD 407) 0, 750, 1500, 3000, 6000 ppm Equal to 0, 172.7, 303.4, 624.4 mg/kg bw/d (males) 0, 179.1, 329.9, 679.3 mg/kg bw/d (females)	RAR B.6.3.1.2., 1999a	**NOAEL = 172.7 (♂)** LOAEL = 303.4 (♂) based on ↓ body weight gain (41.8%); ↓ food consumption throughout all dosing period (>10%), ↓absolute kidneys (18%) weight NOAEL = 329.9 (♀) LOAEL = 679.3 (♀) based on ↓ body weight (17.9%) ↓ body weight gain (95%); ↓ food consumption throughout all dosing period (>14%); ↓absolute ovaries (27.3%) and adrenals (25%) weight
90 days rat oral OECD 408 0, 100, 750, 1500, 3000 ppm equal to 0, 6.54, 47.6, 102, 224 mg/kg bw/d (males) 0, 8.00, 59.9, 137, 333 mg/kg bw/d (females	RAR B.6.3.2.1. Study 1, 1993	**NOAEL = 6.54 (♂)** LOAEL = 47.6 (♂) based on histopathological findings in testes (bilateral elongate spermatid degeneration) and ↑ relative testes weight (10%) NOAEL = 137 (♀) LOAEL = 333 (♀) based on ↓ body weight gain (19.9%), ↓ overall food conversion efficiency (34.9%)
90 days rat oral OECD 408 0, 500, 1000, 2000 ppm equal to 0, 42.6, 85.1, 174.3 mg/kg bw/d (males) 0, 48.1, 97.8, 187.7 mg/kg bw/d	RAR B.6.3.2.1. Study 2., 1999b	**NOAEL = 85.1 (♂)** LOAEL = 174.3 (♂) based on ↓ body weight (11.3%), ↓ body weight gain (14.6%), ↓ food consumption (15.6%), ↑ creatinine (34%), ↑ total bilirubin (85.8%), ↑ relative kidney weight (17.3%), ↑ relative liver weight (15.7%) NOAEL = 97.8 (♀)

(females)		LOAEL = 187.7 (♀) based on ↓ body weight (>10%) during first two weeks, ↓ body weight gain (18-42%) during first three weeks, ↓ food consumption (10-22%) during seven weeks intermittently
90 days mice oral OECD 408 0, 150, 450, 1350 ppm equal to 0, 28.7, 84.4, 256.6 mg/kg bw/d (males) 0, 32.9, 97.3, 302.5 mg/kg bw/d (females)	RAR B.6.3.2.2., 1999b	**NOAEL = 84.4 (♂)** LOAEL = 256.6 (♂) based on ↓ body weight gain (21.4%), ↑ total bilirubin (114.8%), NOAEL = 97.3 (♀) LOAEL = 302.5 (♀) based on ↓ body weight gain (28.6-33.3%) during first five weeks, ↑ total protein (23.7%), ↑ relative liver weight (11.2%)
Two-generation reproduction toxicity study OECD 416 Rat, Hsd Cpb: WU 0, 150, 450, 1350 ppm equal to 0, 10.5, 31.6, 94.0 mg/kg bw/day (F0 males) and 0, 14.9, 42.8, 116.3 mg/kg bw/day (F0 females premating, gestation and lactation)	RAR B.6.6.1.1., 2001 2-generation study in the rat (dietary)	**Parental** **NOAEL: 31.6 (♂) – 42.8 (♀) mg/kg bw/day** LOAEL: 94.0 (♂) – 116.3 (♀) mg/kg bw/ day based on ↓bw gain premating (F0 males: 17%, F1 males: 14%, F0 female: 10%), gestation (F1 female: 20%), lactation (F0 female: 78%) ↓FC premating (F1 males: 10%, F0/F1 female: 9%), gestation (F0/F1 female: 11%/8%), lactation (F0/F1 females: 33%/26%) **Reproductive** **NOAEL: 31.6 (♂) – 42.8 (♀) mg/kg bw/day** LOAEL: 94.0 (♂) – 116.3 (♀) mg/kg bw/day based on F1 generation: ↓ mean number of corpora lutea, ↓mean number of implantations, ↑post-implantation loss (%), ↓mean litter size, ↓ live pups born (%) **Offspring** **NOAEL: 10.5 (♂) – 14.9 (♀) mg/kg bw/day** LOAEL: 31.6 (♂) – 42.8 (♀) mg/kg bw/day based on ↓bw (both sexes combined F1 pups Days 14 and 21: >10%; F2 pups Days 7, 14 and 21: >8%)
Two-generation reproduction toxicity study OECD 416 Rat, Crl:CD®BR 0, 100, 500, 1500 ppm equal to 0, 6.5, 32.1, 97.9 mg/kg bw/day (F0 males) 0, 6.65, 34.7, 103.0 mg/kg bw/day (F0 females gestation)	RAR B.6.6.1.2., 1993 2-generation study in the rat (dietary)	**Parental** **NOAEL: 32.1 – 34.7 mg/kg bw/day** LOAEL: 97.9 – 103 mg/kg bw/day based on clinical signs (end of tail missing, necrotic tip of tail, sore), ↓bw (>10%) premating (F0 males, F0 female), gestation (F0 female) ↓bw gain premating (F0 males: 18%, F1 males: 14%, F0 female: 23%, F1 female: 14%), gestation (F0 female: 12%) ↓FC (>10%) premating (F0/F1 males, F1 female), 1st gestation (F female) **Reproductive** **NOAEL: > 97.9 – 103.0 mg/kg bw/day** LOAEL: Not obtained Did not cause adverse effects at highest dose tested **Offspring** **NOAEL: 6.5 – 6.65 mg/kg bw/day** LOAEL: 32.1 – 34.7 mg/kg bw/day based on ↓viability index (%) of F1 pups (days 1-4) ↓bw (both sexes combined F2B pups, Days 4, 7, 14 and 21: 13-18%)

Developmental (teratogenicity) study (OECD 414) 0, 10, 25, 75, 150 mg/kg bw/day Days 7-16 of gestation, gavage	RAR B.6.6.2.1., 1993 Rat developmental toxicity study	**Maternal:** **NOEL: 10 mg/kg bw/day** LOAEL: 25 mg/kg bw/day based on ↓ body weight gain (45%) over 7-9 days of gestation ↓ food consumption (12%) over 7-9 days of gestation **Developmental:** **NOAEL: 10 mg/kg bw/day** LOAEL: 25 mg/kg bw/day based on ↑incidence of skeletal variations and delayed ossification (partially ossified vertebra, partially ossified skull and wavy ribs) Foetal toxicity at higher dose levels: ↑incidence of skeletal variations (partially ossified sternebra, unossified sternebra, wavy ribs and partially ossified pelvis at 150 mg/kg/day) ↑ incidence of skeletal malformations (hemi vertebra at ≥75 mg/kg/day; exencephalic head and fused ribs at 150 mg/kg/day)
Developmental (teratogenicity) study (OECD 414) 0, 30, 60, 120 mg/kg bw/day Days 6-15 of gestation, gavage	RAR B.6.6.2.2., 1998 Rat developmental toxicity study	**Maternal:** **NOEL: 60 mg/kg bw/day** LOAEL: 120 mg/kg bw/day based on ↓ body weight gain (50%/20%) over 6-15/0-20 days of gestation ↓ food consumption (25%/13%) over 6-15/0-20 days of gestation **Developmental:** NOAEL cannot be established LOAEL: 30 mg/kg bw/day based on ↑incidence of skeletal minor anomalies (dumb-bell shaped thoracic vertebra 6/13) Foetal toxicity at higher dose level: ↑incidence of skeletal variations: delayed ossification (servical vertebra: 7/7 and supraoccipital) and minor anomalies (hypoplasia of sternum: sternebra no. 1/2 and rudimentary 14th rib) at ≥60 mg/kg bw/day
Developmental (teratogenicity) study (OECD 414) 0, 4, 8, 16 mg/kg bw/day Days 6-18 of gestation, gavage	RAR B.6.6.2.3., 1980 Rabbit developmental toxicity study	**Maternal:** **NOAEL: ≥ 16 mg/kg bw/day** LOAEL cannot be established No effects even at the highest dose tested **Developmental:** **NOAEL ≥ 16 mg/kg bw/day** LOAEL cannot be established No effects even at the highest dose tested No treatment related effects regarding maternal toxicity, pregnancy parameters and foetal toxicity (malformations or variations) were observed at any dose level.
Developmental (teratogenicity) study (OECD 414) 0, 8, 16, 32 mg/kg bw/day Days 6-18 of gestation, gavage	RAR B.6.6.2.4., 1981 Rabbit developmental toxicity study	**Maternal:** **NOEL: 8 mg/kg bw/day** LOAEL: 16 mg/kg bw/day based on clinical observations (anorexia/ reduced faecal output) **Developmental:** **NOAEL: 16 mg/kg bw/day** LOAEL: 32 mg/kg bw/day based on

		↑incidences of skeletal malformations (vertebra and/or rib alterations linked with scoliosis)
Developmental (teratogenicity) study (OECD 414) 0, 1, 4, 8, 32 mg/kg bw/day Days 6-18 of gestation, gavage	RAR B.6.6.2.5., 1982 Rabbit developmental toxicity study	**Maternal:** **NOAEL: ≥ 32 mg/kg bw/day** LOAEL cannot be established No effects even at the highest dose tested **Developmental:** **NOAEL: 8 mg/kg bw/day** LOAEL: 32 mg/kg bw/day based on ↑incidences of visceral malformations (cleft palate and hydrocephaly)
Developmental (teratogenicity) study (OECD 414) 0, 5, 15, 25 mg/kg bw/day Days 6-18 of gestation, gavage	RAR B.6.6.2.6., 1999 Rabbit developmental toxicity study	**Maternal:** **NOEL: 15 mg/kg bw/day** LOAEL: 25 mg/kg bw/day based on ↓body weight gain (160%) over 6-18 days of gestation ↓food consumption (17%) over 6-19 days of gestation **Developmental:** **NOAEL: 15 mg/kg bw/day** LOAEL: 25 mg/kg bw/day based on ↑incidences of visceral malformations (dilation of heart ventricles), visceral variants (slight renal pelvis dilation), skeletal variants (incomplete/poor ossification of fore limb), skeletal minor anomalies (accessory floating rib no. 13)

The information presented in the table above was derived from the mammalian toxicological assessment.

From the available **four short-term repeated-dose oral toxicity studies** with rats and mice, the lowest endpoint from the short-term repeated-dose oral toxicity studies with rats, mice were the following: a **NOAEL of 6.54 mg a.s./kg bw/day for males** (RAR B.6.3.2.1. Study 1, 1993; rats, 90-day). The endpoint was based on histopathological findings in testes (bilateral elongate spermatid degeneration) and encrease relative testes weight (10%). Increased elongate spermatid degeneration was observed in three animals of the 47.6 mg/kg bw/day dose group, five of the 102 mg/kg bw/day dose group and seven animals of the 224 mg/kg bw/day dose group: the increased incidence showed a clear dose-relationship and was statistically significant at the highest dose level. In this study NOAEL based on body weight gain was 137 a.s./kg bw/day. The lowest endpoint from other studies was the the NOAEL set at 74.4 mg a.s./kg bw/day, based on a statistically significant effect on body weight gain (17.4%). The endpoints from other short-term repeated-dose oral toxicity studies were higher and mainly baised on body weight.

Effects on histopathological findings in testes were observed only in one study from four short term repeated – dose oral toxicity studies. The changes in the testes and epididymides were not accompanied by toxicologically significant effects on other androgen- and steroidogenesis-related organs such as the seminal vesicles, prostate, uterus and ovaries. The effects in the testes and epididymides appear to be specific, indicating that they likely are not a result of disturbance of the androgen hormonal system.

Additionally, minor effects of cymoxanil on fertility parameters were reported in the F0 and F1 generation parents (for more information see Volume 1, 2.6.6.1.2 and 2.2.2.1 sections).

Therefore, effects on histopathological findings could potentially be considered of low ecotoxicological relevance.

Two 2-generation studies with rats are available. In the study by (RAR B.6.6.1.2., 1993) the NOAEL value were estasblished 6.5 mg/kg bw/d. This endpoint was based on a viability index of F1 pups and decreased body weight of both sexes combined F2 B generation pups. **However, the NOAEL of 6.5 mg/kg bw/d appears to be due to the spacing of dose levels in the study.**

Also a new 2- generation study in rats was generated. From this study (RAR B.6.6.1.1., 2001), a **NOAEL of 10.5 mg a.s./kg bw/day** was derived (10.5 mg/kg bw/day for males and 14.9 mg/kg bw/day for females), based on on reduced body weight of F1 pups (both sexes combined) on days 14 and 21 as well as reduced body weight of F2 pups (both sexes combined) on days 7, 14 and 21. The NOAEL for parental toxicity is proposed at 450 ppm

(equal to **31.6 mg/kg bw/day for males** and 42.8 mg/kg bw/day for females) based on reduced bodyweight gain in the F0 and F1 parental animals; reduced food consumption in the F1 males; reduced food consumption in the F0 and F1 females at the high dose level.

The EU agreed endpoint overall NOAEL of 10.5 mg/kg bw/d (RAR B.6.6.1.1., 2001) was based on parental/offspring effects at 150 ppm. Now the NOAEL for parental toxicity is proposed at 450 ppm (equal to 31.6 mg/kg bw/day for males and 42.8 mg/kg bw/day for females) (for more information see Volume 1, 2.6.6.3.1 section).

For the rat, two developmental toxicity studies are available (**RAR B.6.6.2.1., 1993; RAR B.6.6.2.2., 1998).** The lowest endpoint from these studies was a NOAEL of 10 mg a.s./kg bw/day (RAR B.6.6.2.1., 1993). This endpoint was based on reduced body weight gain and food consumption observed at 25 mg/kg bw/day, the **maternal NOAEL was set at 10 mg/kg bw/day** There were reductions in mean body weight gain by 44.6% and food consumption by 11.7% at 25 mg/kg bw/day dose over the first few days of dosing (7 -9 days of gestation). Maternal adjusted body weight gains were reduced (>20%) at 1-22 days and 7-22 days from 75 mg/kg bw/day only. Mean maternal body weights were significantly reduced (9 - 11%) from day 9 of gestation until the end of the observation period (day 22 of gestation) at the high dose level (150 mg/kg bw/day). The **developmental NOAEL was set at 10 mg/kg bw/day**, based on increased incidences of skeletal variations. It should be noted that increased incidences of some malformations were reported from 75 mg/kg bw/day. There was a statistically significant decrease in the number of male viable foetuses per litter from 75 mg/kg bw/day dose. In the high dose group there was a statistically significant increase in the total number of resorptions/litter, an increase in the number of early resorptions/litter, a reduction in the total number of viable foetuses per litter and a reduction by 16.7% in the mean foetal body weight.

In the second rat developmental toxicity study (RAR B.6.6.2.2., 1998), The NOAEL for maternal toxicity can be established at 60 mg/kg bw/day, based on the findings with respect to body weight gain and food consumption at the highest dose level tested (120 mg/kg bw/day). Since incidences for skeletal minor anomalies (dumb-bell shaped thoracic vertebra 6/13) were shown to be statistically significantly increased and above the HCD even at the lowest dose tested (30 mg/kg bw/day), the developmental NOAEL cannot be established. Only the developmental LOAEL of 30 mg/kg bw/day can be derived in this study.

For the rabbit, four developmental toxicity studies are available (**RAR B.6.6.2.3., 1980; RAR B.6.6.2.4., 1981; RAR B.6.6.2.5., 1982 and RAR B.6.6.2.6., 1999**).

Taking into account the results of the developmental studies available, there is reasonable evidence that cymoxanil can impair foetal development producing also malformations (demonstrated in two developmental toxicity studies in rats and in three out of four studies in rabbits) (see Volume 1, 2.6.6.2.2 section).

In the first rat developmental toxicity study (RAR B.6.6.2.1., 1993) increased incidences of skeletal malformations such as hemi vertebra were reported from 75 mg/kg bw/day and such malformations as exencephalic head and fused ribs were reported at 150 mg/kg bw/day. The **developmental NOAEL was set at 10 mg/kg bw/day.**

In the second rat developmental toxicity study (RAR B.6.6.2.2., 1998), increased incidences of minor anomalies (dumb-bell shaped thoracic vertebra 6/13) from 30 mg/kg bw/day. The Developmental **NOAEL cannot be established. LOAEL: 30 mg/kg bw/day.**

In the first (relevant) rabbit developmental toxicity study (RAR B.6.6.2.4., 1981), there were increases in the incidence of the skeletal malformations such as a vertebral and/or rib alterations (including hemivertebra, absent or fused vertebrae, misaligned vertebral centra/arches, fused/absent ribs, and various degrees of resulting scoliosis) at the high dose of 32 mg/kg bw/day. **Developmental NOAELwas set 16 mg/kg bw/day.**

In the second rabbit developmental toxicity study (RAR B.6.6.2.5., 1982), increased incidences of visceral malformations (hydrocephaly and cleft palates) occurred at the highest dose tested (32 mg/kg bw/day). **The developmental NOAEL was set at 8 mg/kg bw/day.**

In the third developmental toxicity study in rabbits (RAR B.6.6.2.6., 1999), the incidence of visceral malformations such as dilation of heart ventricles of was statistically significantly increased in the highest dose animals (25 mg/kg bw/day). **The developmental NOAEL was set at 15 mg/kg bw/day.**

The lowest endpoint from these studies was a **NOAEL of 8 mg a.s./kg bw/day** from the developmental toxicity study with rabbits (RAR B.6.6.2.4., 1981). It is based on maternal effects (clinical observation, (anorexia/ reduced faecal output) and in another developmental toxicity study with rabbits (RAR B.6.6.2.5., 1982) the most critical

endpoint is a **NOAEL of 8 mg a.s./kg bw/day**, based on foetal effects (increased incidences of visceral malformations-hidrocephaly in two foetuses of the highest dose group). Hydrocephaly was found in two foetuses of the highest dose group; the increased number of foetuses affected was without statistical significance but clearly above the range of historical control data (RAR B.6.6.2.5., 1982). The maternal NOAEL was established to be ≥ 32 mg/kg bw/day (the highest dose) in this study. Also there was no effect of cymoxanil on the pregnancy parameters (e.g. number of implantations, number of corpora lutea, resorption, abortion and litter size) or foetal viability and foetal body weight.

The available data on developmental toxicity reported in rats and rabbits did not show a clear and consistent pattern regarding developmental toxicity following exposure to cymoxanil (see Volume 1, 2.6.6.2.2 section).

Overall, taking into account tests design and biological relevance of effects, the overall lowest maternal and development **NOEL of 10 mg a.s./kg b.w.** based on reduced body weight gain and food consumption and increased incidences of skeletal variations in the rat development toxicity study (RAR B.6.6.2.1., 1993) is proposed as the relevant toxicity endpoint for cymaxonil for the reproductive risk assessment for mammals.

Terrestrial vertebrate wildlife (birds, mammals, reptiles and amphibians)

According to the new data requirements set forth in the Annex to Reg. (EU) No 283/2013, toxicity tests are requested for birds and mammals but not for amphibians and reptiles. There were no studies in the literature search of the toxicity of cymoxanil on amphibians and reptiles.

2.9.2 Summary of effects on aquatic organisms [section 11.5 of the CLH report]

2.9.2.1 Bioaccumulation [equivalent to section 11.4 of the CLH report template]

A study to assess the bioaccumulation of cymoxanil in fish has not been performed as the the measured Log Kow of cymoxanil is < 3 (0.67 - 0.59 at 20 °C). This is below the the cut-off value of 4 given in the CLP Regulation and discussed in the ECHA Guidance on the application of the CLP Criteria (2015) and it indicates a low bioaccumulation potential of cymoxanil.

2.9.2.1.1 Estimated bioaccumulation

Based on the measured log Pow (0.67 - 0.59 at 20 °C) cymoxanil is considered to have a low bioaccumulation potential.

2.9.2.1.2 Measured partition coefficient and bioaccumulation test data

No experimental data are available

2.9.2.2 Acute aquatic hazard [equivalent to section 11.5 of the CLH report template]

2.9.2.2.1 Acute (short-term) toxicity to fish

Table 75: **Summary of relevant information on acute fish toxicity**

Method	Species	Test material	Results[1] (mg/L) $L(E)C_{50}$	Key or Supportive study[1]	Remarks	Reference
Cymoxanil						
OECD 203, ISO 7346-1 Static test GLP	*Oncorhynchus mykiss*	Cymoxanil Batch No 52 Purity 99.1%	26 (im)	Not acceptable as a.s.concentrations were not maintained	96-hour New study	RAR B.9.2.1.1., (2009a) 487930

OECD 203, EPA 72-1 Static test GLP	*Oncorhynchus mykiss*	Cymoxanil Batch No DPX-T3217-113 Purity 97.8%	61 (mm)	Acceptable	96-hour Study from the original DAR	RAR B.9.2.1.2., (1993a) HLR 735-92
OECD 203, EPA 72-1 Static test GLP	*Lepomis macrochirus*	Cymoxanil Batch No DPX-T3217-113 Purity 97.8%	**29 (mm)**	Key Acceptable	96-hour Study from the original DAR	RAR B.9.2.1.3., (1993b) HLR 834-92
EPA 72-3 Flow-through test GLP	*Cyprinodon variegatus*	Cymoxanil Batch No DPX-T3217-113 Purity 97.8%	>47.5 (mm)	Acceptable	96-hour Study from the original DAR	RAR B.9.2.1.4., ,(1996a) HLO 634-96
OECD 203, EEC C.1 Semi-static test GLP	*Brachydanio rerio*	Cymoxanil Batch No 98020 Purity 96.5%	56 (nom)	Not acceptable study as a.s. concentrations were not maintained and not all concentrations were analysed	96-hour New study	RAR B.9.2.1.5., (1998) IMW-98-0018-01
OECD 204, Flow-through test GLP	*Oncorhynchus mykiss*	Cymoxanil Batch No DPX-T3217-113 Purity 97.8%	1.2 (mm) NOEC 0.22 (mm)	Acceptable, should be treated as additional information as this type of studies are not longer conducted, in DAR was used to evaluate chronic toxicity	21 day Study from the original DAR	RAR B.9.2.2.1., (1992) HLR 545-92
metabolites						
OECD 203, EPA 72-1, EEC C.1 Limit test GLP	*Oncorhynchus mykiss*	IN-T4226 Batch No IN-T4226-1 Purity 99.1%	>111 (mm)	Acceptable	96-hour Study from the original DAR	RAR B.9.2.1.7., (2002a) 9386
OECD 203, EEC C.1, ISO 7346-1 Limit test GLP	*Oncorhynchus mykiss*	IN-T4226 Batch No EPP/ISN 62/4 Purity 99.1%	>100 (nom)	Acceptable	96-hour New study	RAR B.9.2.1.8., (2010a) 492653
OECD 203, EEC C.1 Limit test GLP	*Oncorhynchus mykiss*	IN-T4226 Batch No PR08120303JR Purity 99%	>53 (gmm)	Acceptable	96-hour New study	RAR B.9.2.1.9., (2009) 49711230

OECD 203, EPA 72-1, EEC C.1 Limit test GLP	*Oncorhynchus mykiss*	IN-U3204 Batch No IN-U3204-009 Purity 94.4%	>97 (mm)	Acceptable	96-hour Study from the original DAR	RAR B.9.2.1.10., (2002a) 9558
OECD 203, EEC C.1, ISO 7346-1 Limit test GLP	*Oncorhynchus mykiss*	IN-U3204 Batch No EPP/ISN 89.14 Purity 100%	>100 (nom)	Acceptable	96-hour New study	RAR B.9.2.1.11., (2010b) 492652
OECD 203, EEC C.1, Limit test GLP	*Oncorhynchus mykiss*	IN-U3204 Batch No PR0901033/7 Purity 98%	>100 (nom)	Acceptable	96-hour New study	RAR B.9.2.1.12., (2009a) 48672230
OECD 203, EPA 72-1, EEC C.1 Limit test GLP	*Oncorhynchus mykiss*	IN-KQ960 Batch No 25215 Purity 94.6%	>120 (nom)	Acceptable	96-hour Study from the original DAR	RAR B.9.2.1.13., (2002c) 9560
OECD 203, EEC C.1, ISO 7346-1 Limit test GLP	*Oncorhynchus mykiss*	IN-KQ960 Batch No EPP/ISN 1148 Purity 94.9%	>100 (nom)	Acceptable	96-hour New study	RAR B.9.2.1.14., (2010c) 492651
OECD 203, EPA 72-1, EEC C.1 Limit test GLP	*Oncorhynchus mykiss*	IN-KQ960 Batch No PR0901037/41 Purity 98%	**23.923 (mm)**	Key Acceptable	96-hour New study	RAR B.9.2.1.15., (2009b) 49731230
OECD 203 Limit test GLP	*Oncorhynchus mykiss*	IN-R3274 Batch No HHAC-009-01-1 Purity 98.8%	>88 (twa)	Acceptable	96-hour New study	RAR B.9.2.1.16., (2010d) 491259
OECD 203 EEC C.1 Limit test GLP	*Oncorhynchus mykiss*	IN-W3595 Batch No PR090161/3R Purity 95%	>100 (nom)	Acceptable	96-hour New study	RAR B.9.2.1.19., (2009c) 49721230
OECD 203, EEC C.1, ISO 7346-1 Limit test GLP	*Oncorhynchus mykiss*	IN-W3595 Batch No EPP/OZ 098 Purity 100%	>100 (nom)	Acceptable	96-hour New study	RAR B.9.2.1.18., (2010e) 492650
OECD 203, EPA 72-1, EEC C.1 Limit test GLP	*Oncorhynchus mykiss*	IN-W3595 Batch No IN-W3595-004 Purity 98.8%	>130 (mm)	Acceptable	96-hour Study from the original DAR	RAR B.9.2.1.17., (2002b) 9384

OECD 203 EEC C.1 Limit test GLP	*Oncorhynchus mykiss*	IN-KP533 Batch No PR0810782T4 Purity 98.4%	>100 (nom)	Acceptable	96-hour New study	RAR B.9.2.1.20., (2009c) 52551230
OECD 203 EEC C.1 Limit test GLP	*Oncorhynchus mykiss*	M5 (AS999) Batch No PR09094747JR P90 Purity 98%	>83 (mm)	Acceptable	96-hour New study	RAR B.9.2.1.22., (2009d) 52571230
OECD 203, EEC C.1, ISO 7346-1 Limit test GLP	*Oncorhynchus mykiss*	M5 (AS999) Batch No HHAC-010-00-1 Purity 96%	>100 (nom)	Acceptable	96-hour New study	RAR B.9.2.1.21., (2010f) 493512
OECD 203, EEC C.1 Limit test GLP	*Oncorhynchus mykiss*	IN-R3273 Batch No PR090710125 Purity 95%	>100 (nom)	Acceptable	96-hour New study	RAR B.9.2.1.09., (2009e) 52561230

[1] Indicate if the results are based on the measured or on the nominal concentration
Endpoints in **bold** are the lowest endpoints.

Data on acute toxicity of the active substance cymoxanil and its metabolites to fish were previously submitted and evaluated in the context of the original EU review of this active substance. The data were considered acceptable.
Also, new acute toxicity studies on fish with active substance and metabolites were provided (see Volume 3 (AS), B.9.2.1 section). Five acute toxicity tests with cymoxanil were provided. However, two new acute studies with cymoxanil (RAR B.9.2.1.1., 2009a) and (RAR B.9.2.1.5., 1998) and a study with representative formulation Cymoxanil 45 WG (CTF) were not considered valid as the concentrations of active substance could not be sufficiently maintained. No acute fish study was provided with formulation Dauphin 45 (FDJ023) (SFP). A fish acute toxicity study with formulation Rival duo (Agria SA) was provided however, was not considered as valid.
The studies were conducted on fish with all relevant metabolites for the surface water: IN-W3595, IN-KP533, IN-U3204, IN-R3273, IN-KQ960, IN-T4226, IN-JX915, M5 and IN-R3273.
No studies were conducted on fish with metabolite IN-JX915. Overall, available toxicity data indicate that cymoxanil metabolites are less toxic to fish than the parent compound cymoxanil with the exception of IN-KQ960 metabolite (one study of three fish acute toxicity studies provided with this metabolite showed higher toxicity to compare with active substance cymoxanil). For the metabolite IN-KQ960 toxicity value LC_{50} on *Oncorhynchus mykiss* was 23.923 mg a.s./L (RAR B.9.2.1.15., 2009b).
For cymoxanil LC_{50} value on *Lepomis macrochirus* (RAR B.9.2.1.3., 1993a) was determined to be 29 mg a.s./L.
The acute toxicity data for fish exposed to cymoxanil and its metabolites is considered appropriate and sufficient for classification purposes.
As the lowest derived LC_{50} values for cymoxanil and metabolites are higher than 1 mg/L, cymoxanil or its metabolites are not considered hazardous for fish on acute toxicity.

Summaries of the most relevant acute toxicity fish studies with active substance cymoxanil are provided below:

Study 1

Study reference:

RAR B.9.2.1.2., 1993a, Report No HLR 735-92

Study summary and results:

Test type

Static, acute, 96-hour LC50 of DPX-T3217-113 (Cymoxanil) to rainbow trout, Oncorhynchus mykiss

OECD 203, GLP

Test substance

Cymoxanil, Purity: 97.8%, Batch No: DPX-T3217-113

Administration/exposure

The acute toxicity of Cymoxanil (DPX-T3217-113) to juvenile rainbow trout, *Oncorhynchus mykiss*, was determined in a 96-hour, static, unaerated test.
Treatments consisted of a dilution water control, a pH-adjusted control and nominal concentrations of 19, 32, 54, 90, and 150 mg/L.
Observations were made once daily. Dissolved oxygen, pH, and temperature were measured in the water control and pH-adjusted control, and all test concentrations before fish were added at the beginning of the test and daily thereafter. Total alkalinity, EDTA hardness and conductivity of the dilution water were measured before fish were added at the beginning of the test.
Measured concentrations of the active ingredient Cymoxanil were analysed.
The 72- and 96-hour LC_{50}s and their associated confidence limit were calculated using a moving average-angle method. The NOEC was defined as the highest concentration where no significant effect (dark coloration) was observed.

RESULTS AND DISCUSSION

Dark coloration was observed in fish at the 2 highest levels of 79 and 135 mg a.s./L. Mortalities were observed after 48 hours (40% mortality) at 135 mg/L and after 7 hrs (10% mortality) at 79 mg/L. The 96-hr test results are summarised in the table below.

Mean measured concentration (mg a.s./L)	Mortality after 96 hours (%)[a]	Sublethal effects No. affected/No. alive
Water control	0	0/10
pH-adjusted control	0	0/10
17	0	0/10
28	0	0/10
47	0	10[b]/10
79	100	[c]
135	100	[c]

[a] Ten fish per test concentration at test start;
[b] Dark coloration;
[c] No survivors.

Analytical verification

The tested concentrations of cymoxanil were maintained at ±20% of nominal and over 80% for the mean measured.

Validity criteria were met in accordance with OECD guideline no.203.

CONCLUSION

The 96-hour LC_{50} of Cymoxanil for the rainbow trout was 61 mg a.s./L (based on mean measured concentrations). The corresponding NOEC was 28 mg a.s./L, based on the sublethal effects seen at 47 mg a.s./L. This test is acceptable and the 96h-LC_{50} - 61 mg/L based on mean measured concentrations can be used in the risk assessment.

Study 2

Study reference:

RAR B.9.2.1.3., 1993a, Report No HLR 834-92

Study summary and results:

Test type

96-Hour Acute Toxicity Study in Bluegill sunfish (*Lepomis macrochirus)* with Cymoxanil

OECD 203, GLP

Test substance

Cymoxanil, Purity: 97.8%, Batch No: DPX-T3217-113

Administration/exposure

The acute toxicity of Cymoxanil to juvenile bluegill sunfish, *Lepomis macrochirus*, was determined in a 96-hour, static, unaerated test
Treatments consisted of a dilution water control, a pH-adjusted control, and nominal concentrations of 19, 32, 54, 90, and 150 mg/L.
The pH of the dilution water was adjusted to 6.0 in order to maximise the stability of the test substance and this was not considered biologically significant based on the lack of effects seen in the pH-adjusted control.
Observations were made once daily. Dissolved oxygen, pH, and temperature were measured in the water control and pH-adjusted control, and all test concentrations before fish were added at the beginning of the test and daily thereafter. Total alkalinity, EDTA hardness and conductivity of the dilution water were measured before fish were added at the beginning of the test.
Measured concentrations of the active ingredient Cymoxanil were analysed.
The 96-hour LC_{50} and its associated fiducial interval were calculated using a moving average-angle method. The NOEC was defined as the highest concentration where no significant effect (mortality and/or sublethal) was observed.

RESULTS AND DISCUSSION

All chemical and physical parameters were within expected ranges. A summary of cumulative mortality and sublethal effects is shown below. The 96-hour LC_{50}, based on mean measured concentrations and calculated and was 29 mg Cymoxanil /L (with 95% confidence limits of 22 and 36 mg/L). The NOEC, based on the absence of mortality and/or sublethal effects, was 17 mg Cymoxanil /L (measured).

Mean measured concentration (mg a.s./L)	Mortality after 96 hours (%)[a]	Sublethal effects No. affected/No. alive
Water control	0	0/10
pH-adjusted control	0	0/10
17	0	0/10
29	50	5/5[b]
50	100	[c]
82	100	[c]
150	100	[c]

[a] Ten fish per test concentration at test start;
[b] Lying on the bottom of test vessel and exhibited dark colouration;
[c] No survivors.

Analytical verification

Test solutions including water and buffer control solutions, were analysed from aliquots of samples taken on days 0 and 4 of the study.
Nominal test concentrations were 19, 32, 54, 90, and 150 mg/L. Mean, measured concentrations of the active ingredient in Cymoxanil were 17, 29, 50, 82, and 150 mg/L, respectively.

Validity criteria were met in accordance with OECD guideline no.203.

CONCLUSION

The 96-hour LC_{50} of Cymoxanil in the bluegill sunfish was 29 mg a.s./L (based on mean measured concentrations). The corresponding NOEC was 17 mg a.s./L, based on the mortality and sublethal effects seen at 29 mg a.s./L.

This test is acceptable and the 96h-LC_{50} - 29 mg/L based on mean measured concentrations can be used in the risk assessment.

Study 3

Study reference:

RAR B.9.2.1.4., 1996, Report No HLO 634-96

Study summary and results:

Test type

Acute toxicity of DPX T3217 113 (Cymoxanil) to the sheepshead minnow, Cyprinodon variegatus

US EPA 72-3, GLP

Test substance

Cymoxanil, Purity: 97.8%, Batch No: DPX-T3217-113

Administration/exposure

The acute toxicity of Cymoxanil (purity 97.8%) to sheepshead minnow, *Cyprinodon variegatus*, a brackish-water species, was determined in a 96-hour, flow-through, unaerated test. The test was performed under flow-through conditions with five concentrations of test substance, plus the control and solvent control vessels.
Treatments consisted of a dilution water control (filtered seawater adjusted to between 15 and 16‰ salinity), a solvent control (containing 0.5 mL dimethylformamide/L), and nominal concentrations of 7.5, 13, 21, 30, and 50 mg/L.
Observations were made once daily. Dissolved oxygen, pH, and temperature were measured in the water control and pH-adjusted control, and all test concentrations before fish were added at the beginning of the test and daily thereafter. Total alkalinity, EDTA hardness and conductivity of the dilution water were measured before fish were added at the beginning of the test.
Measured concentrations of the active ingredient Cymoxanil were analysed.
Results of the toxicity test could not be interpreted by standard statistical techniques because greater than 50% survival occurred at all tested concentrations. The no observed effect concentration is the highest concentration of test substance that did not cause toxicant related mortalities or sublethal effects.

RESULTS AND DISCUSSION

Summaries of cumulative mortality and sublethal effects are presented in the table below. No mortality or sublethal effects were observed in either of the two controls or at mean measured concentrations up to and including 11.3 mg a.s./L. With the exception of a single mortality at 24 hours in the 18.8 mg/L treatment, all other mortalities were delayed until after the third day of exposure. After 96 hours mortalities were 5%, 5% and 10% at 18.8, 29.0 and 47.5 mg a.s./L, respectively. Sub lethal effects (lethargic fish swimming at the medium surface) were first seen at the two higher treatments after 48 hours exposure and one, seven and 13 fish were affected after 96 hours at 18.8, 29.0 and 47.5 mg a.s./L, respectively. The 96-hour LC_{50} could not be calculated because mortality was below 50% in all treatments. The NOEC, based on the absence of mortality and/or sublethal effects, was 11.3 mg Cymoxanil /L (measured).

Mean, measured concentrations of Cymoxanil (mg/L)	Cumulative mortality (No. dead/20 at test start[a])				Sublethal effects (No. affected[b]/20 at test start)			
	24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h
Water control	0	0	0	0	0	0	0	0
Solvent control	0	0	0	0	0	0	0	0
6.58	0	0	0	0	0	0	0	0
11.3	0	0	0	0	0	0	0	0
18.8	1	1	1	1	0	0	0	1
29.0	0	0	0	1	0	1	1	7
47.5	0	0	0	2	0	9	9	13

[a] Two replicates per treatment, each with 10 fish;
[b] Fish lethargic and at surface of test media.

Analytical verification

The test media were analysed for Cymoxanil by HPLC at the start and end of the test and the mean measured concentrations were 6.58, 11.3, 18.8, 29.0, and 47.5 mg/L.
The measured concentration of exposure was maintained at ±20% of nominal and over 80% for the mean measured.

Validity criteria were met in accordance with OECD guideline no.203.

CONCLUSION

The 96-hour LC_{50} for the sheepshead minnow was >47.5 mg a.s./L based on mean, measured test concentrations. This test is acceptable and the 96h-LC_{50} - >47.5 mg/L based on mean measured concentrations can be used in the risk assessment

Study 4

Study reference:

RAR B.9.2.2.1., 1992, Report No HLR 545-92

Detailed study summary and results:

Test type

Flow-through, 21-day toxicity of DPX-T3217-113 (Cymoxanil) to rainbow trout, Oncorhynchus mykiss

OECD 204 , GLP

Test substance

Cymoxanil, Purity: 97.8%, Batch No: 19,062-02

Administration/exposure

Five fish were added to each replicate (two replicates per concentration; total 10 fish, except 6 fish were added to replicate A, 0.22 mg/L) using random numbers and were fed Purina Trout Chow (Purina Mills, Inc., St. Louis, MO) once daily during the test. Loading was 0.36 g/L passing through the replicate in 24 hours. Positions of each vessel was assigned randomly.
Five test concentrations, a dilution water control, and a DMF control were prepared and maintained using dilution water and a Mount and Brungs Proportional Diluter constructed of glass and Tygon® tubing (for nonexposed surfaces). Nominal test concentrations were 0.26, 0.64, 1.6, 4.0, 10 mg/L. Test solutions were delivered intermittently (about every 17 minutes) to replicate 7-liter glass exposure chambers [20.5 (length) x 21 (width) x 26 (height) cm; 18-cm liquid depth]; the volume of each replicate was exchanged six times daily.
Mortality in fish and sublethal effects were observed daily. Fish length and weight were measured at test start and test end. Dissolved oxygen and pH were measured in the water control and all test replicates before fish were added at the beginning of the test, twice weekly thereafter, and at the end of the test. Temperature was measured in the water control and all test replicates before fish were added at the beginning of the test, daily thereafter, and at the end of the test. Total alkalinity EDTA hardness and conductivity of the dilution control were measured before fish were added at the beginning of the test, weekly, and at the end of the test.
The 21-day L/EC_x values based on mean measured a.s. concentrations were calculated by probit analysis (mortality) or non-linear regression (weight and length) using the statistical program ToxRat Professional 3.3.0. The NOEC values were determined using Dunnett's and Jonckheere's tests or Kruskal-Wallis' test and Dunn's multiple comparison method.

RESULTS AND DISCUSSION
All physical and chemical parameters were within expected ranges over the 21-day test. A summary of cumulative mortality and sublethal effects is shown in the following table. There was no significant difference between the water and solvent control groups and the effects of exposure to Cymoxanil were therefore assessed in relation to the combined controls. Body weight data showed a similar lack of dose-related response, with significant ($p < 0.05$) reductions relative to the combined controls confined to the two mid-range Cymoxanil concentrations.

Mortality and sublethal effects among rainbow trout, *Oncorhynchus mykiss*, exposed to Cymoxanil for 21 days under flow-through conditions

Mean measured concentration (mg/L)	Mortality after 21 days (%)[a]	Sublethal effects No. affected/No. alive (at 21 days)	Standard length (cm)	Wet weight (g)
Water control	0	0/10	5.5	3.03
DMF control	0	0/10	5.4	2.88
0.22	9	0/10	5.6	3.40
0.50	0	0/10	5.0*	2.20*
1.2	50*	0/5	4.8*	1.86*
2.6	70*	0/3	5.0	2.33
6.8	90*	1/1[b]	5.2	1.92

[a] Ten fish per test concentration at test start, except 11 fish used at 0.22 mg/L;
[b] One surviving fish lying on the bottom;
* Statistically significant from the combined control at $p < 0.05$.

Concentration of DPX-T3217-113 (Cymoxanil) in aquatic test solutions was determined by high performance liquid chromatography (HPLC). Test solutions, nominally 0.26 to 10 mg/L, together with H_2O and DMF control solutions were analysed from aliquots of samples taken on days 0, 7, 14 and 21.
Mean measured test concentrations ranged between 65 and 84% of nominal values and all result were based on these mean measured concentrations.

Concentrations of DPX-T3217 (Cymoxanil) in test solutions

Nominal Concentration (mg/L)	Measured Concentration (mg/L) (individual data points reported)						
	Day 0	Day 7	Day 14	Day 21	Average	Overall Mean	% Nominal
Water Control	<0.070 <0.070 <0.070 <0.070	<0.070 <0.070 <0.070 <0.070	<0.070 <0.070 <0.070 <0.070	<0.070 <0.070 <0.070 <0.070	<0.070 <0.070 <0.070 <0.070	<0.070	-
DMF Control	<0.070 <0.070 <0.070 <0.070	<0.070 <0.070 <0.070 <0.070	<0.070 <0.070 <0.070 <0.070	<0.070 <0.070 <0.070 <0.070	<0.070 <0.070 <0.070 <0.070	<0.070	-
0.26	0.26 0.28 0.20 0.23	0.16 0.18 0.18 0.19	030 0.26 0.31 0.23	0.22 0.11 0.15 0.17	0.24 0.21 0.21 0.21	0.22	84
0.64	0.52 0.61 0.54 0.62	0.48 0.45 0.48 0.48	0.53 0.53 0.52 0.59	0.40 0.40 0.41 0.44	0.48 0.50 0.49 0.53	0.50	78
1.6	1.4 1.3 1.4 1.4	1.1 1.1 1.1 1.1	1.2 1.1 1.0 1.1	1.4 1.3 1.3 1.3	1.3 1.2 1.2 1.2	1.2	77
4.0	3.1 3.1 2.9 2.9	2.5 2.3 2.9 2.7	1.9 2.0 1.9 1.8	2.8 2.7 3.1 3.0	2.6 2.5 2.7 2.6	2.6	65
10	8.8 8.9 8.9 8.9	6.0 6.0 6.1 5.9	4.7 4.6 5.1 4.7	7.1 7.1 7.3 7.1	6.7 6.7 6.9 6.7	6.8	68

Toxicity endpoints of the test item Cymoxanil

Effect measurement (mg/L) (mean measured)	NOEC	MATC	LOEC	L/EC_{10}	L/EC_{20}	L/EC_{50}
21 Day survival	0.50	0.77	1.2	0.38 [0.11-0.67]	0.61 [0.25-0.99]	1.5 [0.94-2.7]
Length at Day 21	0.22	0.33	0.50	0.33 [0.20-0.56]	0.52 [0.31-0.88]	1.2 [0.63-2.3]

Weight at Day 21	0.22	0.33	0.50	0.22 [0.023-2.1]	0.62 [0.059-6.9]	4.6 [0.13-142]

[95% confidence limits]

CONCLUSION

The 21-Day NOEC of Cymoxanil for the rainbow trout, based upon effects on standard length and wet weight, was 0.22 mg a.s./L.

Summary of the acute toxicity fish study with metabolite IN-KQ960 is provided below:

Study reference:

RAR B.9.2.1.15., 2009b, Report No 49731230

Detailed study summary and results:

Test type

96-Hour Acute Toxicity Study in Rainbow trout with IN-KQ960

OECD 203 (1992), GLP

Test substance

IN-KQ960, Purity: 98% , Batch No: PR0901037/41

Administration/exposure

The acute toxicity of IN-KQ960 to juvenile rainbow trout, *Oncorhynchus mykiss*, was determined in a 96-hour, static exposure.
Seven rainbow trout per test group were exposed to an untreated control and nominal IN-KQ960 concentrations of 100, 45.5, 20.7, 9.4, 4.3 mg/L.
The test fish were observed after approximately 0, 2, 24, 48, 72 and 96 hours test duration for sublethal effects and mortality. Dead fish were removed at least once daily and discarded. The water temperature, pH-values and the dissolved oxygen concentrations were determined daily in the test media of all treatment groups.

The LC_{50} at the observation times was calculated by Probit analysis. The software used to perform the statistical analysis was ToxRat Professional, Version 2.10, ToxRat® Solutions GmbH, 2009.

RESULTS AND DISCUSSION

In the control one fish had died after 48 hours of exposure. At nominal concentrations of 4.3, 9.4 and 20.7 mg/L, all fish survived until the end of the experiment and showed no sublethal effects. In the two highest test concentrations of nominal 45.5 and 100 mg test item/L (mean measured 35.9 and 90 mg test item/L) all fish had died within 96 hours of exposure. All biological results are presented in the following table:

Nominal concentration (mg/L)	**Mortality**					
	0 hours	**2 hours**	**24 hours**	**48 hours**	**72 hours**	**96 hours**
Control	0	0	0	1	1	1
4.3	0	0	0	0	0	0
9.4	0	0	0	0	0	0
20.7	0	0	0	0	0	0
45.5	0	0	0	0	1	7
100	0	0	0	1	7	7

Analytical verification

Measured test concentrations of IN-KQ960 in the freshly prepared test solutions on Days 0 and 3 showed measured concentrations to range from 77 – 87% of nominal. In the old test samples on Days 1 and 4 there was a decline in measured concentrations to 67 – 78% of nominal values. Given the decline in measured concentrations in the old test samples, the results of the study were based on the mean measured test concentrations.

Validity criteria were met in accordance with OECD guideline no.203.

CONCLUSION

Test conditions were maintained constant throughout the test.

The tested concentrations of metabolite were not maintained at ±20% of nominal during the test. At day 1 and 4 (after 24 hour of exposure) 78% of the nominal values were determined. Therefore the results were based on mean measured concentrations.

This test is acceptable and the 96h-LC_{50} is 23.923 mg IN-KQ960/L based on mean measured concentrations.

2.9.2.2.2 Acute (short-term) toxicity to aquatic invertebrates

Table 76: **Summary of relevant information on acute aquatic invertebrates toxicity**

Method	Species	Test material	Results[1] (mg/L) $L(E)C_{50}$	Key or Supportive study[1]	Remarks	Reference
cymoxanil						
OECD 202, US EPA 72-2 Static test GLP	*Daphnia magna*	Cymoxanil Batch No DPX-T3217-113 Purity 97.8%	**27 (mm)**	Key Acceptable	48-hour Study from the original DAR	Baer (1993c) HLR 736-92
OECD 202, EU C2 Static test GLP	*Daphnia magna*	Cymoxanil Batch No 98020 Purity 97.5%	>100 (nom)	Not Acceptable as test concentrations were not maintained	48-hour New study	Hooftman et al., (1998) V98.145 (IMW-98-0018-02)
FIFRA 72-3(C) Flow-through test GLP	*Mysidopsis bahia*	Cymoxanil Batch No DPX-T3217-113 Purity 97.8%	>44.4 (mm)	Acceptable	96-hour Study from the original DAR	Boeri (1996a) HLO 632-96
US EPA 72-3(b) Flow-through test GLP	*Crassostrea virginica*	Cymoxanil Batch No H-19062-02 Purity 97.8%	> 46.9 (mm)	Acceptable	96-hour Study from the original DAR	Boeri (1996b) HLO 633-96
metabolites						
OECD 202 Semi-static GLP	*Daphnia magna*	IN-T4226 Batch No IN-T4226-1 Purity 99.1%	>116 (mm)	Acceptable	48-hour Study from the original DAR	Boeri (2002c) 9385
OECD 202 Semi-static GLP	*Daphnia magna*	IN-T4226 Batch No PR08120303JR Purity 99%	>37 (gmm)	Acceptable	48-hour New study	Kuhl, Wydra (2009a) 47903220
OECD 202 Semi-static GLP	*Daphnia magna*	IN-T4226 Batch No EPP/ISN 62/4 Purity 99.1%	>100 (nom)	Acceptable	48-hour New study	Migchielsen (2009b) 487931

OECD 202 Static GLP	*Daphnia magna*	IN-U3204 Batch No PR0901033/7 Purity 98%	>100 (nom)	Acceptable	48-hour New study	Kuhl, Wydra (2009b) 51951220
OECD 202 Static GLP	*Daphnia magna*	IN-U3204 Batch No 24985 Purity 97.1%	100 (mm)	Acceptable	48-hour Study from the original DAR	Samel (2002b) 9557
OECD 202 Static GLP	*Daphnia magna*	IN-U3204 Batch No EPP/ISN 89.14 Purity 100%	>100 (nom)	Acceptable	48-hour New study	Bouwman (2009a) 487932
OECD 202 Static GLP	*Daphnia magna*	IN-KQ960 Batch No PR0901037/41 Purity 98%	>100 (nom)	Acceptable	48-hour New study	Kuhl, Wydra (2009c) 4792322
OECD 202 Static GLP	*Daphnia magna*	IN-KQ960 Batch No EPP/ISN 114B Purity 94.9%	>100 (nom)	Acceptable	48-hour New study	Migchielsen (2009d) 487933
OECD 202 Static GLP	*Daphnia magna*	IN-KQ960 Batch No 25215 Purity 94.6%	**0.8 (mm)**	Key Acceptable	48-hour Study from the original DAR	Samel (2002d) 9559
OECD 202 Static GLP	*Daphnia magna*	IN-R3274 Batch No HHAC-009-01-1 Purity 98.8%	>100 (nom)	Acceptable	48-hour New study	Migchielsen (2009g) 491261
OECD 202 Static GLP	*Daphnia magna*	IN-W3595 Batch No PR090161/3R Purity 95%	>101 (nom)	Acceptable	48-hour New study	Kuhl, Wydra (2009e) 47913220
OECD 202 Static GLP	*Daphnia magna*	IN-W3595 Batch No EPP I ISN 76.9 Purity 99.2%	>100 (nom)	Acceptable	48-hour New study	Migchielsen (2009i) 487934

OECD 202 OPPTS 850.1010 EU C.2 Static GLP	*Daphnia magna*	IN-W3595 Batch No IN-W3595-004 Purity 98.8%	>126(mm)	Acceptable	48-hour Study from the original DAR	Boeri (2002c) 9383
OECD 202 EU C2 ISO IS 6341 Static-limit GLP	*Daphnia magna*	M5 (AS999) Batch No HHAC-010-00-1 Purity 96%	>90.5 (mm)	Acceptable	48-hour New study	Migchielsen (2010g) 493513
OECD 202 Semi static GLP	*Daphnia magna*	Cymoxanil 45% WG Batch No S04 Purity 45.2%	>149 mg prep./$L_{(mm)}$ (>67.4 mg/$L_{(mm)}$)	Acceptable	48-hour	Craig (2005a) ENV7080/090419
OECD 202 Semi static GLP	*Daphnia magna*	Cymoxanil 50 g/L + Propamocarb hydrochloride 400 g/L SC Batch No 10/22042012 Purity 5.2%	EC_{50} > 100 mg prep./ $L_{(nom)}$ (equivelant to: >4.74 mg Cymoxanil /L, >37.76 mg propamocarb HCL/L)	Acceptable	48-hour	Desai (2013a) 502-3-07-5084

[1] Indicate if the results are based on the measured or on the nominal concentration
Endpoints in **bold** are the lowest endpoints.

Data on acute toxicity of the active substance Cymoxanil to aquatic invertebrates were previously submitted and evaluated in the context of the original EU review of this active substance. The data were considered acceptable.
One new study was provided (Hooftman et al., 1998), however it was considered as not valid because test concentrations were not maintained during the test. Also data on marine and estuarine species *Mysidopsis bahia and Crassostrea virginica* were provided. These species were less sensitive to cymoxanil than daphnia.
Data on acute toxicity of relevant water metabolites were provided. The studies were conducted on daphnia with such metabolites: IN-W3595, IN-U3204, IN-R3273, IN-KQ960, IN-T4226, M5.
Available toxicity data indicate that cymoxanil metabolites are less toxic to daphnia than the parent compound cymoxanil with exception for IN-KQ960 metabolites, this metabolite showed higher toxicity to compare with cymoxanil. The lowest endpoint for aquatic invertebrates derived from the study with IN-KQ960 to *Daphnia magna* is EC_{50}= 0.8 mg/L (Samel, 2002d). Furthermore, new studies on *daphnia magna* are provided with representative formulations - Cymoxanil 45 WG (CTF) (Craig, 2005a) and Propamocarb hydrochloride 400 g/L + Cymoxanil 50 g/L SC-Rival duo (Agria SA) (Desai, 2013) (see Volume 3 (CP), B.9.3.1 section).

No acute daphnia study was provided with formulation Dauphin 45 (FDJ023) (SFP).

For the metabolite IN-KQ960 toxicity value LC_{50} on *Daphnia magna* was 0.8 mg a.s./L (Samel, 2002d).
For cymoxanil LC_{50} value on *Daphnia magna* (Baer, 1993c) was determined to be 27 mg a.s./L.
The lowest derived LC_{50} values for cymoxanil is higher than 1 mg/L, however its metabolite IN-KQ960 can be considered acutely toxic to aquatic invertebrates.
The acute toxicity data for aquatic invertebrates exposed to cymoxanil and its metabolites is considered appropriate and sufficient for classification purposes.

Summaries of the most relevant acute toxicity aquatic invertebrates studies with active substance cymoxanil are provided below:

Study 1

Study reference:

Baer, K.N., 1993, Report No HLR 736-92

Detailed study summary and results:

Test type

Static, acute 48-hour EC_{50} of DPX-T3217-113 (Cymoxanil) to *Daphnia magna*
OECD 202, GLP

Test substance

Cymoxanil, Purity: 97.8%, Batch No: DPX-T3217-113

Administration/exposure

Daphnids aged less than 24 hours at the start of the test were exposed at five concentrations of the test item for a period of 48 hours under static conditions. 20 daphnids were used for each treated (19, 32 54, 90, and 150 mg/L) and untreated (control) and buffer control groups, divided in 4 replicates of 5 animals each. No feeding occurred during the test.
The appropriate amount of the test substance was added directly to dilution water and used immediately.
The number of immobilised daphnids was assessed after daily and up to 48 hours from the beginning of the test. The criterion for the effect (immobility) was the inability to swim at least two body lengths in any direction within 15 seconds after gentle prodding with a glass rod. Dissolved oxygen concentrations and pH values were measured in all the test groups and the control and buffer control vessels at the beginning and at the end of the test. The temperature and light intensity in the climatic chamber were recorded.
The analysis of the concentration of Cymoxanil was performed for each test concentration. Samples were taken from each test level and were analysed. The samples were collected at 0 hours from fresh test solutions and at the end of the test from the aged test solutions (end value at 48 hours). Analysis was performed by high performance liquid chromatography (HPLC).
The 24-hour EC_{50} and its associated 95% fiducial interval were calculated using the Probit method. The 48-hour EC_{50} and its associated 95% fiducial interval were calculated using the moving average-angle method**.**

RESULTS AND DISCUSSION

The number of immobilized daphnids and the percentage of immobilization at 24 and 48 hours of exposure are presented in the following table.

Percentage of immobilisation after 24 and 48 hours of exposure

Nominal Concentration (mg/L)	Mean measured Concentration* (mg/L)	No. of replicate	No. of *Daphnia* /group	% Immobility							
				24 hr				48 hr			
Control	-	4	20	0	0	0	0	0	0	0	0
Solvent control	-	4	20	0	0	0	0	0	0	0	0
19	15.09	4	20	0	0	0	0	0	0	0	0
32	26.36	4	20	0	0	0	0	80	80	60	40
54	49.36	4	20	40	60	20	40	100	100	80	80
90	83.70	4	20	20	0	40	20	80	60	80	100
150	137	4	20	60	20	40	40	100	100	100	100

*: mean of measured concentrations at 0 and 48 hr

Analysis of the test solutions (fresh and old) was performed for the active substance Cymoxanil 0 and 48 hours after application. The mean measured value was found to be below the range of ±20% of the nominal, therefore, all study results were based on mean measured concentrations.

All validity criteria specified in OECD Test Guideline No 202 were therefore satisfied and the test is considered valid.

CONCLUSION

The 48-hour EC_{50}, based on mean, measured concentrations, and immobility, was 27 mg/L with. a 95% confidence limit of 20 to 34 mg/L.
This study is considered valid and acceptable for use in the risk assessment.

EC_{50} (*Daphnia magna*, 48h) = 27 mg a.s./L (based on mean measured concentrations)

Study 2

Study reference:

Boeri R.L., 1996, Report No HLO 632-96

Detailed study summary and results:

Test type

Acute Toxicity *Mysidopsis bahia*

FIFRA 72-3(c), GLP

Test substance

Cymoxanil, Purity: 97.8%, Batch No: DPX-73217-113

Administration/exposure

The acute toxicity of DPX-T3217-113 (Cymoxanil) to the mysid, *Mysidopsis bahia*, was tested under flow-through conditions with five concentrations of test substance, a solvent control (0.5 mL/L dimethylformamide) and a dilution water control at a temperature of 22 ± 1.0 °C. The number of surviving organisms and the occurrence of sublethal effects were determined visually and recorded initially and after 24, 48, 72, and 96 hours.

Nominal concentrations of Cymoxanil were: 0 mg/L (control and solvent control), 7.5, 13, 21, 31, and 50 mg/L and mean measured were 0, 6.09, 10.4, 17.6, 26.5, and 44.4 mg/L.

Dissolved oxygen (YSI Model 57 meter), pH (Beckman model pHI 12 meter), salinity (refractometer), and temperature (Beckman model pHI 12 meter) were measured and recorded daily in each test chamber. The temperature in one test vessel was recorded continuously during the test.
Analytical determination of DPX-T3217-113 (Cymoxanil) active ingredient was performed with samples collected from each replicate test vessel after 0 and 96 hours.
Results of the toxicity test could not be interpreted by standard statistical techniques because greater than 50% survival occurred at all tested concentrations. The no observed effect concentration is the highest concentration of test substance that did not cause toxicant related mortalities or cause sublethal effects:

RESULTS AND DISCUSSION

One mortality occurred in the 10.4 mg/L concentrations, but it was not considered to be compound related because no mortality occurred in the 17.6 mg/L concentrations.

Nominal Concentration (mg/L)	Mean measured Concentration* (mg/L)	No. of replicate	No. of Mysid /group	% Mortality				
				0 hr	24 hr	48 hr	72 hr	96 hr
Control	-	2	20	0	0	0	0	0
Solvent control	-	2	20	0	0	0	0	0
7.5	6.09	2	20	0	0	0	0	0
13	10.4	2	20	0	0	0	0	5
21	17.6	2	20	0	0	0	0	0
31	26.5	2	20	0	0	5	5	20
50	44.4	2	20	0	0	10	10	25

*: mean of measured concentrations at 0 and 96 hr

One hundred percent survival occurred in the control and solvent control, and no sublethal effects were noted at any concentration during the exposure period.

Validity criteria were met.

CONCLUSION

The concentration of the test substance was maintained over the test period. Mean measured concentrations were 80 to 89% of nominal values.
The study is acceptable. Hence, the results of the study are acceptable and should be used in the risk assessment.

96-hour EC_{50} for Cymoxanil to *Mysidopsis bahia* was >44.4 mg a.s./L. (based on mean measured concentrations)

Study 3

Study reference:

Boeri, R.L., 1996, Report No HLO 633-96

Detailed study summary and results:

Test type

Acute Flow-Through Mollusc Shell Deposition Test with Cymoxanil

FIFRA 72-3, GLP

Test substance: Cymoxanil, Purity: 97.8%, Batch No: H-19062-02

Administration/exposure

The acute toxicity of Cymoxanil to the eastern oyster, *Crassostrea virginica* was tested under flow-through conditions with five concentrations of test substance, a solvent control (0.5 mL/L dimethylformamide) and a dilution water control at a temperature of 20.9ºC.

The number of surviving organisms and the occurrence of sublethal effects were determined visually and recorded initially and after 24, 48, 72, and 96 hours. At the end of the study, oysters were removed from test vessels and the longest finger of new growth was measured to the nearest 0.1 mm with a Manostat® caliper.
Dissolved oxygen, pH, salinity, and temperature were measured and recorded daily in each test chamber. The temperature in one test vessel was recorded continuously during the test.
The LC_{50} and EC_{50} values could not be interpreted by standard statistical techniques because there was 100% survival and greater than 50% of the control new shell growth at all tested concentrations.

RESULTS AND DISCUSSION

One hundred percent survival occurred in the control and solvent control, and no sublethal effects were noted at any concentration during the exposure period. Water quality parameters were within acceptable limits throughout the study.

Nominal Concentration (mg/L)	Mean measured Concentration* (mg/L)	No. of replicate	No. of Oyster /group	% Mortality				
				0 hr	24 hr	48 hr	72 hr	96 hr
Control	-	2	10	0	0	0	0	0
Solvent control	-	2	10	0	0	0	0	0
7.0	6.32	2	10	0	0	0	0	0
12	9.87	2	10	0	0	0	0	0
20	18.6	2	10	0	0	0	0	0
31	28.2	2	10	0	0	0	0	0
50	46.9	2	10	0	0	0	0	0

*: mean of measured concentrations at 0 and 96 hr

Validity criteria were met.

CONCLUSION

The concentration of the test substance was maintained over the test period. Mean measured concentrations were 82 to 94% of nominal values and concentrations were stable throughout the test.
After 96 h of cymoxanil exposure, control and solvent control oysters produced an average, new shell growth of 2.9 and 3.4 mm, respectively.
The RMS is of the opinion that the results of the study are acceptable and can be used in the risk assessment.
96-hour EC_{50} for DPX-T3217-113 to *Crassostrea virginica* was > 46.9 mg a.s./L.

Summary of the acute toxicity *Daphnia magna* study with metabolite IN-KQ960 is provided below:

Study reference:

Samel, A., 2002, Report No 9559

Detailed study summary and results:

Test type

Static, acute 48-hour EC_{50} of IN-KQ960 to *Daphnia magna*
OECD 202, GLP

Test substance

IN-KQ960, Purity: 94.6%, Batch No: 25215

Administration/exposure

Daphnids aged less than 24 hours at the start of the test were exposed at five concentrations of the test item for a period of 48 hours under static conditions. 20 daphnids were used for each treated (0.56, 1.1, 2.1, 4.3 and 8.7 mg/L) and untreated (control) groups. Four replicate test chambers were used per test concentration with 5 daphnids in each chamber (20 daphnids per concentration). Daphnids were not fed during the test.
The appropriate amount of the test substance was added directly to dilution water and used immediately.
Observations of test organisms were made daily. The criterion for the effect (immobility) was a lack of reaction to application of a gentle stimulus.
Dissolved oxygen concentration, pH, and temperature were measured in all replicates of the control and test substance concentrations. These measurements were taken before daphnids were added at test start, and at test end. Total alkalinity, EDTA hardness, and conductivity of the dilution water control and highest test substance concentration were measured before daphnids were added at the beginning of the test. Test solutions were not aerated during the test and were disposed of in an appropriate manner at test end.
The 48-hour EC_{50}s and 95% confidence interval was calculated by the moving average-angle method based on mean measured IN-KQ960 concentrations. The highest mean measured concentration causing no immobility at test end and the lowest mean measured concentration causing 100% immobility at test end were assessed by visual observation.

RESULTS AND DISCUSSION

The number of immobilized daphnids and the percentage of immobilization at 24 and 48 hours of exposure are presented in the following table.

Nominal Concentration (mg/L)	**Mean measured Concentration* (mg/L)**	**No. of replicate**	**No. of *Daphnia* /group**	**Number of Immobile animals (sublethal effects)**							
				24 hr				**48 hr**			
Control	-	4	20	0	0	0	0	0	0	0	0
0.5	**0.56**	4	20	1 (2L)	1 (1L)	0 (2L)	4 (1L)	2 (3L)	2 (2L)	1 (3L)	4 (1L)
1.0	**1.1**	4	20	3 (2L)	0 (3L)	2 (2L)	1 (2L)	5	1 (2L)	4	4
2.0	**2.1**	4	20	4 (1L)	4 (1L)	3 (2L)	3 (1L)	5	5	5	3
4.0	**4.3**	4	20	1 (3L)	2 (2L)	4	3 (1L)	3 (1L, 1F)	4 (1LF)	4	4 (1L)
8.0	**8.7**	4	20	2 (3L)	3 (2L)	2 (2L, 1F)	3 (2L)	5	4	3 (2L)	3 (1L, 1F)

mean of measured concentrations at 0 and 48 hr
(#): number of animals with sublethal effects – F – floating animal, L- Lethargic animal

The mean measured IN-KQ960 concentrations ranged between 111% and 119% of the targeted nominal concentrations. All measured values of IN-KQ960 were within 1.5 times the lowest value for all replicates within a concentration.

All validity criteria were met

CONCLUSION

The test concentrations were maintained within 20% of nominal values throughout the test.
The study is acceptable for use in the risk assessment.
EC_{50} (*Daphnia magna*, 48h) = 0.8 mg IN-KQ960 /L (based on mean measure concentrations)

2.9.2.2.3 Acute (short-term) toxicity to algae or aquatic plants

Table 77: **Summary of relevant information on acute algae toxicity**

Method	Species	Test material	Results[1] (mg/L) L(E)C_{50}	Key or Supportive study[1]	Remarks	Reference
cymoxanil						
OECD 201, US EPA 123-2, EU Method C.3 GLP	*Raphidocelis subcapitata*	Cymoxanil Batch No DPX-T3217 Purity 97.2%	E_rC_{10} = 0.112 E_rC_{50} = 0.167 NOEC = 0.130 (gmm)	Not acceptable as test concentrations were not maintained	96 hour Study from the original DAR	Boeri (1999) 2498
OECD 201, EU Method C.3 GLP	*Raphidocelis subcapitata*	Cymoxanil Batch No 805 Purity 98.8%	E_rC_{10} = 0.306 E_rC_{50} = 0.617 NOEC = 0.218 (gmm)	Not acceptable as test concentrations were not maintained and not all validity criteria are met	72 hour Study from the original DAR	Bell (1996) 107A(a)/950955
OECD 201, EEC C3, EU Method C.3 GLP	*Raphidocelis subcapitata*	Cymoxanil Batch No m/2 F98/-/026 Purity 97.5%	E_rC_{10} = 0.50 E_rC_{50} = 1.37 NOEC = 0.31 (gmm)	Not acceptable as test concentrations were not maintained and not all validity criteria are met	72 hour New study	Hanstveit (1999) IMW-98-0018-03
US EPA-FIFRA Static test GLP	*Navicula pelliculosa*	Cymoxanil Batch No T3217-113 Purity 97.3%	E_rC_{10} = 0.013 **E_rC_{50} = 0.041** NOEC = 0.0148 (gmm)	Not acceptable as test concentrations were not maintained and not all validity criteria are met	96 hour Study from the original DAR	Hughes (1996a) AMR 4112-96
US EPA-FIFRA Static test GLP	*Anabaena flos-aquae*	Cymoxanil Batch No T3217-113 Purity 97.3%	E_rC_{10} = 0.015 E_rC_{50} = 0.046 NOEC = 0.0116 (gmm)	Not acceptable as test concentrations were not maintained and not all validity criteria are met	96 hour Study from the original DAR	Hughes (1996b) AMR 4109-96
US EPA-FIFRA, 40 CFR GLP	*Lemna gibba*	Cymoxanil Batch No DPX-T3217 Purity 97.27%	E_rC_{10} = 0.124 E_rC_{50} = 0.124 NOEC = 0.124	Not acceptable as test concentrations were not maintained	72 hour Study from original DAR	Leva, Sloman (1999) 2498
OECD 201 Static test GLP	*Raphidocelis subcapitata*	Cymoxanil 50 g/L + Propamocarb hydrochloride 400 g/L SC Batch No 10/22042012 Purity 5.2%	39.78 mg prep/$L_{(nom)}$ (equivelant to: E_rC_{50} =1.89 mg Cymoxanil/L 15.02 mg propamocarb HCL/L)	Acceptable	72 hour	Desai (2013b) 501-3-07-5083
metabolites						

OECD 201, ISO 8692, EU Method C.3 GLP	*Raphidocelis subcapitata*	IN-T4226 Batch No EPP/ISN 62/4 Purity 99.1%	E_rC_{10} >76.1 E_rC_{50} >76.1 NOEC = 1.4 (gmm)	Acceptable	72 hour New study	Migchielsen (2009c) 487936
OECD 201, Static test GLP	*Anabaena flos-aquae*	IN-T4226 Batch No IN-T4226-001 Purity 99.1%	E_rC_{10} = 2.5 E_rC_{50} = 70.3 NOEC = 11.6 (gmm)	Not acceptable as test concentrations were not maintained and not all validity criteria are met	96 hour New study	Dengler (2009a) 23924
OECD 201, EU Method C.3 Static test GLP	*Anabaena flos-aquae*	IN-T4226 Batch No PR08120303J R Purity 99%	E_rC_{10} = 1.4 E_rC_{50} = 20 NOEC = 2.1 (gmm)	Not acceptable as test concentrations were not maintained	72 hour New study	Hoofmann, Wydra (2009a) 47904210
US EPA OPPTS 850.5400 Static test GLP	*Anabaena flos-aquae*	IN-T4226 Batch No IN-T4226-1 Purity 99.1%	E_rC_{10} = 7.1 E_rC_{50} = 22 NOEC = 5.5 (gmm)	Not acceptable as validity criteria were not met	96 hour Study from the original DAR	Sloman (2001a) 487936
OECD 201, ISO 8692, EU Method C.3 Static test GLP	*Raphidocelis subcapitata*	IN-U3204	E_rC_{10} = 13.6 E_rC_{50} = 36.4 NOEC = 9.4 (gmm)	Acceptable	72 hour New study	Bouwman (2009b) 488215
OECD 201 Static test GLP	*Anabaena flos-aquae*	IN-U3204 Batch No IN-U3204-009 Purity 94.4%	E_rC_{10} = 0.37 E_rC_{50} = 0.79 NOEC = 0.20 (gmm)	Not acceptable as test concentrations were not maintained and not all validity criteria are met	96 hour New study	Dengler (2010) 23923
US EPA OPPTS 850.5400 Static test GLP	*Anabaena flos-aquae*	IN-U3204 Batch No IN-U3204-6 Purity 97.1%	E_rC_{10} = 0.307 E_rC_{50} = 3.92 NOEC = 0.20	Not acceptable as validity criteria are not met	96 hour Study from the original DAR	Sloman (2002a) 9207
OECD 201, ISO 8692, EU Method C.3 Static test GLP	*Raphidocelis subcapitata*	IN-KQ960 Batch No EPP/ISN 1148 Purity 94.9%	E_rC_{10} > 100 E_rC_{50} > 100 NOEC > 100 (nom)	Acceptable	72 hour New study	Migchielsen (2009f) 488214
US EPA OPPTS 850.5400 Static test GLP	*Anabaena flos-aquae*	IN-KQ960 Batch No IN-KQ960- 002 Purity 94.6%	E_rC_{10} = n.d E_rC_{50} >110 NOEC <110 (nom)	Not acceptable as not all validity criteria were met	96 hour Study from the original DAR	Sloman (2002b) 9206
OECD 201 Static test GLP	*Anabaena flos-aquae*	IN-KQ960 Batch No IN-KQ960- 003 Purity 90%	E_rC_{10} >120 E_rC_{50} >120 NOEC =120 (nom)	Not acceptable as not all validity criteria were met	96 hour New study	Dengler (2008) 23922

OECD 201, ISO 8692, EU Method C.3 Static test GLP	*Raphidocelis subcapitata*	IN-R3274 Batch No HHAC-009-01-1 Purity 98.8%	E_rC_{10} > 38 E_rC_{50} > 38 NOEC > 10 (gmm)	Acceptable	72 hour New study	Migchielsen (2009h) 491260
OECD 201, ISO 8692, EU Method C.3 Static test GLP	*Raphidocelis subcapitata*	IN-W3595 Batch No EPP/ISN 76.9 Purity 99.2%	E_rC_{10} > 100 E_rC_{50} > 100 NOEC > 100 (nom)	Acceptable	72 hour New study	Migchielsen (2009j) 4882016
OECD 201 Static test GLP	*Anabaena flos-aquae*	IN-W3595 Batch No PR090161/3R Purity 95%	E_rC_{10} = 10.9 E_rC_{50} = 109.9 NOEC = 3.2 (nom)	Acceptable	72-hour New study	Hoffmann, Wydra, (2009b) 47914210
US EPA OPPTS 850.5400 Static test GLP	*Anabaena flos-aquae*	IN-W3595 Batch No IN-W3595-3 Purity 94.2%	E_rC_{10} = 15 E_rC_{50} = 20 NOEC = 5 (nom)	Not acceptable as not all validity criteria are met	96-hour Study from the original DAR	Sloman (2001b) 3748
OECD 201 Static test GLP	*Anabaena flos-aquae*	IN-W3595 Batch No IN-W3595-003 Purity 93.7%	E_rC_{10} = 4.1 E_rC_{50} = 24 NOEC = 0.29 (nom)	Not acceptable as not all validity criteria are met	96-hour New study	Dengler (2009b) 23925
US EPA OPPTS 850.5400 Static test GLP	*Anabaena flos-aquae*	IN-KP533 Batch No IN-KP533-003 Purity 99.4%	E_rC_{10} = 11 (gmm) E_rC_{50} = 17 (gmm) NOEC = 15.1 (gmm)	Not acceptable	96-hour Study from the original DAR	Sloman, (2003) 11163
OECD 201 Static test GLP	*Raphidocelis subcapitata*	M5 (AS999) Batch No 103533-862all Purity 95.3%	E_rC_{10} = 25 E_rC_{50} = 71.2 NOEC= 0.623 (gmm)	Acceptable	72 hour New study	Gilberg, Taoudi (2017a) 16EP1AO
OECD 201 Static test GLP	*Raphidocelis subcapitata*	IN-JX915 Batch No IN-JX915-003 Purity 99.8%	E_rC_{10} = 0.268 **E_rC_{50} = 0.931** NOEC= 0.060 (gmm)	Acceptable	72 hour New study	Gilberg, Taoudi (2017b) 16EP2AO
OECD 201 Static test GLP	*Raphidocelis subcapitata*	IN-R3273 Batch No D105082-129-4 Purity 93.2%	E_rC_{10} =51.7 E_rC_{50} =116 NOEC =6.40 (nom)	Acceptable	72 hour New study	Gilberg, Taoudi (2017c) 16EP3AO

[1] Indicate if the results are based on the measured or on the nominal concentration

Data on toxicity of the active substance cymoxanil and its metabolite to algae and *Lemna* were previously submitted and evaluated in the context of the original EU review of this active substance. The data were considered acceptable in the original EU review.

However, during revaluation these studies were not considered valid as the concentrations of active substance could not be sufficiently maintained and also not all validity criteria according the OECD 201 guideline were met for some algae studies (see Volume 3 (AS), B.9.2.6 and B.9.2.7 sections).
The first study with green algae (Boeri, 1999) (120 hour). Analysis of the test solutions was performed at 0, 72 and 120 hour after applications. Concentrations were between 96 and 106% at 0 hour and <LOQ at 72 and 120 hours.
The second study with green algae (Bell, 1996) (72 hour). Analysis of the test solutions was performed at 0 and 72 hour after applications. Concentrations were between 87 and 95% at 0 hour and <LOD at 72 hours. Also not all validity criteria were met.
The 3 rd study with green algae (Hanstveit, 1999) (72 hour). Analysis of the test solutions was performed at 0 and 72 hour after applications. Concentrations were between 49 % at 0 hour and <LOD at 72 hours. No interim measurements were performed. Also not all validity criteria were met.
The study with *Navicula Pelliculosa* (Hughes, 1996a) *(*120 hour). Analysis of the test solutions was performed at 0 and 120 hour after applications. Concentrations were between 98.6 and 110% at 0 hour and <LOD at 120 hours. No interim measurements were performed. The mean coefficient of variation for section-by-section specific growth rates in the control cultures could not be calculated and hence acceptability of the test against this validity criterion could not be established.
The study with *Anabaena flos-aquae* (Hughes, 1996b) *(*120 hour). Analysis of the test solutions was performed at 0 and 120 hour after applications. Concentrations were between 89 and 94% at 0 hour and <LOD at 120 hours. No interim measurements were performed. Also not all validity criteria were met.
The geometric mean measured concentrations were calculated in all these studies. A value of ½ the LOQ or LOD value was substituted to enable calculation of the geometric mean measured concentration.
However, the current opinion of EFSA (EFSA technical report, 2015) is that using LOD or half of the LOQ to calculate a geometric mean concentration can be supported when intermediate measurements (more than one intermediate point or other information) are available. When only initial and final measurements are available and no concentrations were detected at study end, the use of the LOD or half of LOQ is not supported.
Therefore, these algae studies are not valid and are not used in the risk assessment. A new test was required.
Studies with representative formulations Cymoxanil 45 WG (CTF) and Dauphin 45 (SFP) were not considered valid too as the concentrations of active substance could not be sufficiently maintained.

A study with algae with representative formulation Propamocarb hydrochloride 400 g/L + Cymoxanil 50 g/L SC-Rival duo (Agria SA) was provided.

The applicants were asked to provide a new study with algae. The applicants agreed to perform a new green algae study with Cymoxanil and one with representative product. However, these studies were not submitted until finalisation of this draft RAR.
The studies were conducted on algae with all relevant metabolites for the surface water: IN-W3595, IN-KP533, IN-U3204, IN-R3273, IN-KQ960, IN-T4226, IN-JX915, M5 and IN-R3273. Only a study with metabolite IN_KP533 was not valid because the validity criteria were not met.
Data on toxicity of the active substance cymoxanil to aquatic plants *Lemna gibba* were previously submitted and evaluated in the context of the original EU review of this active substance. The data were considered acceptable in the original EU review.
However, during revaluation this study was not considered valid as the concentrations of active substance could not be sufficiently maintained (see volume 3-B.9 (AS)).
For the metabolite IN-JX915 toxicity value ErC_{50} on *Raphidocelis subcapitata* was 0.931 mg a.s./L (Gilberg, Taoudi, 2017b).
For cymoxanil ErC_{50} on *Navicula pelliculosa* (Hughes,1996a) was determined to be 0.041 mg a.s./L, however this study is not considered valid.
The lowest derived ErC_{50} values for cymoxanil metabolite IN-JX915 can be considered acutely toxic to algae.

Summaries of available algae studies with active substance cymoxanil are provided below for the sake of clarity. However, those studies were not considered valid:

Study 1

Study reference:

Boeri, R.L., 1999, Report No Dupont-2498

Detailed study summary and results:

Test type

Cymoxanil Technical: Growth and Reproduction Test with the Freshwater Alga, Selenastrum capricornutum

OECD 201 (1984), GLP

Test substance: Cymoxanil, Purity: 97.2%, Batch No: DPX-T3217

Administration/exposure

A study was performed to assess the inhibitory effect of Cymoxanil technical on the growth of the unicellular freshwater green alga *Raphidocelis subcapitata* (formerly known as *Pseudokirchneriella subcapitata*; reported as *Selenastrum capricornutum)*. Three replicate algal suspensions were each exposed to nominal concentrations of 0.64, 1.3, 2.5, 5.2 and 10 mg/L for 120 hours. Three replicates without test item were used as blank control and two replicates with the highest test item concentration but without algae were used as abiotic stability controls. Observations of cell growth were recorded daily (24, 48, 72, 96 and 120 hours) to determine the potential effect on growth rate and yield relative to the control.
The analysis of the concentration of Cymoxanil was performed for each test concentration from the mixing vessel at the start of the test, after 72 h of inhibition and from pooled replicates at the end of the inhibition test. Achieved concentration was determined by HPLC with LC detection after dilution with AAP media. For the test end samples, centrifugation was undertaken to remove algal cells prior to analysis.

The EC_x values with 95 % confidence limits were calculated by means of probit analysis using the statistical program ToxRat Professional 3.3.0.
The no observed effect concentration (NOEC) was determined using a one-way analysis of variance (ANOVA) and Dunnett's test (TOXSTAT 3.3).

RESULTS AND DISCUSSION

Mean number of healthy cells, area under the curve and growth rates with the corresponding percent inhibition values are presented in the following tables.

Mean number of healthy cells (10^4 cells mL^{-1}) at each observation time

Nominal concentration (mg/L)	Mean number of cells					
	0 hrs	24 hrs	48 hrs	72 hrs	96 hrs	120 hrs
0.0 (control)	0.3	1.1	5.3	46.5	255.3	428.7
0.64	0.3	1.1	3.1	27.7	136.3	412
1.3	0.3	< 1	2.1	6.6	26.5	193.3
2.5	0.3	< 1	< 1	3.7	6.5	35.1
5.2	0.3	< 1	< 1	1.3	3.1	10.1
10	0.3	< 1	< 1	< 1	< 1	< 1

Growth rate, yield and corresponding %inhibition after 72, 96 and 120 hours of exposure

Nominal Concentration (mg/L)	Growth Rate						Yield					
	72 h		96 h		120 h		72 h		96 h		120 h	
	$\bar{x}$	%	$\bar{x}$	%	$\bar{x}$	%	$\bar{x}$	%	$\bar{x}$	%	$\bar{x}$	%
0.0 (control)	1.681	-	1.686	-	1.453	-	46.2	-	255	-	428	-
0.64	1.505	11	1.529	9	1.445	0	27.4	41	136	47	412	4
1.3	1.024	39	1.692	0	1.294	11	6.3	86	264	-4	193	55
2.5	0.838	50	0.759	55	0.952	35	3.4	93	6.2	98	35	92
5.2	0.479	72	0.581	66	0.698	52	1.0	98	2.8	99	9.8	98
10	0.401	76	0.301	82	0.241	83	0.7	99	0.7	100	0.7	100

$\bar{x}$ Mean value
%: Percentage Inhibition

Measured concentrations of Cymoxanil during the test

Nominal Concentration (mg/L)	Measured Concentration			Geometric Mean Measured Concentration
	0 Hours	72 Hours	120 Hours	

	mg/L	%Nominal	mg/L	mg/L	mg/L	%Nominal
0.0 (Control)	ND	-	ND	-	-	-
0.64	0.662	103	<LOQ	<LOQ	0.100	15
1.3	1.380	106	<LOQ	<LOQ	0.130	10
2.5	2.470	99	<LOQ	<LOQ	0.150	6
5.2	5.100	98	<LOQ	<LOQ	0.190	4
10	9.560	96	<LOQ	<LOQ	0.240	2

ND: Not Detectable; Limit of Quantitation (LOQ) = 0.0765 mg/L

Summary of endpoints

Response Variable	Geometric Mean Measured Concentration (mg/L)			
	EC_{10}	EC_{20}	EC_{50}	NOEC
Growth Rate (0 – 96 h)	0.112 [0.085-0.128]	0.129 [0.106-0.142]	0.167 [0.153-0.183]	0.130

[95% confidence limits]
n.d.: not determined either due to mathematical reasons or value is beyond the tested concentrations by more than factor 1000

CONCLUSION

The validity criteria were met. However, the test concentrations were not maintained within 20% of nominal values throughout the test. The concentrations of the newly prepared solutions were between 96% and 106% of the nominal concentration. After 72 and 120 h of exposure the concentrations were below the detection limits.
No more interim analytical measurements were available.
As test concentrations were not maintained, residues were not presented at the end of exposure period and no interim analytical measurements were available this study is not considered acceptable to be used in the risk assessment.

Study 2

Study reference:

Bell, G., 1996, Report No OXN 107A(a)/950955

Detailed study summary and results:

Test type

Cymoxanil Technical: Algal growth inhibition

OECD 201 (1992), GLP

Test substance

Cymoxanil, Purity: 98.8%, Batch No: 805

Administration/exposure

A study was performed to assess the inhibitory effect of Cymoxanil technical on the growth of the unicellular fresh-water green alga *Raphidocelis subcapitata* (formerly known as *Pseudokirchneriella subcapitata*; reported as *Selenastrum capricornutum*). Three replicate algal suspensions were each exposed to nominal concentrations of 1.0, 2.2, 4.6, 10 and 22 mg/L and the blank control for 72 hours. Three replicates without test item were used as control. Observations of cell growth were recorded daily (24, 48 and 72 hours) to determine the potential effect on growth rate and yield relative to the control.
The analysis of the concentration of Cymoxanil was performed for each test concentration at the start of the test and from pooled replicates at the end of the inhibition test. Achieved concentration was determined by HPLC with LC detection. For the test end samples, centrifugation was undertaken to remove algal cells prior to analysis.

The statistical program ToxRat Professional 3.3.0 was used to determine EC_x values. The EC_x values with 95 % confidence limits were calculated by means of probit analysis (growth rate) or non-linear regression (yield).
The "no-observed effect level" (NOEL) was obtained using Williams' test to compare the percentage inhibition in each treated group with that for the control/solvent control cultures.

RESULTS AND DISCUSSION

Mean number of healthy cells, area under the curve and growth rates with the corresponding percent inhibition values are presented in the following tables.

Mean number of healthy cells (10^4 cells mL^{-1}) at each observation time

Nominal concentration (mg/L)	Mean number of cells		
	24 hrs	48 hrs	72 hrs
0.0 (control)	2.5	1.5	5.8
1.0	2.3	1.4	4.4
2.2	1.9	9.0	3.2
4.6	1.7	6.2	2.2
10	1.6	5.0	6.7
22	1.5	1.7	2.4

Growth rate, yield and corresponding %inhibition after 72 hours of exposure

Nominal concentration (mg/L)	Growth rate		Yield	
	Mean value	% inhibition mean value	Mean value	% inhibition mean value
0.0 (control)	1.239	-	56.3	-
1.0	1.189	4	44.7	21
2.2	1.061	14	30.0	47
4.6	0.933	25	20.0	64
10	0.548	56	5.4	90
22	0.208	83	1.1	98

Measured concentrations of Cymoxanil during the test

Nominal Concentration (mg/L)	Measured Concentration				Geometric Mean Measured Concentration	
	0 Hours		72 Hours			
	mg/L	%Nominal	mg/L	%Nominal	mg/L	%Nominal
0.0 (Control)	ND	-	ND	-	<0.05	-
1.0	0.9509	95	ND	-	0.220	22
2.2	1.947	89	ND	-	0.310	14
4.6	4.018	87	ND	-	0.450	10
10	9.102	91	ND	-	0.670	7
22	20.53	93	ND	-	1.010	5

ND: Not Detectable; Limit of Detection (LOD) = 0.05 mg/L

Summary of endpoints

Response Variable	Geometric Mean Measured Concentration (mg/L)			
	EC_{10}	EC_{20}	EC_{50}	NOEC
Growth Rate (0 – 72 h)	0.306 [0.284-0.327]	0.389 [0.368-0.409]	0.617 [0.597-0.639]	<0.218

[95% confidence limits]

CONCLUSION

Not all validity criteria were met.
The test concentrations were not maintained within 20% of nominal values throughout the test. The concentrations of the newly prepared solutions were between 87% and 95% of the nominal concentration. After 72 h of exposure the concentrations were below the detection limits. No interim analytical measurements were available. Therefore this study was not considered acceptable for use in the risk assessment.

Study 3

Study reference:

Hanstveit, A.O., 1999, Report No IMW-98-0018-03

Detailed study summary and results:

Test type

The determination of the effect of Cymoxanil technical on the growth of the alga *Selenastrum capricornutum*

OECD 201, GLP

Test substance

Cymoxanil, Purity: 97.5%, Batch No: m/2 F98/-/026

Administration/exposure

The toxicity of Cymoxanil Technical to the freshwater green alga *Raphidocelis subcapitata* was determined in a three days growth inhibition test (exposure duration: 68.5 h). The nominal concentrations of Cymoxanil tested were 0, 1.0, 1.8, 3.2, 5.7, 10, 32 and 101 mg/L.
The test included the determination of the test substance concentration in samples of the 1.0, 10 and 101 mg/L test concentrations containing no algal cells at the start and the end of the test. The algal growth was determined by electronic particle counting after approximately 21, 44.5 and 68.5 hours of incubation.
The statistical program ToxRat Professional 3.3.0 was used to determine EC_x values. The EC_x values with 95 % confidence limits were calculated by means of probit analysis (growth rate) or non-linear regression (yield).
The 'no-observed-effect-concentration' (NOEC) was estimated by visual comparison of the measured and calculated growth values of the treated algal suspensions with those of the controls

RESULTS AND DISCUSSION

Cell count, growth rate and yield with the corresponding percent inhibition values are presented in the following tables.

Cell count (mean values, 10^3 cells/mL, corrected for background)

Time (h)	Concentration of Cymoxanil (mg/L)								
	0	**0**	**1.0**	**1.8**	**3.2**	**5.7**	**10**	**32**	**101**
0	10.8	10.7	10.7	10.7	10.7	10.7	10.7	10.7	10.7
21	20.6	21.2	21.0	20.2	20.0	18.2	15.6	13.8	12.6
44.5	104.5	109.9	107.3	96.9	92.1	77.3	47.6	15.4	12.5
68.5	649.4	696.9	725.3	640.4	641.7	504.4	164.0	21.1	14.2

Growth rate, yield and corresponding %inhibition after approximately 72 hours of exposure

Nominal concentration (mg/L)	Growth rate		Yield	
	Mean value (μ)	**% inhibition mean value**	**Mean value (10^4 cells/mL)**	**% inhibition mean value**
0.0 (control)	1.3749	-	66.2	-
1.0	1.404	2	71.5	8
1.8	1.362	1	63.0	5
3.2	1.363	1	63.1	5
5.7	1.282	7	49.4	26
10	0.863	37	15.3	77

Nominal concentration (mg/L)	Growth rate		Yield	
	Mean value (µ)	% inhibition mean value	Mean value (10^4 cells/mL)	% inhibition mean value
32	0.226	83.6	1.0	98
101	0.092	93.3	0.3	100

The measured concentrations Cymoxanil at the start of the test varied between 0.49 and 50 mg/L, amounting to approximately 49% of the nominal concentration. After 68.5 h, the measured concentrations Cymoxanil were < 0.1 mg/L.

Measured concentrations of Cymoxanil during the test

Nominal Concentration (mg/L)	Measured Concentration				Geometric Mean Measured Concentration	
	0 Hours		68.5 Hours			
	mg/L	%Nominal	mg/L	%Nominal	mg/L	%Initial
1.0	0.49	49	ND	-	0.2	20
10	4.5	45	ND	-	0.7	7.0
101	50	50	ND	-	2.2	2.2

ND: Not Detectable; Limit of Detection (LOD) = 0.1 mg/L

Summary of endpoints

Endpoint	Nominal Concentration (mg/L)	Geometric Mean Measured Concentration (mg/L)
Growth Rate		
E_rC_{10} (0-72h)	5.2 [3.7-6.5]	0.50 [0.36-0.63]
E_rC_{20} (0-72h)	7.3 [5.7-8.8]	0.71 [0.55-0.85]
E_rC_{50} (0-72h)	14.1 [12-17]	1.37 [1.16-1.65]
NOEC	3.2	0.31

CONCLUSION

Not all validity criteria were met.
The test concentrations were not maintained within 20% of nominal values throughout the test. The concentrations of the newly prepared solutions were between 45% and 50% of the nominal concentration. After 72 h of exposure the concentrations were below the detection limits. No interim analytical measurements were available. Therefore this study was not considered acceptable for use in the risk assessment.

Study 4

Study reference:

Hughes J.S., 1996, Report No AMR 4112-96

Detailed study summary and results:

Test type

Influence on Growth and Reproduction of *Navicula pelliculosa*

EPA 122-2 and 123-2, GLP

Test substance

Cymoxanil, Purity: 97.3%, Batch No: T3217-113

Administration/exposure

The effects of Cymoxanil, on the growth of freshwater green algae, *Navicula pelliculosa*, were determined in a 120-hour static system. Three replicate algal suspensions were each exposed to nominal concentrations of 31.7, 63.5, 127, 254, 496 and 1012 µg/L. Three replicates without test item were used as control. Observations of cell

growth were recorded at 72, 96 and 120 hours to determine the potential effect on growth rate and yield relative to the control.
The analysis of the concentration of Cymoxanil was performed for each test concentration from the mixing vessel at the start of the test and from pooled replicates at the end of the inhibition test. Achieved concentration was determined by HPLC after dilution with AAP media. For the test end samples, centrifugation was undertaken to remove algal cells prior to analysis.
The EC_x values with 95 % confidence limits were calculated by means of non-linear regression analysis using the statistical program ToxRat Professional 3.3.0.
To determine the NOEC value, Bartlett's test for homogeneity of variances, a one-way analysis of variances (ANOVA) and Dunnett's test were performed on the day 5 cell counts.

RESULTS AND DISCUSSION
Mean number of healthy cells, yield and growth rates with the corresponding percent inhibition values are presented in the following tables.

Mean number of healthy cells (10^4 cells mL^{-1}) at each observation time

Nominal concentration (µg/L)	Mean number of cells		
	72 hrs	96 hrs	120 hrs
0.0 (control)	8.5533	67.2797	243.6
31.7	6.5040	42.4127	214.51
63.5	7.5603	54.4820	206.6
127	4.5733	28.7437	161.2367
254	2.6533	10.3453	94.8733
496	1.8623	5.8543	49.2533
1012	0.7493	2.2113	11.7297

Growth rate, yield and corresponding %inhibition after 72, 96 and 120 hours of exposure

Nominal Concentration (µg/L)	Growth Rate						Yield					
	72 h		96 h		120 h		72 h		96 h		120 h	
	$\bar{x}$	%	$\bar{x}$	%	$\bar{x}$	%	$\bar{x}$	%	$\bar{x}$	%	$\bar{x}$	%
0.0 (control)	1.113	-	1.349	-	1.340	-	8.3	-	67	-	243	-
31.7	1.020	8	1.231	9	1.313	2	6.2	25	42	37	214	12
63.5	1.070	4	1.282	5	1.306	3	7.3	12	54	19	206	15
127	0.900	19	1.097	19	1.241	7	4.3	48	28	58	161	34
254	0.722	35	0.693	49	1.150	14	2.4	72	6.4	90	95	61
496	0.602	46	0.738	45	1.008	25	1.6	81	5.6	92	49	80
1012	0.302	73	0.488	64	0.708	47	0.4	95	1.9	97	11	95

$\bar{x}$: Mean value
%: Percentage Inhibition

The analytical results are reported in the following table.

Measured concentrations of Cymoxanil during the test

Nominal Concentration (µg/L)	Measured Concentration				Geometric Mean Measured Concentration	
	0 Hours		120 Hours			
	µg/L	%Nominal	µg/L	%Nominal	µg/L	%Nominal
0.0 (Control)	ND	-	ND	-	-	-
31.7	35	110	ND	-	11	35
63.5	63.3	100	ND	-	14.8	23
127	125	98	ND	-	21	16
254	252	99	ND	-	29.6	12

496	498	100	ND	-	41. 6	8
1012	1012	100	ND	-	59.3	6

ND: Not Detectable; Limit of Detection (LOD) = 3.47 µg/L

Summary of endpoints

Response Variable	Geometric Mean Measured Concentration (µg/L)			
	EC_{10}	EC_{20}	EC_{50}	NOEC
Growth Rate (0 – 96 h)	13 [12-14]	19 [18-20]	41 [38-44]	14.8

[95% confidence limits]
-: No E_yC_x values could be reliably determined

CONCLUSION

As test concentrations were not maintained, residues were not presented at the end of exposure period and no interim analytical measurements were available and is not possible to confirm whether the study meets the mean coefficient variation criteria for section-by-section specific growth rates in the control cultures according OECD 201 (2011) this study is not considered acceptable for use in the risk assessment.

Study 5

Study reference:

Hughes J.S., 1996, Report No AMR 4109-96

Detailed study summary and results:

Test type

Influence on Growth and Reproduction of *Anabaena flos-aquae*

EPA 122-2 and 123-2, GLP

Test substance

Cymoxanil, Purity: 97.3%, Batch No: T3217-113

Administration/exposure

The effects of Cymoxanil, on the growth of freshwater blue-green algae, *Anabaena flos-aquae*, were determined in a 120-hour static system. Three replicate algal suspensions were each exposed to nominal concentrations of 0.038, 0.076, 0.150, 0.300 and 0.600 mg/L. Three replicates without test item were used as control. Observations of cell growth were recorded at 48, 72 and 120 hours to determine the potential effect on growth rate and yield relative to the control.

The analysis of the concentration of Cymoxanil was performed for each test concentration from the mixing vessel at the start of the test and from pooled replicates at the end of the inhibition test. Achieved concentration was determined by HPLC with UV detection after dilution with AAP media. For the test end samples, centrifugation was undertaken to remove algal cells prior to analysis.

The statistical program ToxRat Professional 3.3.0 was used to determine EC_x values. The EC_x values with 95 % confidence limits were calculated by means of non-linear regression.

To determine the NOEC value, Bartlett's test for homogeneity of variances, a one-way analysis of variances (ANOVA) and Dunnett's test were performed on the Day 5 cell counts. All tests of significance were at $\alpha = 0.05$ (except Bartlett's test, which was at $\alpha = 0.01$).

RESULTS AND DISCUSSION

Mean number of healthy cells, yield and growth rates with the corresponding percent inhibition values are presented in the following tables.

Mean number of healthy cells (x 10^4 cells /mL) at each observation time

Nominal concentration (µg/L)	Mean number of cells		
	48 hrs	72 hrs	120 hrs

0.0 (control)	3.4333	20.633	49.667
38	3.1333	17.533	49.267
76	2.9333	14.800	43.067
150	1.7000	8.4333	33.400
300	0.9667	5.3333	22.700
600	0.5000	2.4667	8.2333

Growth rate, yield and corresponding %inhibition after 72, 96 and 120 hours of exposure

Nominal Concentration (µg/L)	Growth Rate						Yield					
	72 h		96 h		120 h		72 h		96 h		120 h	
	$\bar{x}$	%	$\bar{x}$	%	$\bar{x}$	%	$\bar{x}$	%	$\bar{x}$	%	$\bar{x}$	%
0.0 (control)	0.810	-	1.056	-	1.022	-	3.1	-	20	-	49	-
38	0.775	4	1.016	4	1.018	0	2.8	10	17	15	49	1
76	0.759	6	0.970	8	0.991	3	2.6	16	15	29	43	13
150	0.558	31	0.830	21	0.942	8	1.3	59	8.1	60	33	33
300	0.382	53	0.714	32	0.863	16	0.7	79	5.0	75	22	55
600	0.170	79	0.525	50	0.656	36	0.2	94	2.2	89	7.9	84

$\bar{x}$: Mean value
%: Percentage Inhibition

Measured concentrations of Cymoxanil during the test

Nominal Concentration (µg/L)	Measured Concentration				Geometric Mean Measured Concentration	
	0 Hours		120 Hours			
	µg/L	%Nominal	µg/L	%Nominal	µg/L	%Nominal
0.0 (Control)	ND	-	ND	-	<3.99	-
38	34.0	89	ND	-	11.6	31
76	65.2	86	ND	-	16.1	21
150	138	92	ND	-	23.5	16
300	281	94	ND	-	33.5	11
600	563	94	ND	-	47.4	8
ND: Not Detectable; Limit of Detection (LOD) = 3.99 µg/L						

Summary of endpoints

Response Variable	Geometric Mean Measured Concentration (µg/L)			
	EC_{10}	EC_{20}	EC_{50}	NOEC
Growth Rate (0 – 96 h)	15 [9.8-20]	24 [18-29]	46 [41-51]	11.6

[95% confidence limits]

CONCLUSION

Not all validity criteria are met.
The test concentrations were not maintained within 20% of nominal values throughout the test. The concentrations of the newly prepared solutions were between 86% and 94% of the nominal concentration. After 120 h of exposure the concentrations were below the detection limits. No interim analytical measurements were available.

As test concentrations were not maintained, residues were not presented at the end of exposure period and no interim analytical measurements were available and not all validity criteria were met according OECD 201 (2011) this study is not considered acceptable for use in the risk assessment.

Lemna sp. growth inhibition test

Study reference:

Leva, E.S., 1996, Report No AMR 3775-96

Detailed study summary and results:

Test type

Influence on growth and reproduction of *Lemna gibba* G3

EPA 122-2, GLP

Test substance

Cymoxanil, Purity: 97.27%, Batch No: T3217-113

Administration/exposure

The effects of Cymoxanil, on the growth and reproduction of the aquatic monocotyledonous plant, *Lemna gibba*, were investigated in an exposure to nominal concentration of 800 µg a.s./L.
Fronds of *Lemna gibba* were exposed to nominal concentrations of 0 (control) and 800 µg a.s./L for fourteen days without test medium renewal. The effect was expressed in terms of percent inhibition in frond number and biomass relative to the blank control on day fourteen of the study.
The pH of the test media was measured in each test concentration and the control at the start and end of each treatment period, each recovery period and at the end of the test as well as the water temperature. Analysis of achieved concentration for media was undertaken at the start and end of the exposure. Samples were analysed using HPLC.
The computer programs used for the statistical analyses were written and validated within DuPont Agricultural Products. The mean frond number and biomass on Day 14 for the test concentration were expressed relative to the blank control. All calculations were based on the geometric mean measured concentration.

RESULTS AND DISCUSSION
Mean frond numbers and biomass, with corresponding percent inhibition are presented in the following tables.

Number of Healthy Fronds by Exposure Period

Concentration	Rep.	Day 0	Day 2	Day s	Day 7	Day 9	Day 12	Day 14
Blank Control	**1**	15	29	84	181	284	406	556
	2	15	30	85	189	263	397	499
	3	15	31	86	159	245	380	475
	4	15	29	81	136	277	411	500
Mean		**15.00**	**29.75**	**84.00**	**166.25**	**267.25**	**398.50**	**507.50**
Std. Error		**0.00**	**0.48**	**1.08**	**11.91**	**8.61**	**6.82**	**17.17**
800 µg/L	**1**	15	31	91	177	248	402	486
	2	15	33	82	157	242	408	496
	3	15	33	98	187	282	431	543
	4	15	37	110	176	242	386	556
Mean		**15.00**	**33.25**	**95.25**	**174.25**	**253.50**	**406.75**	**520.25**
Std. Error		**0.00**	**1.32**	**5.91**	**6.27**	**9.61**	**9.32**	**17.22**

Mean frond numbers & biomass

Nominal Conc. (µg/L)	No. fronds±s.d.							% I	Mean Biomass (mg) ±s.d.	% I
	Day 0	Day 2	Day 5	Day 7	Day 9	Day 12	Day 14		Day 0-14	
0 (control)	15 ± 0	29.75 ± 0.48	84.00 ± 1.08	166.25 ± 11.91	267.25 ± 8.61	398.50 ± 6.82	507.50 ± 17.17	--	40.73 ± 0.34	--
800	15 ± 0	33.25 ± 1.32	95.25 ± 5.91	174.25 ± 6.27	253.50 ± 9.61	406.75 ± 9.32	520.25 ± 17.22	-2.5%	42.42 ± 0.59	-4.2%

s.d. standard deviation

Mean measured concentrations (µg/L) of Cymoxanil in the exposure solutions

Test item nominal (µg/L)[1]	Day 0		Day 14		Geometric mean (µg/L)
	Mean measured (µg/L)	% Nominal	Mean measured (µg/L)	% Nominal	
0 (control)	<LOD	-	<LOD	-	-
800	700	88	<LOD	n.d.	124.1
800 (control – no Lemna)	n/s [2]	-	<LOD	n.d.	-

[1] DPX-T3217-113 (Cymoxanil) contains 97.3% DPX-T3217

[2] No sample submitted on day 0

The geometric mean was calculated from the available measured test concentrations. For samples whose analyzed test concentration was <LOD, the LOD (22 µg/L) was used in the calculation.

Summary of endpoints

Parameter	Cymoxanil, µg/L (geometric mean measured)			
	Growth Rate (frond no.)	Yield (frond no.)	Growth Rate (dry weight)	Yield (dry weight)
EC_{50}	>124	>124	>124	>124
EC_{20}	>124	>124	>124	>124
EC_{10}	>124	>124	>124	>124
NOEC	124	124	124	124

CONCLUSION

The validity criteria were met.
However, the test concentrations were not maintained within 20% of nominal values throughout the test. The concentration of the newly prepared solutions was 80% of the nominal concentration. After 14 day of exposure the concentrations were below the detection limits. No interim analytical measurements were available. Therefore this study is not considered acceptable to be used in the risk assessment.

Summary of algae study with metabolite IN-JX915 is provided below:

Study reference:

Gilberg D., Taoudi, M., 2017, Report No 16EP2AO

Detailed study summary and results:

Test type

The determination of the effect of IN-JX915 on the growth of the alga *Selenastrum capricornutum*

OECD 201, GLP

Test substance

IN-JX915, Purity: 99.8%, Batch No: IN-JX915-003

Administration/exposure

The effects of IN-JX915-003 metabolite on the growth of freshwater green algae, *Raphidocelis subcapitata (*former name: *Pseudokirchneriella subcapitata)*, were determined in a 72-hour static system. Three replicates each of nominal concentrations of IN-JX915-003 metabolite were tested: 0.04, 0.127, 0.40, 1.27 and 4 mg/L. Additionally, the test organisms were exposed under control conditions (six replicates with non-treated test medium). Growth was determined daily based on determination of the cell number per volume test solution (cell concentration).

Analysis of the test solutions (fresh and spent) for the determination of the content of IN-JX915-003 metabolite was performed in samples taken at 0, 24, 48 and 72 hours after application. Achieved concentrations were between 78.6 and 91.9% in the fresh samples and decrease after 72 hours exposure at between 28.8 – 33.2%. The substance was determined to be not stable under the test conditions. Therefore, the evaluation of effect for the test item was based on the geometric mean measured concentrations.

In this study the effect EC_x values with respect to growth rate and yield were evaluated by the Shapiro-Wilk's Test and Range-to-standard-deviation-ratio Test for normal distribution for normal distribution, and by Levene's Test for homogeneity of variances. The Williams Multiple Sequential t-test and the Welch-t test for inhomogeneous variances was applied to find out whether there were significant differences between the growth of algae in the controls and the algae exposed to the test item concentration.

Probit and Weibull analyses using linear maximum likelihood regression was used to determine the concentration-response function. The statistical software package ToxRat 2.10 Professional was used for these calculations.

RESULTS AND DISCUSSION

Cell count, growth rate and yield with the corresponding percent inhibition values are presented in the following tables.

Mean number of healthy cells (10^4 cells mL^{-1}) at each observation time

Nominal concentration (mg/L)	Mean number of cells		
	24 hrs	48 hrs	72 hrs
0.0 (control)	2.96	26.29	129.51
0.040	2.96	25.93	118.19
0.127	2.72	24.52	124.15
0.400	2.9	22.42	104.98
1.27	2.55	10.08	15.19
4.00	1.47	1.62	1.75

Growth rate, yield and corresponding %inhibition after 72 hours of exposure

Nominal concentration (mg/L)	Growth rate		Yield	
	Mean value ± std. deviation	% inhibition mean value	Mean value ± std. deviation	% inhibition mean value
0.0 (control)	1.8519 ± 0.01759	0.00	129.01 ± 6.802	0.00
0.040	1.8207 ± 0.03352	1.69	117.69 ± 12.053	8.77
0.127	1.8373 ± 0.03076	0.79	123.65 ± 11.141	4.15
0.400	1.7820 ± 0.01593	3.77	104.48 ± 5.007	19.01
1.27	1.1167 ± 0.14311	39.70	14.69 ± 6.877	88.51
4.00	0.4165 ± 0.02200	77.51	1.25 ± 0.116	99.03

Measured concentrations of IN-JX915-003 metabolite during the test

Nominal Concentration (mg/L)	Measured Concentration				Geometric Mean Measured Concentration	
	0 Hours		72 Hours			
	mg/L	%Nominal	mg/L	%Nominal	mg/L	%Nominal

0.0 (Control)	ND	-	ND	-	<0.05	-
0.040	0.0334	84	0.0115	29	0.0196	49
0.126	0.0990	79	0.0364	29	0.0600	48
0.400	0.352	88	0.118	30	0.204	51
1.27	1.16	91	0.420	33	0.698	55
4.00	3.43	86	1.17	29	2.00	50

ND: Not Detectable; Limit of Detection (LOD) = 0.0012 mg/L

Summary of endpoints

Endpoint	**Effect concentration (geometric mean measured) mg/L**	**72 hours**
Growth Rate	EC_{10} (95% confidence intervals)	0.268 (0.214 – 0.320)
	EC_{20} (95% confidence intervals)	0.411 (0.349 – 0.469)
	EC_{50} (95% confidence intervals)	0.931 (0.852 -1.02)
	LOEC	0.204
	NOEC	0.060

CONCLUSION

The validity criteria were met.
The test concentrations were not maintained within 20% of nominal values throughout the test. The concentrations of the newly prepared solutions were between 79 - 91% of the nominal concentration. At 72 hours there was a significant decline in measured test concentrations to 29 – 33% of nominal. Therefore, the test item concentration was based on the geometric mean measured concentration.

This study is considered acceptable for use in the risk assessment.

2.9.2.2.4 Acute (short-term) toxicity to other aquatic organisms

Cymoxanil and its metabolites are not likely to partition into sediments. As neither cymoxanil nor any of its metabolites partitioned significantly to sediment (see Volume 3 (AS), B.8 section), therefore no toxicity test with the sediment dwelling midge *Chironomus* spp. was deemed necessary for cymoxanil and its metabolites.

2.9.2.3 Long-term aquatic hazard [equivalent to section 11.6 of the CLH report template]

2.9.2.3.1 Chronic toxicity to fish

Table 78: **Summary of relevant information on chronic fish toxicity**

Method	**Species**	**Test material**	**Results[1] (mg/L)**		**Key or Supportive study[1]**	**Remarks**	**Reference**
			NOEC	**L(E)C_{10}**			
OECD 210, EPA 72-4 Flow-through test GLP	*Oncorhynchus mykiss*	Cymoxanil Batch No DPX-T3217-113 Purity 97.3%	> 0.12 (mm)	> 0.12 (mm)	Not acceptable as not all validity criteria are met	ELS Study from the original DAR	RAR B.9.2.2.3., (1997) HLO 1013-96
EPA 72-4 Flow-through test	*Cyprinodon variegatus*	Cymoxanil Batch No DPX-	0.0942 (mm)	0.093 (mm)	Acceptable	ELS Study from the original	RAR B.9.2.2.5., (1996)

GLP		T3217-113 Purity 97.8%				DAR	HLO 913-96
OECD 210, Semi-static test GLP	*Brachydanio rerio*	Cymoxanil Batch No m/2 F98/-026 Purity 97.5%	0.86 (mm)	0.2 (mm)	Not acceptable as test concentrations were not maintained	ELS New study	RAR B.9.2.2.7., (1998) IMW-98-0018-05
OECD 210, EPA 72-4, EPA-540/9-86-138 Flow-through test GLP	*Oncorhynchus mykiss*	Cymoxanil Batch No DPX-T3217-113 Purity 97.3%	**0.044 (mm)**	-	Key Acceptable	ELS Study from the original DAR	RAR B.9.2.2.9., (1996) HLR 411-96

[1] Indicate if the results are based on the measured or on the nominal concentration
Endpoints in bold are the lowest endpoints used for classification purpose.

Data on chronic toxicity of the active substance cymoxanil to fish were previously submitted and evaluated in the context of the original EU review of this active substance. The data were considered acceptable.
Also, a new chronic toxicity study on *Brachydanio rerio* (RAR B.9.2.2.7., 1998) with active substance was provided (see Volume 3 (AS), B.9.2.2 section). However, this study is not valid and not used in the risk assessment.

The lowest endpoint for fish is derived from the ELS study with *Oncorhynchus mykiss* (RAR B.9.2.2.9., 1996).

NOEC (*Oncorhynchus mykiss*, 90-day) = 0.044 mg cymoxanil/L.
The long-term toxicity data for fish exposed to cymoxanil is considered appropriate and sufficient for classification purposes.

Summaries of available Fish early-life stage (FELS) toxicity studies with active substance cymoxanil are provided below

Study 1

Study reference:

RAR B.9.2.2.3., 1997, Report No HLO 1013-96

Detailed study summary and results:

Test type

Early life-stage toxicity to rainbow trout, Oncorhynchus mykiss

OECD 210, GLP

Test substance

Cymoxanil, Purity: 97.3%, Batch No: DPX-T3217-113

Administration/exposure

The early life-stage toxicity of DPX-T3217-113 (Cymoxanil) to the rainbow trout, *Oncorhynchus mykiss*, was conducted under unaerated, flow-through conditions with six concentrations of test substance and a dilution water control at 10 ± 2°C.
Nominal concentrations of the active ingredient were tested: 0 µg/L (control), 1.0, 2.5, 6.5, 16, 40, and 120 µg/L DPX-T3217-l 13 (Cymoxanil). Mean measured concentrations of test substance were ND (not detected at or above the limit of detection of 0.065 µg/L; control), 0.98, 2.4, 5.7, 15, 38, and 120 µg/L. The test media were analysed for Cymoxanil by HPLC/UV at test initiation, every 7 days, and at test termination. Two replicates, each containing 40 fertilised embryos (80 total), were used per concentration, and the volume of each replicate (approximately 15 L) was exchanged on average 7.6 times daily. The test was conducted at a temperature of 9.0 to 11.8°C and a photoperiod of 16 hours light. Dissolved oxygen, pH, temperature, and conductivity were

measured daily in each replicate test vessel. On Day 35, larvae were thinned to 15 larvae/replicate (two replicates/treatment) and the fish were fed three times daily with newly-hatched brine shrimp (*Artemia* sp.). The number of surviving organisms and the occurrence of sublethal effects were determined daily. In addition, body length and wet weight of all surviving fish were measured at the end of the test.
In order to determine the NOEC, data sets were initially subjected to Shapiro Wilks and Bartlett's tests to confirm normal distribution and homogeneous variances. Where both requirements were satisfied, one-way ANOVA was applied, followed by Dunnett's test to compare the effects of Cymoxanil treatments against the control. Otherwise, a non-parametric analysis (Kruskal and Wallis' test) was applied.
L/EC_x values were estimated following inspection of the generated data.

RESULTS AND DISCUSSION
Water quality parameters were within acceptable limits throughout the test. Hatching, survival, and growth data are summarised in the following table. No sublethal effects were observed at any time during the test. Exposure to Cymoxanil had no effect on the onset or duration of hatching, on the timing of swim-up or on survival at hatch and at the end of the test. The most sensitive endpoint (*i.e*. the only endpoint where statistically significant ($p < 0.05$) differences were identified between the control and any of the Cymoxanil treatments), was the total mean length of surviving fish, although the highest concentration tested caused only a slight (4%) reduction relative to the control group. The concentration at which there was no statistically significant impact on body length was 0.98 µg a.s./L. However, the biologically significant NOEC is considered to be higher than this because:
1) There was no evidence of effects at the population level following continuous 97-day exposure to all concentrations up to and including 120 µg Cymoxanil /L;
2) There was no evidence of a monotonic relationship between reduced mean body length and increasing concentration from 2.4 to 120 µg a.s./L. Fish exposed to 2.4 µg a.s./L (mean length: 47.2 mm, reduced by 6% compared to the control) were shorter than those at 120 µg a.s./L (mean length: 48.4 mm).
3) Despite the slight reductions in body length, there were no corresponding reductions of mean wet weights: mean wet weights at all Cymoxanil treatment levels were equal to or up to 10% greater than the mean control value. None of the mean wet weights was significantly different from the control group and the highest mean weights were recorded at the two highest exposure concentrations, indicating that there was no treatment-related impact on overall fish size.
In addition, the apparent effect on mean body length was not reflected in either the 21-day test with juvenile *O. mykiss* or the early life-stage test with *L. macrochirus* in which both species were exposed to higher Cymoxanil concentrations.

Effects of exposure to Cymoxanil on survival and growth (hatch to test end) of rainbow trout, *Oncorhynchus mykiss*, in a 97-day early life stage test

Mean measured concentration (µg a.s./L)	Day of hatch[a]		First day swim-up[a]	Survival (%)[a]		Total length[b] (mm)	Wet weight[b] (g)
	Start	End		At hatch	At test end		
Control	32	35	45	67.5	100	50.4	1.41
0.98	33	35	45	66.3	100	49.0	1.45
2.4	33	35	44	62.5	100	47.2*	1.44
5.7	33	35	44	66.3	100	47.2*	1.45
15	32	35	45	61.3	100	48.6*	1.41
38	32	34	45	63.8	100	48.4*	1.55
120	32	33	45	66.3	100	48.4*	1.54

[a] Mean of 2 replicates.
[b] Mean values from pooled replicate data.
* Significantly different from control at $p < 0.05$.

In view of the very slight reduction in total length, that was unrelated to dose over a 60-fold concentration range and unaccompanied by any other indication of significant overall reduction of fish size, combined with the weight of evidence provided by other studies, the NOEC based on biologically significant effects is considered to be 120 µg Cymoxanil /L, the highest concentration tested.
Based on the data generated, L/EC_{10}, L/EC_{20} and L/EC_{50} values for hatchability, swim-up, survival, total length and wet weight were all estimated to be greater than 120 µg Cymoxanil /L, the highest concentration tested.
Test solutions (nominally 1.0 to 120 µg/L) including water control solutions, were analysed from aliquots of samples taken on days 0 to 97 (and for every 7 days) of the study and the mean results are shown in the table below.

Mean measured concentrations of Cymoxanil during the test

Nominal test item concentration (µg/L)	Mean measured concentration (µg/L)	% Nominal
Control	ND[a]	-
1.0	0.98	98%
2.5	2.4	96%
6.5	5.7	88%
16	15	94%
40	38	95%
120	120	100%

[a] ND = none detected at or above the analytical detection limit (0.65 µg/L)

Toxicity endpoints

Biological Endpoint	Mean Measured Concentration (µg/L)					
	NOEC	LOEC	MATC	L/EC_{10}	L/EC_{20}	L/EC_{50}
Survival of embryos at hatch	120	>120	>120	>120	>120	>120
Time to hatch	120	>120	>120	>120	>120	>120
Time to swim up and first feeding	120	>120	>120	>120	>120	>120
Surviving fish at test end	120	>120	>120	>120	>120	>120
Healthy fish at test end	120	>120	>120	>120	>120	>120
Total Length	120	>120	>120	>120	>120	>120
Wet Weight (blotted)	120	>120	>120	>120	>120	>120

CONCLUSION

Validity criteria were not fulfilled. Consequently, the study is not considered acceptable.

Study 2

Study reference:

RAR B.9.2.2.5., 1996, Report No HLO 913-96

Detailed study summary and results:

Test type

Early life-stage toxicity with the sheepshead minnow

USEPA 72-4, GLP

Test substance

Cymoxanil, Purity: 97.8%, Batch No: DPX-T3217-113

Administration/exposure

The early life-stage toxicity of DPX-T3217-113 (Cymoxanil) to the sheepshead minnow (*Cyprinodon variegatus*), was conducted under unaerated, flow-through conditions with five concentrations of test substance and a dilution water control at 30 ± 1°C.

Nominal concentrations of the active ingredient were tested: 0 mg/L (control), 0.058, 0.10, 0.20, 0.40, and 0.80 mg/L DPX-T3217-l 13 (Cymoxanil). Mean measured concentrations of test substance were ND (not detected at or above the limit of detection of 0.0400 mg/L; control), 0.0581, 0.0942, 0.178, 0.364 and 0.767 mg/L. The test media were analysed for Cymoxanil by HPLC/UV at test initiation, every 7 days, and at test termination. Two replicates, each containing 40 fertilised embryos (80 total), were used per concentration, and the volume of each replicate (approximately 7 L) was exchanged on average 22 times daily. The test was conducted at a temperature of 29.2 to 31.0°C and a photoperiod of 16 hours light. Dissolved oxygen, pH, temperature, and conductivity were measured daily in each replicate test vessel. On Day 4, larvae were thinned to 15 larvae/replicate (two replicates/treatment) and the fish were fed two or three times daily with newly-hatched brine shrimp (*Artemia* sp.). The number of surviving organisms and the occurrence of sublethal effects were determined daily. In addition, body length and wet weight of all surviving fish were measured at the end of the test.

Results of the toxicity test were interpreted by standard statistical techniques (U.S. EPA, 1988), when warranted. Computer methods (TOXSTAT 3.3; Gulley, et. al., 1990) were used to analyse the data.
A Chi-square test was used to determine that the data were normally distributed and a one-way analysis of variance (ANOVA) and Dunnett's "t" test were used to compare treatment and control means. Survival and sublethal effects data were arc sine [square root(Y)] transformed prior to statistical analysis.
The L/EC_x values based on mean measured a.s. concentrations were calculated by probit analysis or non-linear regression using the statistical program ToxRat Professional 3.3.0.

RESULTS AND DISCUSSION
Water quality parameters were within acceptable limits throughout the test. Hatching, survival, and growth data are summarised in the following table:

Summary of hatch, survival, and growth (hatch to test end) of Cymoxanil in an early life stage test with sheepshead minnow, *Cyprinodon variegatus*

Mean measured concentration (mg/L)	Day of hatch[a]		Survival (%)[a]						Total length[b] (mm)	Wet weight[b] (mg)
	Start	End	At hatch	+ 7 d	+ 14 d	+ 21 d	+ 28 d	+ 32 d		
Control	3	4	98	100	97	97	97	93	20.6	160
0.0581	3	4	100	97	90	90	90	90	19.6	140
0.0942	3	4	96	90	83	80	80	80	19.1	142
0.178	3	4	100	67*	67*	60*	60*	60	20.7	187
0.364	3	4	96	57*	33*	30*	27*	23*	21.3	212
0.767	3	4	94	27*	7*	7*	7*	7*	21.6†	205†

[a] Mean of 2 replicates.
[b] Mean values from pooled replicate data (except the 0.767 mg/L treatment where only one replicate contained live fish at test-end).
* Live normal fish significantly different from control at $p < 0.05$.
† Excluded from statistical analysis due to disparate survival in the two replicates of this treatment.

No mortalities occurred after 48 hours of exposure in all replicates at all tested concentrations. Time to hatch, start and end, was also the same for each treatment and the control, so statistical analyses of these endpoints were not warranted.
The mortality of test organisms after 48 hours of exposure was not statistically analysed due to 100% survival in all replicates at all tested concentrations. The time to first feeding was not statistically analysed because all fish fed when first presented with food.
Because no survival occurred in one of the two replicates of the highest treatment (0.767 mg/L), data from this treatment were assumed to be different from the control and not included in the statistical analyses of the length and weight data. Statistically significant dose-response related reductions in survival were observed in the 0.178 and 0.364 mg/L test concentrations 7, 14, 21 and 28 Days after hatching.
No statistically significant dose-response related reductions in length or weight data were observed at the test concentrations 0.0581, 0.0942, 0.178 or 0.364 mg/L.
No sublethal effects were observed in the control or the 0.0581 mg/L treatments. A lack of equilibrium, lethargy and erratic swimming were observed intermittently, but were confined to four fish of the 0.767 mg/L treatment group on Day 4 and a single fish among the 0.364 mg/L treatment group on the day of termination. Otherwise, observations of sublethal effects were confined to subjective assessments of reduced size by comparison with the control fish in the 0.0942 – 0.767 mg/L test groups. However, measurements made at termination showed there were no statistically significant differences in mean length or weight of survivors of the Cymoxanil treatments, compared to the control group.
The most sensitive biological endpoint was survival, and the NOEC, based on significant effects on survival on Days 7, 14, 21 and 28, was 0.0942 mg/L.
The determination of the content of Cymoxanil in the test solutions showed a recovery range of 84% to 114% with the exception of the 0.20 mg/L Replicate 2, 0.40mg/L Replicate 2 and 0.80 mg/L Replicate 1 samples on Day 0 which gave a recovery of 75%, 76% and 74% respectively. These slightly low measured concentrations were considered not to affect the integrity of the study given that the other replicate vessel concentrations were within the acceptable range of 80% - 120% of nominal values and that the overall mean measured concentrations for these vessels were also within the acceptable range. The analytical results are reported in the following table.

Measured concentrations of Cymoxanil during the test

Nominal concentration of Cymoxanil (mg/L)		Measured concentration of Cymoxanil (mg/L)								
		Day 0	Day 7	Day 14	Day 21	Day 28	Day 35	Day 36	Mean	Nominal
0.058	R_1[a]	0.0626	0.0567	0.0583	0.0584	0.0608	0.0507	0.0527	0.0581	100%
	R_2	0.0573	0.0574	0.0590	0.0651	0.0660	0.0553	0.0527		
0.10	R_1	0.0901	0.0909	0.0928	0.0994	0.104	0.0945	0.0882	0.0942	94%
	R_2	0.0873	0.0910	0.0964	0.0973	0.101	0.0886	0.0966		
0.20	R_1	0.176	0.174	0.173	0.183	0.193	0.178	0.182	0.178	89%
	R_2	0.149	0.167	0.180	0.183	0.194	0.175	0.181		
0.40	R_1	0.339	0.359	0.353	0.363	0.388	0.370	0.359	0.364	91%
	R_2	0.303	0.346	0.373	0.376	0.410	0.382	0.379		
0.80	R_1	0.589	0.756	0.757	0.785	-[b]	-	-	0.767	96%
	R_2	0.767	0.769	0.784	0.804	0.805	0.804	0.814		

[a] $R_1 – R_2$ = Replicates 1 and 2

[b] - no analysis due to 100% mortality in test replicate

Toxicity endpoints for the test item Cymoxanil

Endpoint	Effect concentration (mean measured)	mg/L
Percent hatch	EC_{10}	>0.767
	EC_{20}	>0.767
	EC_{50}	>0.767
	NOEC	0.767
32-day post hatch survival	LC_{10}	0.093 [0.012-0.165]
	LC_{20}	0.149 [0.050-0.286]
	LC_{50}	0.370 [0.208-2.90]
	NOEC	0.0942
	LOEC	0.178
	MATC	0.129
Total length	EC_{10}	0.717
	EC_{20}	0.735
	EC_{50}	0.771
	NOEC	0.767
Wet weight	EC_{10}	0.737
	EC_{20}	0.750
	EC_{50}	0.775
	NOEC	0.767
Most sensitive endpoint: Survival	NOEC	0.0942
	LOEC	0.178
	LC_{10}	0.093 [0.012-0.165]

[95% confidence limits]

CONCLUSION

The validity criteria were met

Analytical measurement of the test concentrations confirmed that test concentrations were maintained within 20% of nominal values throughout the test with the exception of the 0.20 mg/L Replicate 2, 0.40 mg/L Replicate 2 and 0.80 mg/L Replicate 1 samples on Day 0 which gave a recovery of 75%, 76% and 74% respectively. Consequently, the study is considered acceptable.

Study 3

Study reference:

RAR B.9.2.2.7., 1998, Report No IMW-98-0018-05

Detailed study summary and results:

Test type

Semi-Static Early Life Stage Test with Cymoxanil Techn. and *Brachydanio Rerio*

OECD 210, GLP

Test substance

Cymoxanil, Purity: 97.5%, Batch No: m/2 F98/-/026

Administration/exposure

The early life-stage toxicity of Cymoxanil to the zebra fish, *Brachydanio Rerio*, was conducted under aerated, semi-static conditions with six concentrations of test substance and a dilution water control at 25 ± 1°C.
Nominal concentrations of the active ingredient were tested: 0 mg/L (control), 0.032, 0.10, 0.32, 1.0, 3.2 and 10 mg Cymoxanil/L. The test media were analysed for Cymoxanil by HPLC/DAD at test initiation, every 7 days, and at test termination. Measured concentrations of Cymoxanil in the freshly prepared test solutions were shown to range from 81% to 94% of nominal values. However, due to the known instability of Cymoxanil in aqueous test medium, analysis of the expired test solutions (48 hours after preparation) showed the concentration of Cymoxanil to be below the analytical detection limit (0.1 mg/L) with only trace amounts being detected in the 10 mg/L test sample. The results of the test have therefore been presented based on the geometric mean measured test concentrations.
Four replicates, each containing 20 fertilised embryos (80 total), were used per concentration, and the volume of each replicate (approximately 800 mL) was exchanged on a semi-static basis with renewal three times per week (Monday, Wednesday and Friday). The test was conducted at a temperature of 24.9 to 25.9°C and a photoperiod of 16 hours light/8 hours dark with a 30-minute transition period. pH and dissolved oxygen concentrations were measured in each replicate test vessel at the start and end of the test, as well as weekly in the fresh and expired test solutions. Temperature was measured in one of the control vessels at the start and end of the test, as well as in the fresh and expired control solution at each replacement time. Immediately after hatching, fish were fed abundantly with rotifers and brine shrimp (*Artemia* sp.). The number of surviving organisms and the occurrence of sublethal effects were determined daily until hatching was complete, and then at each test media renewal time. In addition, body length and dry weight of all surviving fish were measured at the end of the test.
Statistical significance for mortality was determined with a binomial test at the 95% significance level, combining the results of the quadruplicates.
Statistical significance for growth was determined with the two-tailed Dunnett-test with a 95% and 99% significance level. In both cases the observations at each concentration were compared with those of the control. In case of significance at the 99% level only that significance is given. The NOEC for condition was not determined statistically.
The L/EC_x values were calculated by probit analysis using the statistical program ToxRat Professional 3.3.0.

RESULTS AND DISCUSSION

Water quality parameters were within acceptable limits throughout the test. Hatching, survival, and growth data are summarised in the following table.
Exposure to Cymoxanil had no effect on the onset or duration of hatching. At the highest nominal test concentration of 10 mg/L there was a significant effect on hatching with only 50 of the 80 exposed eggs hatching after 4 days. No effects on the number of hatched eggs were observed at any of the other test concentrations.
The survival during the 34-day exposure period at the nominal test concentrations of 0.032, 0.10 and 1.0 mg/L was not significantly different from the control, statistically significant effects were however observed at the nominal test concentrations of 3.2 and 10 mg/L ($p = 0.01$). A statistically significant effect on survival was also observed at the nominal test concentration of 0.32 mg/L ($p = 0.05$), however as this was an extremely low effect (6% reduction in survival as compared to the control) and was within typical mortality rates allowable in the Test Guideline, this was not considered to be attributable to test item exposure and hence was excluded from the determination of the NOEC based on survival. The NOEC and LOEC values for survival were therefore determined to be 1.0 and 3.2 mg/L respectively based on nominal test concentrations.
In terms of growth measurements, there were no statistically significant effects on length at all concentrations, and the only effect on weight was a slight increase at the highest nominal concentration tested of 10 mg/L.

Given the low number of surviving fish at the nominal test concentration of 10 mg/L (five) it is considered likely that the increase in average weight for this test group was an artefact of the weighing process rather than a true effect. As growth increase is not considered as an adverse effect and that it is unlikely that fish would survive at higher test item concentrations, the nominal concentration of 10 mg/L is considered as the NOEC for growth (length and weight).

At the nominal test concentration of 10 mg/L some fish with a slow and/or a disturbed swimming behaviour were observed during the test; some small fish were also observed. However, at the end of the test the remaining fish at 10 mg/L were observed to be in good condition with even an increase in weight over that of the control fish. In the control and all other test concentrations, surviving larvae swam and fed actively throughout the exposure period and no malformations were observed with the exception of a few small fish. These small fish were considered as incidental cases which are a part of normal biological development of zebra fish in a test system and were therefore not considered significant.

Effects of exposure to Cymoxanil on survival and growth (hatch to test end) of zebra fish, *Brachydanio Rerio*, in a 34-day early life stage test

Nominal Concentration (mg/L)	% of eggs hatched after 4 days	% survival after 34 days	Growth measurements		
			Number of surviving fish	Mean Length (cm)	Mean Dry weight (mg)
Control	100	100	80	1.63	4.71
0.032	100	99	79	1.58	3.93
0.10	100	96	77	1.60	4.39
0.32	100	94[1]	75	1.59	4.73
1.0	100	96	77	1.62	4.87
3.2	3.2	86[2]	69	1.64	5.09
10	10	6[2]	5	1.74	10.9[3]

[1] Live fish significantly different from control (p = 0.05)
[2] Live fish significantly different from control (p = 0.01)
[3] Significantly higher than control (p = 0.01)

Measured concentrations of Cymoxanil during the test

Nominal Concentration (mg/L)	Measured Concentration (mg/L)										Geometric Mean Measured Concentration	
	D1[f]	D3[s]	D8[f]	D10[s]	D15[f]	D17[s]	D22[f]	D24[s]	D29[f]	D31[s]	mg/L	%Nom
0.32	0.28	<LOD	0.27	<LOD	0.27	<LOD	0.28	<LOD	0.28	<LOD	0.17	52
1.0	-	-	-	-	-	-	-	-	0.91	<LOD	0.3	30
3.2	2.9	<LOD	2.8	<LOD	2.8	<LOD	2.9	<LOD	2.9	<LOD	0.53	17
10	-	-	-	-	-	-	-	-	8.9	<LOD	0.94	9

Limit of Detection (LOD) = 0.1 mg/L
-: no sample taken
f: fresh sample
s: spent sample

Toxicity endpoints

Endpoint	**Effect concentration (geometric mean measured concentrations)**	**mg/L**
Percent hatch	EC_{10}	1.7 [1.14-2.06]
	EC_{20}	2.3 [1.83-2.82]
	EC_{50}	4.1 [3.20-7.36]
	NOEC	0.86
	LOEC	2.7
34-day post hatch survival	LC_{10}	0.2 [0.02-1.88]
	LC_{20}	0.4 [0.07-2.41]
	LC_{50}	1.7 [0.19-14.83]
	NOEC	0.27
	LOEC	0.86
Total length	EC_{10}	>27
	EC_{20}	>27
	EC_{50}	>27
	NOEC	27
Wet weight	EC_{10}	>27
	EC_{20}	>27
	EC_{50}	>27
	NOEC	27
Most sensitive endpoint: Survival	NOEC	0.27
	LOEC	0.86
	LC_{10}	0.2 [0.02-1.88]

[95% confidence limits]

The validity criteria were met.

CONCLUSION

As test concentrations were not maintained and residues were not presented at the end of the renewal period and at the end of exposure period, this study is not acceptable to be used in the risk assessment.

Study 4

Study reference:

RAR B.9.2.2.9., 1996, Report No HLR 411-96

Detailed study summary and results:

Test type

Early life-stage toxicity to rainbow trout, Oncorhynchus mykiss

OECD 210, GLP

Test substance

Cymoxanil, Purity: 97.3%, Batch No: DPX-T3217-113

Administration/exposure

The early life-stage toxicity of DPX-T3217-113 (Cymoxanil) to the rainbow trout, *Oncorhynchus mykiss*, was conducted under unaerated, flow-through conditions with six concentrations of test substance and a dilution water control at 10 ± 2°C.
Nominal concentrations of the active ingredient were tested: 0 µg/L (control), 1.0, 2.5, 6.5, 16, 40, and 120 µg/L DPX-T3217-l 13 (Cymoxanil). Mean measured concentrations of test substance were ND (not detected at or above the limit of detection of 0.065 µg/L; control), 0.98, 2.4, 5.7, 15, 38, and 120 µg/L. The test media were analysed for Cymoxanil by HPLC/UV at test initiation, every 7 days, and at test termination. Two replicates, each containing 40 fertilised embryos (80 total), were used per concentration, and the volume of each replicate

(approximately 15 L) was exchanged on average 7.6 times daily. The test was conducted at a temperature of 9.0 to 11.8°C and a photoperiod of 16 hours light. Dissolved oxygen, pH, temperature, and conductivity were measured daily in each replicate test vessel. On Day 35, larvae were thinned to 15 larvae/replicate (two replicates/treatment) and the fish were fed three times daily with newly-hatched brine shrimp (*Artemia* sp.). The number of surviving organisms and the occurrence of sublethal effects were determined daily. In addition, body length and wet weight of all surviving fish were measured at the end of the test.

Exposure to DPX-T3217-113 (Cymoxanil technical) had no effect on first day of hatching. Statistically significant differences in percent hatch, survival and abnormalities from hatching to thinning were found at 1.5 mg/L. Delays in last day of hatching and first day of swim-up were significant at 0.59 mg/L and 1.5 mg/L.

100% mortality in fingerlings occurred in the two highest test concentrations (0.59 and 1.5 mg/L) by the end of the test. Survival was significantly reduced at 0.25 mg/L at the end of the test, and abnormalities were significantly higher at 0.11 mg/L.

Mean length and wet weight were significantly reduced at all test concentrations, except 0.59 and 1.5 mg/L where 100% mortality was observed. These values should be treated with caution as (i) the control length data were on average higher than previous ELS studies conducted at the test facility, (ii) excluding control data, there was no significant downward trend in either mean weight or length in the test concentrations themselves.

Statistics

The data were analysed to determine the no observed effect concentration (NOEC), the lowest observed effect concentration (LOEC) and the maximum acceptable toxicant concentration (MATC) with respect to the first and last day of hatching, percent hatch, first day of swim-up, number of dead and abnormal larvae from hatching to thinning (alevin stage), number of dead and abnormal larvae from thinning to test end (fingerling stage), standard length and wet weight (blotted dry) of surviving larvae after 90 days (test end). All analyses were based on mean measured test concentrations.

Embryo viability, larval survival and abnormalities was evaluated using the Cochran-Armitage test in a sequential (step-down) manner i.e. the test was applied to all data from all concentrations and if no significant difference was noted the testing stopped. If a significant difference was found, the highest test concentration data was deleted and the test repeated.

Length and weight data were analysed using ANOVA, considering both between replicate and within replicate variability.

Time to hatching, and time to swim-up data were evaluated using one-way ANOVA.

Results And Discussion

Exposure to DPX-T3217-113 (Cymoxanil technical) had no effect on first day of hatching. Statistically significant differences in percent hatch, survival and abnormalities from hatching to thinning were found at 1.5 mg/L. Delays in last day of hatching and first day of swim-up were significant at 0.59 mg/L and 1.5 mg/L.

100% mortality in fingerlings occurred in the two highest test concentrations (0.59 and 1.5 mg/L) by the end of the test. Survival was significantly reduced at 0.25 mg/L at the end of the test, and abnormalities were significantly higher at 0.11 mg/L.

Mean length and wet weight were significantly reduced at all test concentrations, except 0.59 and 1.5 mg/L where 100% mortality was observed. These values should be treated with caution as (i) the control length data were on average higher than previous ELS studies conducted at the test facility, (ii) excluding control data, there was no significant downward trend in either mean weight or length in the test concentrations themselves.

Water quality parameters were within acceptable limits throughout the test. Hatching, survival, and growth data are summarised in the following table.

Effects of exposure to DPX-T3217-113 (Cymoxanil technical) on survival and growth of rainbow trout, *Oncorhynchus mykiss*, in a 90--day early life stage test

Mean measured concn (mg/L)	Day of hatch[a]		% hatch[a]	Swim-up (1st day)
	Start	End		
Control	29	30	89	42
Control (pH adjusted	28	29	83	42
0.031	28	29	90	42
0.044	28	30	86	41
0.11	28	30	86	42
0.25	29	30	88	45
0.59	29	31*	79	46*
1.5	28	30*	76*	-

Mean	Hatch to thinning[a]	Thinning to test end	Total length[b]	Wet weight[b]

measured concn (mg/L)	**Survival (%)**	**Abnormal[b] (%)**	**Survival (%)**	**Abnormal[b] (%)**	**(cm)**	**(g)**
Control	100	0	100	0	3.6	0.7046
Control (pH adjusted	98	0	100	0	3.6	0.7861
0.031	99	0	100	0	3.3*	0.6534**
0.044	99	4.4	100	0	3.2*	0.6628**
0.11	100	0	97	14*	3.3*	0.6125**
0.25	99	1.4	67*	50*	3.1*	0.6370**
0.59	98	3.3	0*	-	-	-
1.5	71*	4.7*	0*	-	-	-

[a] Mean of 2 replicates
[b] Determined as no. affected/no. alive (%)
* Significantly different from combined controls (<0.05)
** Significant difference from pH adjusted control (p<0.05)

Analytical verification

Analysis of stock solutions gave results in the range 80 to 113% of nominal. Samples of test solutions were taken on days –1, 0, 7, 14, 21, 28, 35, 42, 49, 56, 63, 70, 76, 84 and 90. The results of the lowest nominal test concentration (0.0075 mg/L) were consistently high, despite several adjustments to the test solution delivery system. It was concluded that the diluter was not delivering the correct volume of stock solution despite adjustments.

The mean measured concentrations of DPX-T3217-113 (cymoxanil technical) are shown in the table below.

Mean measured concentrations of DPX-T3217-113 (Cymoxanil technical) during the test

Nominal test item concentration (mg/L)	**Mean measured concentration (mg/L)**	**% Nominal**
Control	ND[a]	-
Control (pH adjusted to 6.9)	ND[a]	-
0.0075	0.031	413
0.038	0.044	116
0.096	0.11	115
0.24	0.25	104
0.60	0.59	98
1.5	1.5	100

[a] ND = none detected at or above the analytical detection limit (0.00098 mg/L). Limit of quantification (LoQ) was determined to be 0.0033 mg/L

Toxicity endpoints

Biological Endpoint	**Mean Measured Concentration (mg/L)**			
	NOEC	**LOEC**	**L/EC_{50}**	**95% CI**
Time to 1st hatch	1.5	>1.5	-	-
Time to last hatch	0.25	0.59	-	-
Time to 1st swim-up[a]	0.25	0.59	-	-
Cumulative no. dead eggs	0.59	1.5	3.8[b]	2.3-15
Survival (no. dead larvae hatch to thinning)	0.59	1.5	1.9[b]	1.7-2.4
No. abnormal larvae (hatch to thinning)	0.59	1.5	NC	-
Survival (no. dead larvae thinning to test end)	0.11	0.25	0.29	0.25-0.36
No. abnormal larvae (thinning to test end)	0.044	0.11	0.24	0.18-0.42
Total length, surviving fingerlings	<0.031[c]	0.031	-	-
Wet weight (blotted), surviving fingerlings	<0.031[c]	0.031	-	-

a No observations in highest test concentration or in 2 of 4 replicates in 0.59 mg/L test concentration.
b Extrapolated beyond the range of concentrations used in this test. Result should be treated with caution
c No data at 0.59 and 1.5 mg/L due to 100% mortality
NC Not calculated as <5% larvae in high dose group exhibited abnormalities

The validity criteria were met.

CONCLUSION

Mean length and weight of surviving fingerlings at test end were significantly lower than control mean values at test concentrations at and above 0.031 mg/L except 0.59 and 1.5 mg/L, where data were not available due to total mortality. However, there was no dose-response relationship for weight and length data. All the test concentration means were significantly lower than the pooled control mean, but excluding control data, there was no significant downward trend in either mean weight or length at the surviving test concentrations.
The report "DPX-T3217-113 (cymoxanil): Early-life stage toxicity to Rainbow trout, *Oncorhynchus mykiss.* Comparison to historical control data" was provided.
A graphical representation of the historical control data and the data gathered in the Kraemer study was generated using Microsoft® Excel.
Statistical analysis of the historical control data and the data generated in the Kraemer study was undertaken using the Two-sample Mann-Whitney U-test Procedure. All statistical analysis was performed using the ToxRatPro® Version 3.3.0 software package.
Length and weight data from control and solvent control groups from three previous Fish Early-Life Stage studies performed at the same laboratory that conducted the study in question were collated to give a total of 147 distinct measurements for length and weight. This historical control data was compared to the control and lowest test concentration data generated from Kraemer (1996).
Both the length and weight data for the controls from the Kraemer study were shown to be statistically different to the historical control data ($\alpha = 0.05$). Data from the lowest test concentration (0.031 mg/L) was shown not to be statistically different from the historical control data.
Taking this information into account, a NOAEC of 0.044 mg/L as considered in the DAR rev.1 (April 2008) is therefore considered a reasonable ecotoxicologically relevant endpoint for this study.

2.9.2.3.2 Chronic toxicity to aquatic invertebrates

Table 79: **Summary of relevant information on chronic aquatic invertebrates toxicity**

Method	**Species**	**Test material**	**Results[1] (mg/L)**		**Key or Supportive study[1]**	**Remarks**	**Reference**
			NOEC	**L(E)C_{10}**			
cymoxanil							
OECD 211 Semi-static test GLP	*Daphnia magna*	Cymoxanil Batch No 160418F Purity 98.4%	0.145 (twa)	**0.0619 (twa)**	Key Acceptable	21 day New study	Kümmich (2017) S17-03158
OECD 202, EPA 72-2 Semi-static test GLP	*Daphnia magna*	Cymoxanil Batch No DPX-T3217-113 Purity 99.9%	0.15 (gmm)	0.14 (gmm)	Acceptable	21 day Study from the original DAR	Baer (1993) HLR 354-93
OECD 202, EPA 72-2 Semi-static test GLP	*Daphnia magna*	Cymoxanil Batch No m/2 F98/-/026 Purity 97.5%	0.31 (gmm)	0.5 (gmm)	Not acceptable as test concentrations were not maintained	21 day New study	Hooftman, Borst (1998) IMW-98-0018-04
metabolites							
OECD 202 Semi-static test GLP	*Daphnia magna*	IN-KQ960 Batch No PR0901037/41 Purity 98%	17.1 (gmm)	**0.9 (gmm)**	Key Acceptable	21 day New study	Kuhl, Wydra (2009) 47925221

OECD 211, OPPTS 850.1300 Static-renewal test GLP	*Daphnia magna*	IN-KQ960 Batch No 25579 Purity 96.2%	0.3 (nom)	>0.3 (nom) The highest concentration tested	Acceptable	21 day Study from the original DAR	Samel (2003) 11971
OECD 211, ISO IS 10706:2000 Semi-static test GLP	*Daphnia magna*	IN-KQ960 Batch No EPP/ISN 114B Purity 94.9%	10 (nom)	>10 (nom) The highest concentration tested	Acceptable	21 day New study	Migchielsen, (2009e) 487935

[1] Indicate if the results are based on the measured or on the nominal concentration

Data on chronic toxicity of the active substance Cymoxanil and its metabolite IN-KQ960 to aquatic invertebrates were previously submitted and evaluated in the context of the original EU review of this active substance. The data were considered acceptable (see volume 3-B.9 (AS)).
Two new daphnia chronic toxicity studies have been submitted. One study is not valid (see Volume 3 (AS), B.9.2.5 section). Furthermore, two new studies on *daphnia magna* are provided with metabolite IN-KQ960. Available data indicate that metabolite IN-KQ960 is less toxic to *daphnia magna* than the active substance cymoxanil.
The lowest endpoint for IN-KQ960 derived from the study with *Daphnia magna* is $EC_{10\ (reproduction)}$ = 0.9 mg/L (Kuhl, Wydra, 2009d).
The lowest endpoint for aquatic invertebrates is derived from the study with *Daphnia magna* is $EC_{10\ (reproduction)}$ = 0.0619 mg/L (Kümmich, 2017).
The long-term toxicity data for invertebrates exposed to cymoxanil and its metabolites is considered appropriate and sufficient for classification purposes.

Summaries of available chronic toxicity to aquatic invertebrates studies with active substance cymoxanil are provided below:

Study 1

Study reference:

Kümmich F., 2017, Report No S17-03158

Detailed study summary and results:

Test type

Chronic toxicity of Cymoxanil to *Daphnia magna*: semi-static test system for 21 days.
OECD 211 (2012)

Test substance

Cymoxanil, Purity: 98.4%, Batch No: 160418F

Administration/exposure

The objectives of this study were to determine the effects of Cymoxanil Tech. on the survival and reproduction of the water flea *Daphnia magna* under worst case exposure conditions, the No Observed Effect Concentration (NOEC), the Lowest Observed Effect Concentration (LOEC) and the lethal concentrations / effect concentrations causing 50, 20 and 10 % of inhibition ($LC_{50,20,10}$/$EC_{50,20,10}$), if possible. 1st instar juveniles < 24 h old were exposed in a semi-static test system for 21 days.
The reproduction test was performed in a semi-static system, included 10 vessels per test concentration and 20 vessels for the untreated control groups. Each of the vessels contained one neonate (<24h old) *Daphnia magna* in 50 mL test medium. The nominal Cymoxanil concentrations tested were 0.00149, 0.00477, 0.0153, 0.0488, 0.156, 0.500 and 1.60 mg/L.
The initial measured content of Cymoxanil was between 101 % and 134 % of nominal with a mean of 117 % and the aged measured content was between 70 % and 91 % of nominal with a mean of 80 %. The toxicological endpoints were therefore evaluated using nominal concentrations of the test item and actual concentrations of Cymoxanil based on the time weighted average of each concentration level which were determined to be

0.00150, 0.00465, 0.0147, 0.0447, 0.145, 0.476 and 1.51 mg/L.
Temperature, pH-value, oxygen concentration and total hardness of the test solutions (all concentrations and controls), measured every weekday are reported. Light intensity was measured at test start. Additionally, the temperature was continuously monitored via the monitoring system.
NOEC and LOEC were determined by using a multiple comparison method, the values for $EC_{10,20,50}$/$LC_{10,20,50}$ were determined by Probit analysis.

RESULTS AND DISCUSSION

The first offspring in the buffered control was observed on day 8. At test item concentrations of 0.00149, 0.00477, 0.0153, 0.0488 and 0.156 mg/L first offspring was observed on day 8. The first offspring at the concentration level of 0.500 mg/L was observed on day 9. The first offspring in the unbuffered control was observed on day 10. The first offspring in highest test item concentration of 1.60 mg/L was observed on day 13.

Cumulative mortality of parental daphnids during the 21-day exposure period.

Concentration (mg/L)	No of daphnids	Cumulative number of dead parental daphnids on day:												Mortality %
		10	11	12	13	14	15	16	17	18	19	20	21	
Buffered Control	10	0	0	0	0	0	0	0	0	0	0	0	0	0
Unbuffered Control	10	0	0	0	0	0	0	0	0	0	1	1	1	10
0.00149	10	0	0	0	0	0	0	0	0	0	0	0	0	0
0.00477	10	0	1	1	1	2	2	2	2	2	2	2	2	20
0.0153	10	0	0	1	1	1	1	1	1	1	1	1	2	20
0.0488	10	1	1	1	2	2	2	2	2	2	2	2	2	20
0.156	10	0	1	1	1	1	1	1	1	1	1	1	1	10
0.500	10	0	0	0	0	0	0	0	0	0	0	0	0	0
1.60	10	0	0	0	0	0	0	0	0	0	0	0	0	0

* Statistically significant from controls at p<0.05.

Average body lengths (mm) of parental daphnids with the standard deviation (SD) per concentration measured at the end of the test and the percentage reduction of body length relative to the control.

Nominal Concentration (mg/L)	Mean measured Concentration (mg/L)	Adult observation		Reproduction parameters	
		Mortality (%)	Length (mm)	Mean of alive offspring / alive adult at test end	Time to first Brood (days)
Control (buffered)	-	0	4.408	141.3	8
Control (unbuffered)	-	10	4.542	115.9[1)]	10
0.00149	0.00150	0	4.466	176.3[1)]	8
0.00477	0.00465	20	4.470	118.4[1)]	8
0.0153	0.0147	20	4.619	135.5	8
0.0488	0.0447	20	4.454	123.9	8
0.156	0.145	10	4.413	118.6	8
0.500	0.476	0	3.904	63.0*	9
1.60	1.51	0	3.280	2.2*	13

*: statistically significant difference ($p < 0.05$) from pooled control
[1)] was not included for the EC_x calculation

Measured concentrations of Cymoxanil during the test

Nominal Concentration (mg/L)	Mean Measured (mg/L)	Time weighted average [%]
buffered control	-	-
unbuffered control	-	-
0.00149	0.00150	102
0.00477	0.00465	99
0.0153	0.0147	98
0.0488	0.0447	93
0.156	0.145	94
0.500	0.476	97
1.60	1.51	96

- = not calculated; LOQ = 0.000500 mg/L Cymoxanil; LOD = 0.0001500 mg/L Cymoxanil

Toxicity endpoints of the test item Cymoxanil

	Effect concentration of Cymoxanil [mg/L]							
	Mortality of Adults		Alive offspring per adult from test start		Alive offspring per adult from test end		Body length	
	nominal	measured*	nominal	measured*	nominal	measured*	nominal	measured*
NOEC	1.60[1)]	1.51[1)]	0.156[3)]	0.145[3)]	0.156[3)]	0.145[3)]	0.156[3)]	0.145[3)]
LOEC	-	-	0.500[3)]	0.476[3)]	0.500[3)]	0.476[3)]	0.500[3)]	0.476[3)]
LC_{10} /EC_{10}	> 1.60[2)]	> 1.51[2)]	0.0663[4)]	0.0619[4)]	0.190[5)]	0.178[5)]	0.597[5)]	0.565[5)]
LC_{20} /EC_{20}	> 1.60[2)]	> 1.51[2)]	0.133[4)]	0.125[4)]	0.253[5)]	0.238[5)]	1.12[5)]	1.06[5)]
LC_{50} /EC_{50}	> 1.60[2)]	> 1.51[2)]	0.384[4)]	0.362[4)]	0.439[5)]	0.415[5)]	> 1.60[6)]	> 1.51[6)]

- not applicable
[1)] observed value
[2)] Due to no clear dose response relationship no statistical evaluation was performed
[3)] Following Dunnetts-t-test (left-sided, $p<0.05$)
[4)] Probit analysis following Gompertz distribution
[5)] Probit analysis following normal distribution
[6)] Was not statistically evaluated since the inhibition was below 50 %
* Based on the time weighted average of each concentration level of the active ingredient

CONCLUSION

The validity criteria were met.
The concentration of the test substance was maintained over the test period. The initial measured concentrations were between 101% and 91% of nominal and the aged measured concentration was between 70 % and 91 % of nominal with a mean of 80 %. The toxicity was evaluated using nominal concentrations and actual concentrations of cymoxanil based on the time weighted average of each concentration level.
The RMS is of the opinion that the results of the study are acceptable and can be used in the risk assessment.
21 d NOEC for cymoxanil to *Daphnia* was 0.145 mg/L (time-weighted average), EC_{10} for cymoxanil to *Daphnia* was 0.0619 mg/L (time-weighted average).

Study 2

Study reference:

Baer, K.N. 1993, Report No HLR 354-93

Detailed study summary and results:

Test type

Chronic toxicity of DPX-T3217-113 (Cymoxanil) to *Daphnia magna*: 24-hour renewal
OECD 202, US EPA 72-4, GLP

Test substance

Cymoxanil, Purity: 99.9%, Batch No: DPX-T3217-113

Administration/exposure

Chronic toxicity was evaluated by exposing *Daphnia magna* neonates (less than 24 old) to DPX-T3217-113 (Cymoxanil) in a semi-static, unaerated test with test solution renewal daily for 21 days. Nominal concentrations of DPX-T3217-113 (Cymoxanil) were 0.039, 0.078, 0.16, 0.31, 0.63, 1.3, and 2.5 mg/L.
Measured concentrations in freshly prepared test solutions ranged from 87 to 127% of nominal values, however there was a significant decline in measured concentrations in the old test preparations resulting in concentrations between 54 and 91% of nominal. It was therefore considered appropriate to base the results of the test on the geometric mean measured test concentrations which were determined to be 0.03, 0.06, 0.15, 0.30, 0.50, 1.0 and 1.9 mg/L.
Within the 21-day reproduction test, effects on immobility and reproductive outputs were evaluated daily. The length of each surviving adult at test conclusion was measured by use of a calibrated ocular micrometer in the eyepiece of a Wild Heerbrugg M5A dissecting microscope.
Dissolved oxygen and pH were measured daily. Temperature was measured in one replicate of the water and buffer controls plus all test concentrations daily.
The statistical program ToxRat Professional 3.3.0 was used to determine EC_x values. The EC_x values with 95 % confidence limits were calculated by means of probit analysis (immobility) or non-linear regression (reproduction).

RESULTS AND DISCUSSION

A summary including percent adult survival, first day of reproduction, total live young produced, total live young produced per surviving adult, total immobile young produced, and length of surviving adults is presented in the table below:

Summary data for daphnids during the 21-day exposure period to Cymoxanil.

Geomean measured Concentration (mg/L)	No of daphnids	No of immobile *Daphnia*	Mortality %	Day of first brood	Total Young	Young/ Survived Parent	Total Immobile Young	Adult Length (mm)
Water Control	40	2	5%	8	217	58	0.3	3.8
Buffered Control	40	3	8%	8	236	64	5.2	3.8
0.03	40	3	8%	7	234	63	2.4	3.8
0.06	40	5	13%	7	238	67	3.3	3.8
0.15	40	12*	30%	9*	190	65	2.8	3.8
0.3	40	20*	50%	10*	134*	61	3.8	3.7
0.5	40	35*	88%	12*	14*	7.0*	14	3.9
1.0	40	27*	68%	13*	14*	5.1*	8.1	3.4*
1.9	40	34*	85%	16*	0*	0*	0.4*	2.9*

* Statistically significant from controls at $p<0.05$.

Geometric mean, measured concentrations of the active ingredient in DPX-T3217-113 (Cymoxanil) in test solutions were calculated and summarised in the table below:

Measured concentrations of Cymoxanil during the test

Nominal Concentration (mg/L)	Measured Concentration (mg/L) [% Nominal]						Geometric Mean Measured Concentration (mg/L) [% Nominal]
	Day 0	Day 7		Day 14		Day 21	
	New	New	Old	New	Old	Old	
Buffered Control	-	-	-	-	-	-	-
Unbuffered Control	-	-	-	-	-	-	-
0.039	0.0435 [112]	0.0495 [127]	0.0315 [81]	0.0345 [88]	0.021 [54]	0.0245 [63]	0.03 [84]
0.078	0.0785 [101]	0.0955 [122]	0.0625 [80]	0.0745 [96]	0.0445 [57]	0.046 [59]	0.06 [83]
0.16	0.15	0.19	0.14	0.19	0.12	0.145	0.15

	[94]	[119]	[88]	[119]	[75]	[91]	[96]
0.31	0.29	0.34	0.235	0.335	0.21	0.24	0.3
	[94]	[110]	[76]	[108]	[68]	[77]	[87]
0.63	0.55	0.7	0.505	0.64	0.375	0.42	0.5
	[87]	[111]	[80]	[102]	[60]	[67]	[82]
1.3	1.15	1.4	0.925	1.3	0.75	0.79	1.0
	[88]	[108]	[71]	[100]	[58]	[61]	[79]
2.5	2.3	2.5	1.7	2.4	1.4	1.5	1.9
	[177]	[192]	[131]	[185]	[108]	[115]	[147]

- = not calculated; LOD = 0.00089 mg/L Cymoxanil

Measured toxicity endpoints of Cymoxanil

Endpoint	Geometric Mean Measured Concentration (mg/L)	
	Adult Survival	Reproduction
NOEC	0.06	0.15
LOEC	0.15	0.3
EC_{10}	0.23	0.14 [0.11-0.19]
EC_{20}	0.38	0.18 [0.14-0.24]
EC_{50}	1.02	0.29 [0.20-0.41]

[95% confidence limits]

CONCLUSION

The validity criteria were met.

The initial measured concentrations were between 80% and 127% of nominal and the aged measured concentration was between 54% and 80% of nominal. Therefore, ECx values were based on geometric mean measured concentrations.
Thw study is acceptable.
21 d NOEC for cymoxanil to *Daphnia* was 0.15 mg/L (reproduction) (based on geometric mean measured), EC_{10} for cymoxanil to *Daphnia* was 0.14 mg/L (based on geometric mean measured).

Study 3

Study reference:

Hooftman, R.N., Borst, B., 1998, Report No IMW-98-0018-04

Detailed study summary and results:

Test type

Semi-static reproduction test with Cymoxanil Techn. and the crustacean species Daphnia magna
OECD 202, US EPA 72-2, GLP
Test substance

Cymoxanil, Purity: 97.5%, Batch No: m/2 F98/-/026

Administration/exposure

Cymoxanil Techn. was tested for inhibiting effects on reproduction of *Daphnia magna.* A number of 10 *Daphnia* (individually exposed) was used for each test substance concentration and the control. The exposure duration was 21 days and the test was carried out as a semi-static test with replacement of the test solutions three times per week. The nominal concentrations tested were 0.18, 0.32, 0.56, 1.0, 1.8 and 3.2 mg/L DSWL-E was used as control medium.
Actual concentrations of Cymoxanil in the test solutions just after dosing were between 93.8% and 100% of the nominal concentrations (average 98%). To test the stability, cymoxanil concentrations were also analysed in the spent solution just before replacement. They appeared to be below the detection limit (0.1 mg/L). The results of the test were therefore based on the geometric mean measured test concentrations.
Dissolved oxygen and pH were measured daily. Temperature was measured in one replicate of the water and buffer controls plus all test concentrations daily.
The statistical program ToxRat Professional 3.3.0 was used to determine EC_x values. The EC_x values with 95 % confidence limits were calculated by means of probit analysis or non-linear regression.

RESULTS AND DISCUSSION
A summary of the biological results is given in the following table:

Number of surviving daphnia and average cumulative number of young per living female daphnia after 21 days

Nominal concentration Cymoxanil Techn. mg/L	Surviving Daphnia	Cumulative number of young per living female	
		Determined	% of control
0	9	128	100
0.18	9	126[1]	98
0.32	9	103[2]	80
0.56	8	134[1]	104
1.0	9	112[1]	87
1.8	8	93[3]	72
3.2	3	10[3]	8

1: Not significantly less from the control reproduction (two-tailed Dunnett-test; p = 0.05)
2: Significantly less (two-tailed Dunnett-test; p = 0.05) than control reproduction.
3: Significantly less (two-tailed Dunnett-test; p = 0.01) than control reproduction

Measured concentrations of Cymoxanil during the test

Nominal Concentration (mg/L)	Measured Concentration (mg/L)						Geometric Mean Measured Concentration	
	Day 5 (fresh)	Day 7 (spent)	Day 12 (fresh)	Day 14 (spent)	Day 19 (fresh)	Day 21 (spent)	mg/L	%Nom
0.32	0.15	<LOD	0.30	<LOD	0.31	<LOD	0.16	48
1.0	0.48	<LOD	0.97	<LOD	1.0	<LOD	0.28	28
3.2	1.6	<LOD	3.2	<LOD	3.2	<LOD	0.50	16

Limit of Detection (LOD) = 0.1 mg/L

Toxicity endpoints

Endpoint	**Geometric Mean Measured Concentration (mg/L)**	
	Adult Survival	**Reproduction**
NOEC	**0.56**	**0.31**
LOEC	**0.99**	**0.56**
EC_{10}	**0.1 [0-0.21]**	**0.5 [0.43-0.58]**
EC_{20}	**0.2 [0.06-0.4]**	**0.6 [0.49-0.64]**
EC_{50}	**0.8 [0.43-5.89]**	**0.7 [0.58-0.86]**

[95% confidence limits]

The validity criteria were met.

CONCLUSION

The test concentrations were not maintained within 20% of nominal values throughout the test. The concentrations of the newly prepared solutions were between 47% and 100% of the nominal concentration. After 48 h of exposure the concentrations were below the detection limits. Also, not all test concentrations were analytical measured. As test concentrations were not maintained and residues were not presented at the end of the renewal period and at the end of exposure period, this study is not acceptable to be used in the risk assessment.

Summary of the chronic toxicity *Daphnia magna* study with metabolite IN-KQ960 is provided below:

Study reference:

Kuhl, R., Wydra, V., 2009d, Report No 47925221

Detailed study summary and results:

Test type

Semi-static reproduction test with IN-KQ960 and the crustacean species Daphnia magna
OECD 211 (2008)

Test substance

Cymoxanil, Purity: 98%, Batch No: PR0901037/41

Administration/exposure

A prolonged toxicity study was conducted to determine the effect of IN-KQ960 on survival and reproduction of *Daphnia magna*. 1st instar juveniles < 24 h old were exposed in a semi-static test system for 21 days. The nominal concentrations of IN-KQ960 tested were 1.0, 3.2, 10, 32 and 100 mg test item/L.
A total of 10 replicates, each containing one, less than 24-hour old neonate, was tested per concentration (10 neonates/concentration) and the dilution water control. The test media of all test concentrations and of the control were renewed on Monday, Wednesday and Friday. Before each renewal, the test medium was freshly prepared.
In the freshly prepared test media 103 % of the nominal test concentration was found (average for all test concentrations). In the aged test media 33 % of the nominal value was determined (average for all test concentrations) during the test medium renewal periods of 48 and 72 hours. Therefore, the biological results were based on the geometric mean measured test concentrations.
Dissolved oxygen and pH were measured daily. Temperature was measured in one replicate of the water and buffer controls plus all test concentrations daily.
The statistical program ToxRat Professional 3.3.0 was used to determine EC_x values. The EC_x values with 95 % confidence limits were calculated by means of probit analysis or non-linear regression.

RESULTS AND DISCUSSION

A summary of the biological results is given in the following table:

Number of surviving daphnia and average cumulative number of young per living female daphnia after 21 days

Exposure Day	Nominal Concentration (mg/L)					
	Control	**1.0**	**3.2**	**10**	**32**	**100**
21	693	793	718	686	599	464
No. surviving adults	**9**	**10**	**10**	**10**	**8**	**10**
Average number of offspring per surviving adult	**77**	**79.3**	**71.9**	**68.6**	**74.9**	**46.4**

Measured concentrations of Cymoxanil during the test

In the freshly prepared test media 103 % of the nominal test concentration was found (average for all test concentrations). In the aged test media 33 % of the nominal value was determined (average for all test concentrations) during the test medium renewal periods of 48 and 72 hours. Therefore, the biological results were based on the geometric mean measured test concentrations.

Toxicity endpoints of metabolite IN-KQ960

Endpoint	Geometric Mean Measured Concentration (mg/L)	
	Adult Survival	**Reproduction**
NOEC	62.4	17.1
LOEC	>62.4	62.4
EC_{10}	>62.4	0.9 [0-19.5]
EC_{20}	>62.4	5.2 [0.2-131.6]
EC_{50}	>62.4	>62.4

[95% confidence limits]

The validity criteria were met.

CONCLUSION

The study is acceptable and can be used in the risk assessment.

21 d NOEC for IN-KQ960 to *Daphnia* was 17.1 mg/L (based on geometric mean measured), EC_{10} for IN-KQ960 to *Daphnia* was 0.9 mg/L (based on geometric mean measured).

2.9.2.3.3 Chronic toxicity to algae or aquatic plants

Table 80: **Summary of relevant information on chronic algae toxicity**

Method	Species	Test material	Results[1] (mg/L)		Key or Supportive study[1]	Remarks	Reference
			NOEC	L(E)C_{10}			
cymoxanil							
OECD 201, US EPA 123-2, EU Method C.3 GLP	*Raphidocelis subcapitata*	Cymoxanil Batch No DPX-T3217 Purity 97.2%	NOEC = 0.130 (gmm)	E_rC_{10} = 0.112 (gmm)	Not acceptable as test concentrations were not maintained	96 hour Study from the original DAR	Boeri (1999) 2498
OECD 201, EU Method C.3 GLP	*Raphidocelis subcapitata*	Cymoxanil Batch No 805 Purity 98.8%	NOEC = 0.218 (gmm)	E_rC_{10} = 0.306 (gmm)	Not acceptable as test concentrations were not maintained and not all validity criteria are met	72 hour Study from the original DAR	Bell (1996) 107A(a)/950955
OECD 201, EEC C3, EU Method C.3 GLP	*Raphidocelis subcapitata*	Cymoxanil Batch No m/2 F98/-/026 Purity 97.5%	NOEC = 0.31 (gmm)	E_rC_{10} = 0.50 (gmm)	Not acceptable as test concentrations were not maintained and not all validity criteria are met	72 hour New study	Hanstveit (1999) IMW-98-0018-03
US EPA-FIFRA Static test GLP	*Navicula pelliculosa*	Cymoxanil Batch No T3217-113 Purity 97.3%	NOEC = 0.0148 (gmm)	E_rC_{10} = 0.013 (gmm)	Not acceptable as test concentrations were not maintained and not all validity criteria are met	96 hour Study from the original DAR	Hughes (1996a) AMR 4112-96
US EPA-FIFRA Static test GLP	*Anabaena flos-aquae*	Cymoxanil Batch No T3217-113 Purity 97.3%	NOEC = 0.0116 (gmm)	E_rC_{10} = 0.015 (gmm)	Not acceptable as test concentrations were not maintained and not all validity criteria are met	96 hour Study from the original DAR	Hughes (1996b) AMR 4109-96
US EPA-FIFRA, 40 CFR GLP	*Lemna gibba*	Cymoxanil Batch No DPX-T3217 Purity 97.27%	NOEC = 0.124	E_rC_{10} = 0.124	Not acceptable as test concentrations were not maintained	72 hour	Leva, Sloman (1999) 2498
metabolites							
OECD 201, ISO 8692, EU Method C.3 GLP	*Raphidocelis subcapitata*	IN-T4226 Batch No EPP/ISN 62/4 Purity	NOEC = 1.4 (gmm)	E_rC_{10} >76.1 (gmm)	Acceptable	72 hour New study	Migchielsen (2009c) 487936

		99.1%					
OECD 201, Static test GLP	*Anabaena flos-aquae*	IN-T4226 Batch No IN-T4226-001 Purity 99.1%	NOEC = 11.6 (gmm)	E_rC_{10} = 2.5 (gmm)	Not acceptable as test concentrations were not maintained and not all validity criteria are met	96 hour New study	Dengler (2009a) 23924
OECD 201, EU Method C.3 Static test GLP	*Anabaena flos-aquae*	IN-T4226 Batch No PR08120303JR Purity 99%	NOEC = 2.1 (gmm)	E_rC_{10} = 1.4 (gmm)	Not acceptable as test concentrations were not maintained	72 hour New study	Hoofmann, Wydra (2009a) 47904210
US EPA OPPTS 850.5400 Static test GLP	*Anabaena flos-aquae*	IN-T4226 Batch No IN-T4226-1 Purity 99.1%	NOEC = 5.5 (gmm)	E_rC_{10} = 7.1 (gmm)	Not acceptable as validity criteria were not met	96 hour Study from the original DAR	Sloman (2001a) 487936
OECD 201, ISO 8692, EU Method C.3 Static test GLP	*Raphidocelis subcapitata*	IN-U3204	NOEC = 9.4 (gmm)	E_rC_{10} = 13.6 (gmm)	Acceptable	72 hour New study	Bouwman (2009b) 488215
OECD 201 Static test GLP	*Anabaena flos-aquae*	IN-U3204 Batch No IN-U3204-009 Purity 94.4%	NOEC = 0.20 (gmm)	E_rC_{10} = 0.37 (gmm)	Not acceptable as test concentrations were not maintained and not all validity criteria are met	96 hour New study	Dengler (2010) 23923
US EPA OPPTS 850.5400 Static test GLP	*Anabaena flos-aquae*	IN-U3204 Batch No IN-U3204-6 Purity 97.1%	NOEC = 0.20	E_rC_{10} = 0.307	Not acceptable as validity criteria are not met	96 hour Study from the original DAR	Sloman (2002a) 9207
OECD 201, ISO 8692, EU Method C.3 Static test GLP	*Raphidocelis subcapitata*	IN-KQ960 Batch No EPP/ISN 1148 Purity 94.9%	NOEC > 100 (nom)	E_rC_{10} > 100 (nom)	Acceptable	72 hour New study	Migchielsen (2009f) 488214
US EPA OPPTS 850.5400 Static test GLP	*Anabaena flos-aquae*	IN-KQ960 Batch No IN-KQ960-002 Purity 94.6%	NOEC <110 (nom)	E_rC_{10} = n.d (nom)	Not acceptable as not all validity criteria were met	96 hour Study from the original DAR	Sloman (2002b) 9206

OECD 201 Static test GLP	*Anabaena flos-aquae*	IN-KQ960 Batch No IN-KQ960-003 Purity 90%	NOEC =120 (nom)	E_rC_{10} >120 (nom)	Not acceptable as not all validity criteria were met	96 hour New study	Dengler (2008) 23922
OECD 201, ISO 8692, EU Method C.3 Static test GLP	*Raphidocelis subcapitata*	IN-R3274 Batch No HHAC-009-01-1 Purity 98.8%	NOEC > 10 (gmm)	E_rC_{10} > 38 (gmm)	Acceptable	72 hour New study	Migchielsen (2009h) 491260
OECD 201, ISO 8692, EU Method C.3 Static test GLP	*Raphidocelis subcapitata*	IN-W3595 Batch No EPP/ISN 76.9 Purity 99.2%	NOEC > 100 (nom)	E_rC_{10} > 100	Acceptable	72 hour New study	Migchielsen (2009j) 4882016
OECD 201 Static test GLP	*Anabaena flos-aquae*	IN-W3595 Batch No PR090161/3R Purity 95%	NOEC = 3.2 (nom)	E_rC_{10} = 10.9 (nom)	Acceptable	72-hour New study	Hoffmann, Wydra, (2009b) 47914210
US EPA OPPTS 850.5400 Static test GLP	*Anabaena flos-aquae*	IN-W3595 Batch No IN-W3595-3 Purity 94.2%	NOEC = 5 (nom)	E_rC_{10} = 15 (nom)	Not acceptable as not all validity criteria are met	96-hour Study from the original DAR	Sloman (2001b) 3748
OECD 201 Static test GLP	*Anabaena flos-aquae*	IN-W3595 Batch No IN-W3595-003 Purity 93.7%	NOEC = 0.29 (nom)	E_rC_{10} = 4.1(nom)	Not acceptable as not all validity criteria are met	96-hour New study	Dengler (2009b) 23925
US EPA OPPTS 850.5400 Static test GLP	*Anabaena flos-aquae*	IN-KP533 Batch No IN-KP533-003 Purity 99.4%	NOEC = 15.1 (gmm)	E_rC_{10} = 11 (gmm)	Not acceptable	96-hour Study from the original DAR	Sloman, (2003) 11163
OECD 201 Static test GLP	*Raphidocelis subcapitata*	M5 (AS999) Batch No 103533-862all Purity 95.3%	NOEC= 0.623 (gmm)	E_rC_{10} = 25 (gmm)	Acceptable	72 hour New study	Gilberg, Taoudi (2017a) 16EP1AO
OECD 201 Static test GLP	*Raphidocelis subcapitata*	IN-JX915 Batch No IN-JX915-003 Purity 99.8%	**NOEC= 0.060 (gmm)**	**E_rC_{10} = 0.268 (gmm)**	Key Acceptable	72 hour New study	Gilberg, Taoudi (2017b) 16EP2AO

OECD 201 Static test GLP	*Raphidocelis subcapitata*	IN-R3273 Batch No D105082-129-4 Purity 93.2%	NOEC =6.40 (nom)	E_rC_{10} =51.7 (nom)	Acceptable	72 hour New study	Gilberg, Taoudi (2017c) 16EP3AO

[1] Indicate if the results are based on the measured or on the nominal concentration
Endpoints in **bold** are the lowest endpoints.

Data on toxicity of the active substance cymoxanil to algae and *Lemna* were previously submitted and evaluated in the context of the original EU review of this active substance. The data were considered acceptable in the original EU review. The lowest endpoint for algae was derived from the study conducted with cymoxanil on *Navicula pelliculosa* -EC_{10} = 0.013 mg/L (Hughes, 1996a). However, during revaluation these studies were not considered valid as the concentrations of active substance could not be sufficiently maintained and also not all validity criteria according the OECD 201 guideline were met for some algae studies (see Volume 3 (AS), B.9.2.6 and B.9.2.7 sections).
No algae studies were considered valid for the use in the risk assessment and also for classification.
The studies were conducted on algae with all relevant metabolites for the surface water: IN-W3595, IN-KP533, IN-U3204, IN-R3273, IN-KQ960, IN-T4226, IN-JX915, M5 and IN-R3273. Some studies with metabolites were not considered valid because the concentrations of the metabolites could not be sufficiently maintained or the validity criteria were not met (see the table above).
The most sensitive endpoint for metabolites was derived for metabolite IN-JX915 from the study on *Raphidocelis subcapitata* of NOEC = 0.06 mg/L (Gilberg, Taoudi, 2017b).
Data on toxicity of the active substance cymoxanil to aquatic plants *Lemna gibba* were previously submitted and evaluated in the context of the original EU review of this active substance. The data were considered acceptable in the original EU review. However, during revaluation this study was not considered valid as the concentrations of active substance could not be sufficiently maintained (see volume 3-B.9 (AS)).

2.9.2.3.4 Chronic toxicity to other aquatic organisms

Cymoxanil and its metabolites are not likely to partition into sediments. As neither cymoxanil nor any of its metabolites partitioned significantly to sediment (see Volume 3 (AS), section B.8) section, therefore no toxicity test with the sediment dwelling midge *Chironomus* spp. was deemed necessary for cymoxanil and its metabolites.

2.9.2.4 Comparison with the CLP criteria

2.9.2.4.1 Acute aquatic hazard

Table 81: **Summary of information on acute aquatic toxicity relevant for classification**

Method	Species	Test material	Results[1](mg/L)	Remarks	Reference
OECD 203, EPA 72-1 Static test GLP	*Lepomis macrochirus*	Cymoxanil Batch No DPX-T3217-113 Purity 97.8%	LC_{50}=29 (mm)	96-hour Study from the original DAR	RAR B.9.2.1.3(1993b) HLR 834-92
OECD 203, EPA 72-1, EEC C.1 Limit test GLP	*Oncorhynchus mykiss*	IN-KQ960 Batch No PR0901037/41 Purity 98%	LC_{50}=23.923 (mm)	96-hour New study	RAR B.9.2.1.15, (2009b) 49731230
OECD 202, US EPA 72-2 Static test GLP	*Daphnia magna*	Cymoxanil Batch No DPX-T3217-113 Purity 97.8%	EC_{50}=**27 (mm)**	48-hour Study from the original DAR	Baer (1993c) HLR 736-92

OECD 202 Static test GLP	*Daphnia magna*	IN-KQ960 Batch No 25215 Purity 94.6%	**EC_{50}=0.8 (mm)**	48-hour Study from the original DAR	Samel (2002d) 9559
OECD 201 Static test GLP	*Raphidocelis subcapitata*	IN-JX915 Batch No IN-JX915-003 Purity 99.8%	**E_rC_{50} = 0.931 (gmm)**	72 hour New study	Gilberg, Taoudi (2017B) 16EP2AO

Reliable acute aquatic data on cymoxanil is available for two tropic level, for fish and aquatic invertebrates. Data for algae were disregarded due to instability of cymoxanil in the test system.Classification proposal is based on studies coducted with cymoxanil and also on studies conductes with metabolites as metabolites IN-KQ960 and IN-JX915 showed higher acute toxicity to fish and aquatic invertebrates than the parent substance. Also metabolite IN-JX915 shows similar toxicity to algae as metabolite IN-KQ960 to aquatic invertebrates. Therefore, they were taken into account further in the classification proposal.

According to CLP classification criteria, the acute aquatic hazard is based on the EC_{50} = 0.8 mg/L of *daphnia magna* 48-hour toxicity test for metabolite IN-KQ960 as most sensitive taxa. The lowest acute toxicity EC50 ≤ 1mg/L. On this basis, the following classification and labelling of Cymoxanil is proposed:

Aquatic Acute 1 H400 (Very toxic to aquatic life); as the lowest EC is between 0.1 and 1 mg/L the associated M-factor is 1.

2.9.2.4.2 Long-term aquatic hazard (including bioaccumulation potential and degradation)

Table 82: **Summary of information on long-term aquatic toxicity relevant for classification**

Method	Species	Test material	Results[1](mg/L)	Remarks	Reference
OECD 210, EPA 72-4, EPA-540/9-86-138 Flow-through test GLP	*Oncorhynchus mykiss*	Cymoxanil Batch No DPX-T3217-113 Purity 97.3%	NOEC = 0.044 (mm)	ELS Study from the original DAR	RAR B.9.2.2.9, (1996) HLR 411-96
OECD 211 Semi-static test GLP	*Daphnia magna*	Cymoxanil Batch No 160418F Purity 98.4%	EC_{10}=0.0619 (twa)	21 day New study	Kümmich (2017) S17-03158
OECD 201 Static test GLP	*Raphidocelis subcapitata*	IN-JX915 Batch No IN-JX915-003 Purity 99.8%	E_rC_{10} = 0.268 (gmm)	72 hour New study	Gilberg, Taoudi (2017b) 16EP2AO

Reliable chronic aquatic data on cymoxanil is available for two tropic level, for fish and aquatic invertebrates. Classification proposal is based on studies conducted with cymoxanil although there are chronic studies available for metabolite IN-KQ960 for daphnia and the studies for all relevant aquatic metabolites for algae. The lowest and the most reliable endpoint values for classification purpose were obtained from the studies with the parent substance cymoxanil.

According to CLP classification criteria, the chronic aquatic hazard is based on the NOEC (0.044 mg a.s./L) of *Oncorhyncus mykis* ELS toxicity test for cymoxanil as most sensitive taxa. Cymoxanil according to the CLP criteria is considered as not rapidly degradable (see 2.8.2). On this basis, the following classification and

labelling of Cymoxanil is proposed:

Aquatic Chronic 1 H410 (Very toxic to aquatic life with long lasting effects); as the lowest NOEC is between 0.01 and 0.1 mg/L the associated M-factor is 1.

Bioaccumulation

A study to assess the bioaccumulation of cymoxanil in fish has not been performed as the the measured Log Kow of cymoxanil is 0.67 - 0.59 at 20 °C. This is below the the cut-off value of 4 given in the CLP Regulation and discussed in the ECHA Guidance on the application of the CLP Criteria (2015) and it indicates a low bioaccumulation potential of cymoxanil.

2.9.2.5 Conclusion on classification and labelling for environmental hazards

On the basis of the above information on acute and chronic toxicity, the following classification and labelling of Cymoxanil is proposed:

For the acute toxicity to aquatic organisms the proposed classification is **Acute Category 1** with the Hazard statement H400. A acute **M-factor of 1** is applicable.

For the chronic toxicity to aquatic organisms the proposed classification is **Chronic Category 1** with the Hazard statement H410. A chronic **M-factor of 1** is applicable.

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier Submitter's proposal

Cymoxanil has gone through the following regulatory processes:

(i) an initial risk assessment provided by the Rapporteur Member State Austria (Draft Assessment Report (DAR) published in 2007)

(ii) CLH process

A harmonised classification and labelling for cymoxanil has been adopted by the ECHA Committee for Risk Assessment (RAC) on 14 September 2012 (ECHA/RAC/CLH-O-0000002970-73-01/F). The substance was classified as Aquatic Acute 1, H400, M=1 based on *Anabaena flos-aquae* with E_rC_{50} = 0.254 mg/L and Aquatic Chronic 1, H410, M=1 based on *Oncorhynchus mykiss* with 90d NOEC = 0.044mg/L, non-rapid degradable and low bioaccumulation potential.

(iii) a renewal of the approval of the active substance (Renewal Assessment Report (RAR) prepared according to the Commission Regulation (EC) No 1107/2009 by Lithuania and Finland in February 2020)

(iv) current CLH process

The substance is currently listed in Annex VI of Regulation (EC) No 1272/2008 with a classification for environment hazard as Aquatic Acute 1, H400, M=1 and Aquatic Chronic 1, H410, M=1 based on the data indicated above (see point (ii)). Based on re-evaluation of all relevant old and new acute and chronic aquatic toxicity studies that were provided for the renewal of the approval of the cymoxanil same classification of the substance was proposed by the RMS/DS. The DS proposed to classify the substance as Aquatic Acute 1, H400, M=1 based on 48h EC_{50} value of 0.8 mg/L for *Daphnia magna* for metabolite IN-

KQ960 and Aquatic Chronic 1, H410, M=1 based on lack of rapid degradation and a 90d NOEC value of 0.044 mg/L for the *Oncorhynchus mykiss* for cymoxanil.

Degradation

Hydrolysis of cymoxanil was investigated in sterile buffer solutions at pH 4, 5, 7 and 9 in two independent studies, which gave consistent results. Cymoxanil undergoes extensive hydrolysis strongly depending on the pH of the solution, leading to the formation of numerous metabolites. Cymoxanil is considered stable at a pH of 4, half-life times at pH 5, 7 and 9 were 144, 1.1 and 0.02 days at 25°C and at 20°C half-life times at pH 7 and 9 were determined to be 2.1 and 0.04 days. At pH 5 only minor metabolites were observed, no major metabolites were found. The major metabolites formed under sterile conditions at pH 7 and 9 were: IN-U3204 (60.8% AR), IN-JX915 (11.0% AR), IN-W3595 (41.5% AR), IN-KP533 (57.4% AR), IN-R3273 (10.2% AR), IN-KQ960 (14.1% AR). The metabolites IN-W3595, IN-KQ960, IN-R3273, IN-KP533 were considered stable under the conditions of sterile hydrolysis at both pHs. Metabolite IN-U3204 is highly unstable in aqueous solutions, rapidly degrading into IN-KP533, IN-T4226 and IN-KQ960. IN-T4226 is a further transient hydrolysis metabolite rapidly degrading into IN-KP533. Metabolites IN-KQ960 and IN-KP533 have to be considered stable under the conditions of sterile hydrolysis. Metabolite IN-JX915 rapidly degrades into IN-R3273, which in turn slowly degrades into IN-T4226. The parent cleavage product IN-W3595 is considered rather stable under the conditions of hydrolysis in sterile buffer solutions. Ethyl urea, which is likely to be formed together with IN-W3595, was never quantified in environmental fate studies, since the labelling of the parent (cyanoacetamide position) does not allow to follow the fate of this cleavage product. Nevertheless, ethyl urea has to be considered a major degradation product of the hydrolysis of cymoxanil in sterile buffer solutions at neutral and alkaline pH, too. Hydrolysis half-life of the transient metabolites IN-U3204, IN-JX915 and IN-T4226 at pH 7 and pH 9 were estimated to be 2.5 and 0.5 days, 0.7 and 1.7 days, and 7.2 and 2.0 days, respectively. The metabolites IN-W3595, IN-KQ960, IN-R3273 and IN-KP533 have to be considered rather stable under the conditions of sterile hydrolysis at each pH, their amounts remained almost stable once the hydrolysis process has finished (which occurred by approx. 15 DAT at pH 7 and by 7 DAT at pH 9).

In the Oxon study (Slangen and Willams, 2003) several degradation products, not exceeding 10% of AR individually, remained unidentified.

Another hydrolysis study (Anand, 2007) showed that cymoxanil is stable at pH 5 and very quickly hydrolyses under basic conditions with half-life values of 0.02 days at pH 7 and 1.4 days at pH 9. Under acidic conditions, cymoxanil slowly hydrolyses with half- life value of 148 days at pH 4 at 25°C. At pH 7 and 25°C, degradation of IN-U3204 was observed up to 52.7% of AR after 2 days, IN-W3595 up to 16.2% of AR after 15 days, IN-KP533 up to 57.0% of AR after 30 days. At pH 9 and 25°C, degradation of IN-W3595 was observed up to 39.0% of AR after 3 days, IN-KP533 up to 31.6% of AR up to 10 days, IN-U3204 up to 60.8% of AR after 0.17 days and IN-KQ960 up to 13.5% of AR after 7 days.

The photolytic degradation of cymoxanil in water has been investigated under sterile conditions in acetate buffer solutions at pH 5 for up to 30 days. Cymoxanil degraded rapidly under photolytic conditions at pH 5. Two major degradants were seen at > 10% IN-JX915 and IN-R3273, plus a further two metabolites IN-KP533 and IN-T4226 were seen at 7.9 and 6.7% AR respectively, several additional minor components were also

seen.

The DT_{50} for cymoxanil under continuous irradiation was 1.8 days. Under the impact of irradiation, degradation of cymoxanil owing to photolysis is strongly driven by formation of metabolite IN-JX915 (52.6% of AR), which rapidly further degrades to IN-R3273 (35.4% of AR). No other major metabolites were observed. This pathway is clearly the major degradation route of cymoxanil in acidic solutions exposed to irradiation. The alternative hydrolysis processes (cyclisation to IN-U3204 and cleavage of the parent to form IN-W3595) were almost negligible at the investigated pH value. In the dark control samples almost no degradation of cymoxanil was observed.

Net photolysis half-life time of cymoxanil in sterile buffer solution at pH 5 was calculated to be 1.7 and 3.0 days. The experimental net photolysis of cymoxanil ranged between 4.3 and 12.1 days under environmental conditions (midsummer day, approx. 40 °N). As demonstrated in one additional experiment, conducted in non-sterile pond water at pH 7.0, the impact of irradiation on the overall dissipation of cymoxanil in aquatic ecosystems loses its significance at neutral and alkaline conditions owing to the extensive abiotic hydrolysis of cymoxanil at higher pH values. Quantum yield of cymoxanil was calculated in two studies to range between 0.0052 and 0.00058.

The DT_{50} of IN-JX915, owing to the influence of photolysis and hydrolysis, was calculated to be approx. 6.6 days at the investigated pH of 5.0. However, owing to the highly transient character of IN-JX915 during hydrolysis under neutral and alkaline conditions (hydrolysis DT_{50} < 2 days) it is expected that levels of photolytically formed IN-JX915 will be significantly lower in aquatic systems under environmental conditions (without considering biotic degradation).

Degradation half-life of IN-R3273 at pH 5.0, owing to the influence of photolysis and hydrolysis, was calculated to be 32.7 days, no reliable half-life time could be calculated for IN-R3273. Further minor photolysis products (< 10% of AR) were IN-T4226 and IN-KP533 which derive from the degradation of IN-JX915 and IN-R3273.

There are three ready biodegradation studies available on cymoxanil. In the first study (Luit, 2001) the biodegradation of the cymoxanil was determined with modified Sturm test (OECD TG 301B) over 10 days at 10 mg TOC/L and 21 – 23.5°C. No significant degradation (< 10%) of cymoxanil technical was observed under test conditions. Also the second study (Desmares-Koopmans, 2008) which was performed with modified Sturm test (OECD TG 301B) but over 28 days at 12 mg TOC/L and 21.3 – 22.6°C in darkness indicated no significant degradation (< 20%) of cymoxanil. Third study (Feil, 2009) carried out using Manometric respirometry test (OECD TG 301F) show no significant degradation (23% based on $ThOD_{NO3}$) of cymoxanil over 28 days at 21 – 22°C in darkness. Based on these studies, the DS concluded that cymoxanil is not readily biodegradable.

In an aerobic mineralisation study (Irmer, 2019) after 61 days the mineralisation of cymoxanil to CO_2 was observed in amounts up to 24.1% AR and 29.0% AR for the low (9.4 µg/L) and high (96.0 µg/L) test concentration, respectively. Degradation was very rapid. The DT_{50} values for cymoxanil were 2.52 hours (9.4 µg/L) and 2.42 hours (96.0 µg/L). The DT_{90} values were 10.8 hours (9.4 µg/L) and 99.1 hours (96.0 µg/L). Calculated DT_{50} for cymoxanil in surface water were 0.09-0.15 days in non sterile system. Six major metabolites were detected: IN-KP533 (25.8% AR), IN-W3595 (30.3% AR), IN-U3204 (34.3% AR), IN-R3274 (15.0% AR), IN-KQ960 (21.5% AR) and IN-R3273

(7.3% AR). Two major metabolites (M125 and M103) could not be identified. The metabolite M125 was very transient and its instability did not allow identification. M103 was sufficiently stable but it is very high polarity indicated a very small molecular structure. Due to this, it was not possible to identify M103 but it is assumed that it consists of oxalic acid.

Aerobic water/sediment studies were conducted in five aerobic/water systems (pH of water phase 6.7 to 8.9). Cymoxanil dissipated from the water phase to the sediment very rapidly.

Seven metabolites (> 5% AR) were observed in water phase: IN-W3595, IN-KP533, IN-U3204, IN-KQ960, AS999 (M5), IN-JX915 and IN-T4226. The maximum level of cymoxanil in the sediment phase never reached above 5% in any studies. No metabolite was observed < 5% AR in the sediment phases of all test systems investigated. Degradation of cymoxanil in the whole system was fast with DT_{50} values in a range of 0.056-1.6 days following SFO kinetics with a geometric mean of 0.268. Since transfer of cymoxanil into sediment layer was negligible, dissipation in the water layer is almost consistent to degradation in the entire system. Based on the entire system, the following metabolites are considered major (> 10% of AR): IN-U3204 (maximum occurrence 25.4% of AR by 1 DAT), IN-W3595 (24.6% of AR by 4 DAT), IN-KQ960 (14.25% of AR by 10 DAT), IN-JX915 (11.5% of AR by 2 DAT), IN-KP533 (21.6% of AR by 8 DAT), IN-T4226 (12.0% of AR by 3 DAT), M5/ASS999 (29.2% of AR by 3 DAT). None of the observed metabolites in the water/sediment studies was persistent. In the Table 66 of the CLH report the whole system DT50s are presented for cymoxanil and its metabolites. Whole system DT50s for major metabolites were between 0.794 and 160 days.

Based on the available data, the DS concluded that cymoxanil is considered as not rapidly degradable for classification purposes.

Bioaccumulation

No experimental data on bioaccumulation of cymoxanil in fish has been performed as the measured octanol-water partition coefficient (log K_{OW}) is < 3 (0.67 - 0.59 at 20 °C). Based on the data presented DS concluded that cymoxanil has a low potential for bioaccumulation as log K_{OW} of cymoxanil is below the cut-off value of 4 given in the CLP Regulation.

Aquatic Toxicity

For cymoxanil, reliable aquatic toxicity data are available for two tropic levels, fish and aquatic invertebrates. In addition to aquatic toxicity studies using cymoxanil, reliable aquatic toxicity studies on fish, invertebrates and algae using different metabolites are presented in the CLP report. The summary of the relevant information on aquatic toxicity for cymoxanil and its metabolites are provided by the DS in Tables 75 - 80 of the CLP report.

<u>Acute toxicity</u>

Three valid acute toxicity studies with three different fish species (*Oncorhynchus mykiss, Lepomis macrochirus* and *Cyprinodon variegatus*) using cymoxanil and seventeen studies with one fish species (*Oncorhynchus mykiss*) using all relevant metabolites for the surface water (IN-W3595, IN-KP533, IN-U3204, IN-R3273, IN-KQ960, IN-T4226, M5 and IN-R3273) with exception IN-JX915 were available. The lowest acute endpoint for

cymoxanil for fish is mean measured 96h LC_{50} value of 29 mg/L for *Lepomis macrochirus*. Based on the available data (summarised in the Table 75 of CLH report) DS concluded that metabolites are less toxic to fish than the parent compound with the exception of IN-KQ960 metabolite with mean measured 96h LC_{50} value of 23.923 mg/L for *Oncorhynchus mykiss*. As the lowest acute endpoints for cymoxanil and metabolites are higher than 1 mg/L, cymoxanil or its metabolites are not considered hazardous for fish on acute toxicity.

Three valid acute toxicity studies with three different invertebrate species (*Daphnia magna*, *Mysidopsis bahia* and *Crassostrea virginica*) using cymoxanil and fourteen studies with invertebrate *Daphnia magna* using relevant water metabolites (IN-W3595, IN-U3204, IN-R3273, IN-KQ960, IN-T4226, M5) were provided. The lowest acute endpoint for invertebrates is mean measured 48h EC_{50} value of 27 mg/L for *Daphnia magna* using cymoxanil*.* Based on the available data (summarised in the Table 76 of CLH report) DS concluded that metabolites are less toxic to daphnia than the parent compound cymoxanil with exception of IN-KQ960 (48h LC_{50} = 0.8 mg/L) which showed higher toxicity to *Daphnia magna* in comparison with cymoxanil. The lowest derived LC_{50} value for cymoxanil is higher than 1 mg/L, however its metabolite IN-KQ960 can be considered acutely toxic to aquatic invertebrates.

In the CLH report, aquatic toxicity studies with algae and aquatic plants are available for cymoxanil but are considered not valid by the DS as the concentrations of cymoxanil could not be sufficiently maintained and also not all validity criteria according the OECD TG 201 guideline were met for some algae studies. The toxicity studies on algae with relevant metabolites (IN-W3595, IN-U3204, IN-R3274, IN-KQ960, IN-T4226, IN-JX915, M5 and IN-R3273) for the surface water are provided. The lowest acute endpoint for algae is the result from the study using fresh water green algae *Raphidocelis subcapitata* with geometric mean measured 72h E_rC_{50} value of 0.931 mg/L using metabolite IN-JX915.

DS based the classification proposal on studies conducted with cymoxanil and metabolites as metabolite IN-KQ960 showed higher acute toxicity to fish and aquatic invertebrates than the cymoxanil. Furthermore metabolite IN-JX915 shows similar toxicity to algae as metabolite IN-KQ960 to aquatic invertebrates. From the available aquatic toxicity data, invertebrates are the most acutely sensitive trophic group, therefore the acute aquatic classification proposed by the DS was based on *Daphnia magna* toxicity study (48h EC_{50} of 0.8 mg/L) with metabolite IN-KQ960. The lowest acute toxicity value of 0.8 mg/L is lower than classification threshold value of 1 mg/L, therefore the substance should be classified as Aquatic Acute 1, H400 with M-factor of 1 ($0.1 < L(E)C_{50} \leq 1$ mg/L).

<u>Chronic toxicity</u>

Two chronic toxicity studies with two different fish species (*Oncorhynchus mykiss* and *Cyprinodon variegatus*) were available. The lowest chronic endpoint for cymoxanil for fish is mean measured 90d NOEC value of 0.044 mg/L for *Oncorhynchus mykiss.*

Two valid chronic toxicity studies using cymoxanil and three studies using metabolite IN-KQ960 were provided for *Daphnia magna*. The lowest chronic endpoint for invertebrates is 21d EC_{10} value of 0.0619 mg/L based on time-weighted average for *Daphnia magna* using cymoxanil. Based on the available data (summarised in the Table 79 of CLH report)

DS concluded that metabolite IN-KQ960 is less toxic to *Daphnia magna* than the cymoxanil.

In the CLH report, aquatic toxicity studies with algae and aquatic plant are available for cymoxanil but are considered not valid by the DS as the concentrations of cymoxanil could not be sufficiently maintained and also not all validity criteria according the OECD TG 201 guideline were met for some algae studies. Toxicity studies using relevant metabolites for the surface water (IN-W3595, IN-U3204, IN-R3274, IN-KQ960, IN-T4226, IN-JX915, M5 and IN-R3273) were provided for algae. The lowest endpoint for algae was derived for metabolite IN-JX915 from the study on *Raphidocelis subcapitata* with geometric mean measured 72h NOEC value of 0.06 mg/L and geometric mean measured 72h E_rC_{10} value of 0.268 mg/L.

DS based the classification proposal on aquatic toxicity studies conducted with cymoxanil although there are chronic toxicity studies available for metabolite IN-KQ960 for daphnia and toxicity studies for all relevant aquatic metabolites for algae. The lowest and the most reliable endpoint values for classification purpose were obtained from the studies with the parent substance cymoxanil. The results of long-term aquatic toxicity studies indicate that fish are the most sensitive taxon therefore chronic aquatic classification proposed by DS was based on the fish *Oncorhynchus mykiss* toxicity study (90d NOEC = 0.044 mg/L). The DS proposed Aquatic Chronic 1, H410 with an M-factor = 1 (0.01 < NOEC ≤ 0.1 mg/L) along with the understanding that the substance is not rapidly degradable.

Comments received during consultation

Two MSCAs and one National Authority provided comments. Both MSCA agreed with the proposed classification for environmental hazards by DS.

One MSCA pointed out that proposed classification is based only on reliable data for fish and invertebrates, while the not acceptable algae studies (e.g. Hughes 1996) suggested that algae could be the most sensitive taxon to cymoxanil. Therefore, the new study on algae with cymoxanil, which was not submitted until finalisation of the draft RAR, could be vital for the final classification of the substance and defining study for setting M-factors. MSCA indicated that both M-factors should be updated as soon as the new study results are available. DS confirmed that new algae study has been submitted and agreed with commenting MSCA regarding M-factors.

National Authority asked for clarification regarding the proposed current environmental classification as it is the same as harmonised classification for cymoxanil that was agreed by RAC in 2011. DS clarified that current classification was proposed based on re-evaluation of all relevant aquatic toxicity old and new studies provided for the renewal of the approval of cymoxanil. National Authority agreed that the substance is not rapidly degradable due to the toxicity of the degradation products.

In the view of the National Authority it would be useful to consider this to support the conclusion on the degradation potential of the parent substance and the relevance of new degradant ecotoxicity data. DS explained that cymoxanil is considered as not readily biodegradable based on three ready biodegradation studies. However, cymoxanil degrades very rapidly in water under neutral and alkaline conditions. The degradation is mainly driven by hydrolysis. Due to the rapid conversion of cymoxanil to metabolites and higher toxicity of some metabolites than the parent, toxicity data of metabolites are

considered in the classification of the substance.

National Authority also commented that current proposal considers available algal toxicity data for cymoxanil not reliable, however the validity criteria were met in the algal study by Boeri (1999) which was considered by DS not acceptable because test concentrations were not maintained and below the LOQ after 72 hours. The DS referenced an EFSA technical report (2015) to support this view that the use of half the LOD or LOQ is not acceptable to calculate geometric mean measured concentrations where intermediate measured concentrations are not available. National Authority referred to ECHA's Guidance on Application of CLP Criteria (2017) section I.4.1 where is indicated that the use of half the LOQ or LOD is suitable for hazard classification. Therefore, reliable chronic and acute toxicity endpoints should be derived for the 72h endpoint from this study. These endpoints would be within the same concentration range as the key endpoints used for the proposed classification and would therefore support this proposal. DS agreed with National Authority in regard to CLP guidance and use of half the LOQ or LOD and added that new algae study will be submitted.

In this section the key information from the new algae study generated during the procedure for renewal of the approval of cymoxanil in accordance with Commission Implementing Regulation (EU) No 844/2012 is presented.

New OECD TG 201 *Raphidocelis subcapitata* growth inhibition test with cymoxanil

Test was carried out in accordance with OECD TG 201 and Method C.3 of Commission Regulation (EC) No 761/2009. Green algae *Raphidocelis subcapitata* (formerly known as *Pseudokirchneriella subcapitata*) was exposed to cymoxanil (purity 98.1%) for 72 hours under static exposure conditions to the nominal concentrations of 0.010, 0.032, 0.10, 0.32, 1.0 and 3.2 mg/L. Analysis of the test preparations at 0 hours showed measured test concentrations to range from 0.0094 to 3.0 mg/L (80% to 94% of the nominal concentrations). Analysis of the test preparations at 72 hours showed measured test concentrations to range from 0.0088 to 2.7 mg/L (70% to 88% of nominal). The validity criteria were met. The results of the study are presented in the table below.

Table: Summary of toxicity endpoints

Endpoint	Geometric mean measured concentration (mg/L)
Growth Rate	
E_rC_{10} (0-72h)	0.051
E_rC_{50} (0-72h)	0.69
NOEC	0.0091
Yield	
E_yC_{10} (0-72h)	0.019
E_yC_{50} (0-72h)	0.095
NOEC	0.0091

RAC notes that a new algae study generated during the procedure for renewal of the approval of cymoxanil did not affect the acute and chronic classification of the cymoxanil

as the values are in the same range as the key values used for classification by DS.

Assessment and comparison with the classification criteria

Degradation

The substance does not undergo rapid abiotic degradation at pH 4 (DT_{50} = 148 days at 25°C) and pH 5 (DT_{50} = 144 days at 25°C) but undergoes extensive hydrolysis with increasing alkalinity (DT_{50} are 2.1 days (pH 7) and 0.04 days (pH 9) at 20°C and 1.1 days (pH 7) and 1.4 days (pH 9) at 25°C). Several major hydrolysis metabolites were formed. Data on hydrolysis might be considered for classification purposes only when the longest half-life determined within the pH range 4 - 9 is less than 16 days (corresponding to a degradation of > 70% within 28 days). Accordingly, cymoxanil is hydrolytically stable.

In the ready biodegradability tests (OECD TG 301B and OECD TG 301F) no significant degradation was observed, indicating that cymoxanil is not readily biodegradable.

Limited mineralisation after 61 days was observed for cymoxanil in an aerobic mineralisation study. Degradation of cymoxanil was very rapid with DT_{50} values of 2.52 hours (9.4 µg/L) and 2.42 hours (96.0 µg/L). Eight major metabolites were formed (IN-KP533, IN-W3595, IN-U3204, IN-R3274, IN-KQ960, IN-R3273, M125 and M103). Calculated DT_{50} for cymoxanil in surface water were 0.09-0.15 days in non sterile system.

In an aerobic water/sediment simulation studies, whole system DT_{50} for the cymoxanil was between 0.056 and 1.6 days following SFO kinetics with a geometric mean of 0.268. Seven major metabolites were observed (IN-W3595, IN-KP533, IN-U3204, IN-KQ960, AS999 (M5), IN-JX915 and IN-T4226). Whole system DT50s for major metabolites were between 0.794 and 160 days. Classification information for metabolites is lacking. Based on the acute toxicity data available in CLH report the metabolite IN-KQ960 is more toxic to fish and invertebrates than cymoxanil. In line with CLP guidance (version 5, June 2017, section II.3.4.) data on primary degradation may be used for demonstrating rapid degradability only when it can be satisfactory demonstrated that the degradation products formed do not fulfil the criteria for classification as hazardous to the aquatic environment.

Overall, the substance does not pass the ready biodegradability tests, the available abiotic and biotic degradation information does not indicate that cymoxanil is ultimately degraded in the aquatic environment with a half-life of < 16 days (corresponding to a degradation of > 70% within 28 days) or that it is transformed to non-classifiable products. Consequently, RAC considers the substance to be not rapidly degradable for the purpose of environmental classification.

Bioaccumulation

RAC agrees with the DS conclusion to consider cymoxanil having low potential for bioaccumulation based on the estimated log K_{OW} values of 0.67 - 0.59 which is below the decisive CLP Regulation threshold of 4.

Aquatic toxicity

In the CLH report, no reliable toxicity data were reported for algae and aquatic plants.

During the opinion development process, additional toxicity study with algae was provided by the DS. EC_{50}, NOEC and EC_{10} values based on geometric mean measured concentrations were reported for toxicity study carried out on green algae *Raphidocelis subcapitata*. The algae study meets all validity criteria according to the current version of OECD TG 201 and is considered acceptable by the RMS/DS. RAC is of the opinion that it is appropriate to consider this data relevant for classification of the substance. According to current CLP Guidance (Version 5.0, July 2017), the endpoint based on growth rate reduction is preferred for algae. Therefore the 72h E_rC_{50} of 0.69 mg/L, 72h NOE_rC of 0.0091 mg/L and 72h E_rC_{10} of 0.051 mg/L were selected to be used for classification by RAC. In addition, in line with the current CLP Guidance (Version 5.0, July 2017), the preference is given to EC_{10} value over the NOEC value, therefore RAC considers that the E_rC_{10} should take precedence over NOE_rC. According to recent scientific developments, E_rC_{10} values are preferred as these are statistically derived from the entire dataset, and less dependent on test design considerations than the NOEC.

Acute toxicity

RAC is of the opinion that in case of cymoxanil, reliable acute toxicity data are available for all three trophic levels. Also studies with different metabolites were available for all three trophic levels. The toxicity studies with different degradation products generally derive effect values higher (namely, much lower toxicity) than for cymoxanil with exception of IN-KQ960 which was more acutely toxic to fish and daphnia than parent compound. Based on all available data algae are the most acutely sensitive group, and the lowest result is a 72h E_rC_{50} value of 0.69 mg/L for *Raphidocelis subcapitata* using cymoxanil. Cymoxanil shows similar toxicity to algae (0.69 mg/L) as metabolite IN-JX915 (0.931 mg/L). The lowest acute toxicity value of 0.69 mg/L is below the classification threshold value of 1 mg/L, RAC concludes that a classification as Aquatic Acute 1, H400, is justified. As $0.1 < E_rC_{50} \leq 1$ mg/L, the acute M-factor is 1.

Chronic toxicity

Reliable long-term aquatic toxicity data on cymoxanil are available for all three trophic levels while studies with different metabolites are available only for invertebrates and algae. The toxicity studies with different degradation products derive effect values higher (namely, much lower toxicity) than for cymoxanil. The lowest chronic effect value corresponds to a test with fish *O. mykiss* with mean measured 90 d NOEC value of 0.044 mg/L for cymoxanil. As the value is below the classification threshold value of 1 mg/L and the substance is considered not rapidly biodegradable, RAC concludes that a classification as Aquatic Chronic 1, H400, is justified. As $0.01 < NOEC \leq 0.1$ mg/L, the chronic M-factor is 1.

In summary, on the basis of the available data, RAC considers that cymoxanil should be classified according to CLP as:

Aquatic Acute 1, H400, M-factor = 1 and

Aquatic Chronic 1, H410, M-factor = 1

This is consistent with the conclusion of the Dossier Submitter.These recommendations are in line with RAC's previous evaluation (RAC, 2012) of Cymoxanil.

2.9.3 Summary of effects on arthropods

Bees

One study on the acute oral and contact toxicity of cymoxanil to honey bees was previously submitted in the EU and was summarized in the original DAR Volume 3 Ecotoxicology. Also new studies have been submitted. In addition, a study on the acute toxicity of cymoxanil to bumble bees has also been submitted by CTF. The new chronic adult toxicity studies with cymoxanil and 22- day repeated exposure laboratory studies with honeybee larvae have been submitted by each applicant (CTF, Agria and SFP).

Table 83: Summary of toxicity data to bees

Test Item	Test Species	Time-scale Test type / substrate	Endpoint	Data point Author, year
Cymoxanil	Honey bee *Apis mellifera* L.	48 h Acute oral	LD_{50} > 106.6 µg a.s./bee	CA 8.3.1.1.1-01 Schmitzer S., 2007 (CTF)
		48 h Acute contact	LD_{50} > 100 µg a.s./bee	
		48 h Acute oral	LD_{50} > 104 µg a.s./bee	CA 8.3.1.1.1-02 (CTF) CA 8.3.1.1.1-01 (SFP) Sekine T., 2009
		48 h Acute contact	LD_{50} > 100 µg a.s./bee	
		48 h Acute oral	**LD_{50} > 85.59 µg a.s./bee**	CA 8.3.1.1.1-03 Schur A., 1999 (DAR 2007)
		48 h Acute contact	**LD_{50} > 100 µg a.s./bee**	
		72 h Larval acute oral	NOED > 49.7 µg a.s./larva LD_{50} > 99.3 µg a.s./larva	CA 8.3.1.1.1-04 Kleebaum K., 2014 (CTF)
	Bumble bee *Bombus terrestris* L.	48 h Acute oral	**LD_{50} > 100 µg a.s./bee**	CA 8.3.1.1.1-05 Colli M., 2017a (CTF)
		48 h Acute contact	**LD_{50} > 100.00 µg a.s./bee**	
	Honey bee *Apis mellifera* L.	22 d Larval toxicity	NOED = 1.93 µg a.s./larva/test	CA 8.3.1.3-01 Wilkins S., 2018 (CTF)
			NOED = 9.34 µg a.s./larva/test	CA 8.3.1.3-01 Couture, E., 2018 (Agria) 237SRFR17C02
			NOED = 0.241 µg a.s./larva/test	CA 8.3.1.3-01 Couture, E., 2018 (SFP) 207SRFR17C01
		10 d Chronic oral	LDD_{50} = 8.47 µg a.s./bee/day	CA 8.3.1.2-01 Colli M., 2017b (CTF)
			LDD_{50} = 6.25 µg a.s./bee/day	CA 8.3.1.2-01 Deslandes L. , 2018 (Agria)
			LDD_{50} = 4.879 µg a.s./bee/day	CA 8.3.1.2-01 Lenoir J.-C, 2019 (SFP)

Test Item	Test Species	Time-scale Test type / substrate	Endpoint	Data point Author, year

Note: When more than one endpoint is available for a substance for the same taxonomic group and study type, the lowest endpoint is in **bold** is used in the risk assessment

Studies on non-target arthropods were performed with the representative formulations, containing the active substance cymoxanil. The available data with formulated products are indicated in the respective Volume 3 CP B.9.

CTF and SFP

Table 84: Summary of toxicity data to non-target arthropods

Test species	Time scale - Substrate	Test material	Endpoint - Effect	Data point Author, year
Aphidius rhopalosiphi	48 h – glass plate (2D)	Cymoxanil 45 WG	LR_{50} > 960 g a.s./ha ER_{50} (reproduction) > 960 g a.s./ha	CP-10.3.2.1-01 Moll, M. 2008a (CTF)
Aphidius rhopalosiphi	48 h – glass plate (2D)	Cymoxanil 45 WG	LR_{50} > 420 g a.s./ha ER_{50} (reproduction) > 420 g a.s./ha	CP-10.3.2.1-02 Moll, M. 2011 (CTF) and (SFP)
Typhlodromus pyri	7 d – glass plate (2D)	Cymoxanil 45 WG	LR_{50} > 480 g a.s./ha ER_{50} (reproduction) > 480 g a.s./ha	CP-10.3.2.1-03 Moll, M. 2008b (CTF)
Typhlodromus pyri	7 d – glass plate (2D)	Cymoxanil 45 WG	LR_{50} > 420 g a.s./ha ER_{50} (reproduction) > 26.3 g a.s./ha	CP-10.3.2.1-04 Schwarz, A. 2011 (CTF) and (SFP)
Typhlodromus pyri	7 d – extended laboratory – 3 bioassays (fresh and aged residues) (3D)	BCP 308 F*	LR_{50} > 394.12 g a.s./ha (fresh residues, 3-d aged residues & 6-d aged residues) ER_{50} (reproduction) > 394.12 g a.s./ha (fresh residues, 3-d aged residues & 6-d aged residues)	CP-10.3.2.2-01 Schwarz, A., 2012 (CTF)
Chrysoperla carnea	30 d – extended laboratory (3D)	Cymoxanil 45 WG	LR_{50} > 480 g a.s./ha ER_{50} (reproduction) > 480 g a.s./ha	CP-10.3.2.2-02 Moll, M. 2008c (CTF)
Poecilus cupreus	14 d – extended laboratory (3D)	Cymoxanil 45 WG	LR_{50} > 480 g a.s./ha	CP-10.3.2.2-03 Schmitzer, S. 2008 (CTF)
Predatory mites in vineyard	Field study	Cymbal 45 WG	4 applications at 180 g a.s./ha: 41 days after the last application < 50% effects	CP 10.3.2.4-01 Rosenkranz, B., Schabio, S., 2009 (CTF)

* The formulation name is equivalent to the Cymoxanil 45WG product

Agria

Table 85: Summary of toxicity data to non-target arthropods

Test species	Time scale - Substrate	Test material	Endpoint - Effect	Data point Author, year
Aphidius rhopalosiphi	48 h – glass plate (2D)	Propamocarb HCL 400 g/l + Cymoxanil 50 g/l SC	LR_{50} = **924** ml product/ha (equivelant to:46.75 g Cymoxanil /ha, 369.97 g propamocarb HCL/ha) ER_{50} = 174 ml product/ha (8.80 g cymoxanil/ha, 69.67 g propamocarb HCl/ha)	CP 10.3.2.1/05 Malecki, S., 2018
Typhlodromus pyri	7 d – glass plate (2D)	Propamocarb HCL 400 g/l + Cymoxanil 50 g/l SC	LR_{50} = >4375 ml product/ha (equivelant to:>221.4 g Cymoxanil /ha, >1751.8 g propamocarb HCL/ha) ER_{50} = 2635ml product/ha (133.3 g cymoxanil/ha, 1055 propamocarb-HCL/ha	CP 10.3.2.1/04 Malecki, S., 2018
Typhlodromus pyri	7 d – extended laboratory (3D)	Propamocarb HCL 400 g/l + Cymoxanil 50 g/l SC	LR_{50} = >2500 ml product/ha (equivelant to:>126.25 g Cymoxanil /ha, >1002.5 g propamocarb HCL/ha) LOER - 500 ml product/ha (200.5 g Propamocarb HCl, 25.25 g Cymoxanil/ha)	CP 10.3.2.1/01 CP 10.3.2.1/02 Cockroft, D., 2014 plus Amendment 1
Aphidius rhopalosiphi	48 h – extended laboratory (3D)	Propamocarb HCL 400 g/l + Cymoxanil 50 g/l SC	LR_{50} = >2500 ml product/ha ER_{50} > 2500 ml prod./ha (equivelant to:>126.25 g Cymoxanil /ha, >1002.5 g propamocarb HCL/ha)	CP 10.3.2.1/03; Gamblin, C., 2014

2.9.4 Summary of effects on non-target soil meso- and macrofauna

CTF

Table 86: Summary of toxicity data to earthworms

Test item	Test species	Duration	Endpoint	Data point/Author, year
Cymoxanil tech.	*Eisenia fetida*	Long-term 56 days	**$NOEC_{rep}$ = 12.8 mg a.s./kg dws** EC_{10} > 12.8 mg a.s./kg dws EC_{20} > 12.8 mg a.s./kg dws EC_{50} > 12.8 mg a.s./kg dws	CA 8.4.1-01 Lührs, U., 2008 CA 8.4.1-02 Mead, C., 2019ae
Cymoxanil (DPX-T3217) 60 WG*	*Eisenia fetida*	Long-term 56 days	$NOEC_{rep}$ = 95.3 mg form./kg dws (56 mg a.s./kg dws) EC_{50} = 311.89 mg form./kg dws (185 mg a.s./kg dws)	CA 8.4.1-03 Shanmugasundaram, R., 2013

Cymoxanil 25% WP*	*Eisenia fetida*	Long-term 56 days	$NOEC_{rep}$ = 208 mg form./kg dws (55 mg a.s./kg dws) EC_{10}, EC_{20}, EC_{50} > 208 mg form./kg dws (> 55 mg a.s./kg dws)	CA 8.4.1-04 Witte, B., 2009
IN- T4226	*Eisenia fetida*	Long-term 56 days	**$NOEC_{rep}$ = 500 mg met./kg dws** EC_{10} (95% CI) = 531.9 (409.4-614.4) mg met./kg dws EC_{20} (95% CI) = 619.3 (508.3-693.7) mg met./kg dws EC_{50} (95% CI) = 828.6 (753.2-893.4) mg met./kg dws	CA 8.4.1-05 Bandow, C., Senn, L., 2018a
IN-U3204	*Eisenia fetida*	Long-term 56 days	**$NOEC_{rep}$ = 250 mg met./kg dws** EC_{10} (95% CI) = 288.8 (219.5 – 342.3) mg met./kg dws EC_{20} (95% CI) = 367.4 (301.3 – 418.8) mg met./kg dws EC_{50} (95% CI) = 582.6 (525.7 – 646.7) mg met./kg dws	CA 8.4.1-06 Bandow, C., Senn, L., 2018b
IN-KQ960	*Eisenia fetida*	Long-term 56 days	**$NOEC_{rep} \geq$ 1000 mg met./kg dws** EC_{10} , EC_{20}, EC_{50} (95% CI) = (ND) mg met./kg dws	CA 8.4.1 -07 Bandow, C., Senn, L., 2018c
IN-R3273	*Eisenia fetida*	Long-term 56 days	**$NOEC_{rep}$ = 31.25 mg met./kg dws[1]** EC_{10} (95% CI) = (ND) mg a.s./kg dws[1] EC_{20} (95% CI) = 21.0 (7.5 – 32.5) mg met./kg dws[1] EC_{50} (95% CI) = 54.6 (36.7 – 73.5) mg met./kg dws[1]	CA 8.4.1-08 Bandow, C., Senn, L., 2018d
IN-W3595	*Eisenia fetida*	Long-term 56 days	$NOEC_{rep}$ = 31.25 mg met./kg dws[1] **EC_{10} (95% CI) = 28.2 (10.7 – 47.0) mg met./kg dws[1]** EC_{20} (95% CI) = 56.9 (29.9 – 307.3) mg met./kg dws[1] EC_{50} (95% CI) = 219 (166 – 307) mg met./kg dws[1]	CA 8.4.1–09 Bandow, C., Senn, L., 2017
IN-JX915	*Eisenia fetida*	Long-term 56 days	**$NOEC_{rep}$ = 40 mg met./kg dws**	CA 8.4.1-10 Bandow, C., Senn, L., 2018e

dws = dry weight soil
Note: In line with EFSA Supporting publication 2015:EN-924, where a reliable media EC_{10} can be calculated, the lower value between this value and the NOEC is used in the risk assessment; values in bold used for risk assessment
* Studies presented *in lieu* of a study performed on the representative formulation. The formulations used in these studies contained the active substance as the only active ingredient. It was considered that none of the co-formulants would have a significant effect on the toxicity of the active substance
[1]: Corrected for Log P_{ow} >2

Agria

Table 87: Summary of toxicity data to earthworms

Data Point	Species	Substance	Exposure System	Results	Reference
CA 8.4/01	*Eisenia fetida*	Cymoxanil	14 d, acute	LC_{50} = >1000 mg/kg dw.soil	EFSA, 2008
	Eisenia fetida	Propamocarb-HCL	14 d, acute	LC_{50} = >660 mg/kg dw.soil	EFSA, 2006.
CP 10.4.1/01	*Eisenia fetida*	Cymoxanil 50 g/L + Propamocarb 400 g/L	14 d, acute	LC_{50} = >5000 mg f.p. /kg dw.soil	Desai Y.P., 2012,

Data Point	Species	Substance	Exposure System	Results	Reference
		hydrochloride		(equivelant to: >237.01 mg Cymoxanil /kg >1887.9 mg propamocarb HCL/kg)	
	Eisenia fetida	Cymoxanil	Long-term 56 days	**$NOEC_{rep}$ = 12.8 mg a.s./kg dw. soil** EC_{10}, EC_{20}, EC_{50} > 12.8 mg a.s./kg dw.soil	CA 8.4.1-01 Lührs, U., 2008 (CTF study)
CA 8.4.1/01	*Eisenia fetida*	Cymoxanil/ Famoxadone 50 WG	56 d, chronic	NOEC=6.6mg cymoxanil-equiv/kg dw. soil	EFSA, 2008
	Eisenia fetida	Propamocarb	56 d, chronic	NOEC 362 mg a.s./kg dw. soil	EFSA, 2006
CP 10.4.1.1/01	*Eisenia fetida*	Cymoxanil 50 g/L + Propamocarb 400 g/L hydrochloride SC	56 d, chronic	**$NOEC_{rep}$ 257 mg f.p./kg dw. soil** (equivelant to: 12.63 mg Cymoxanil /kg, 93.71 mg propamocarb HCL/kg) EC_{10} (95% CI) = 404.4 (251.1-651.4) mg f.p./kg dws EC_{20} (95% CI) = 629 (447.4-884.4) mg f.p./kg dws EC_{50} (95% CI) = 1464 (1170-1834) mg f.p./kg dws	Lührs, U., 2018a,

SFP

Table 88: Summary of toxicity data to earthworms

Data Point	Species	Substance	Exposure System	Results	Reference
	Eisenia fetida	Cymoxanil	14 d, acute	LC_{50} = >1000 mg/kg dw.soil	EFSA, 2008
CA 8.4/01	*Eisenia fetida*	Cymoxanil	14 d, acute	LC_{50} = 319 mg/kg dw.soil	CA 8.4/01 Van Erp, Y.H.M., 1995
	Eisenia fetida	Cymoxanil	Long-term 56 days	**$NOEC_{rep}$ = 12.8 mg a.s./kg dw. soil** EC_{10}, EC_{20}, EC_{50} > 12.8 mg a.s./kg dw.soil	CA 8.4.1-01 Lührs, U., 2008 (CTF study)

Data Point	Species	Substance	Exposure System	Results	Reference
CA 8.4.1/01	*Eisenia fetida*	Cymoxanil 25% WP	Long-term 56 days	$NOEC_{rep}$ = 208 mg form./kg dw.soil (55 mg a.s./kg dws) EC_{10}, EC_{20}, EC_{50} > 208 mg form./kg dw.soil	CA 8.4.1/01 Witte, B., 2009
	Eisenia fetida	Cymoxanil/ Famoxadone 50 WG	Long-term 56 days	NOEC = 6.6mg cymoxanil-equiv/kg dw.soil	EFSA, 2008

Chronic toxicity studies with non-target soil meso- and macrofauna were conducted for the active subtstance cymoxanil. No studies are available with the formulation Cymoxanil 45 WG.

A summary of the relevant endpoints for the effects of Cymoxanil on non-target soil meso- and macrofauna (other than earthworms) are listed in the Table: 113.

Table 89: Summary of toxicity data to non-target soil meso- and macrofauna (other than earthworms)

Test item	Test species	Duration	Endpoint	Data point/Author, year
Cymoxanil	*Folsomia candida*	Long-term 28 d	$NOEC_{rep}$ = 57.4 mg a.s./kg dws EC_{10} (95% CI) = 72.3 (51.5-101) mg a.s./kg dws EC_{20} (95% CI) = 86.0 (61.7-120) mg a.s./kg dws EC_{50} (95% CI) = 119 (78.9-179) mg a.s./kg dws	CA 8.4.2.1-01, Wong, J., 2017a (CTF)
	Folsomia candida	Long-term 28 d	**NOECrep = 52.9 mg/kg dws** EC_{10} (95% CI) = 100 (77.6-128.9) mg a.s./kg dws EC_{20} (95% CI) = 125.6 (103.9-151.9) mg a.s./kg dws EC_{50} (95% CI) = 194 (171.8-219.4) mg a.s./kg dws	Lührs, U., 2017, Ibacon Report No.122071016 (Agria)
	Hypoaspis aculeifer	Long-term 14 d	$NOEC_{rep}$ = 308.6 mg a.s./kg dws **EC_{10} (95% CI) = 279.1 (195.0-344.9) mg a.s./kg dws** EC_{20} (95% CI) = 379.3 (295.7– 444.1) mg a.s./kg dws EC_{50} (95% CI) = 682.4 (604.2–781.5) mg a.s./kg dws	Lührs, U., 2017, Ibacon Report No.122071089 (Agria)
	Hypoaspis aculeifer	Long-term 14 d	NOEC ≥ 984 mg cymoxanil/kg soil dw EC_{20} > 984 mg cymoxanil/kg soil dw EC_{50} > 984 mg cymoxanil/kg soil dw	CA 8.4.2.1-02, Wong, J., 2017b (CTF)

dws = dry weight soil

Note: In line with EFSA Supporting publication 2015:EN-924, where a reliable media EC_{10} can be calculated, the lower value between this value and the NOEC is used in the risk assessment; values in **bold** used for risk assessment

Co-RMS FI is of the opinion that EC_{10} value is preferred over a NOEC value if EC_{10} value is a valid value. Therefore, FI suggest using EC_{10} -72.3 mg a.s./kg dws in the risk assessment. RMS is of the opinion that the lowest one should be used in the risk assessment. Therefore, as EC_{10}> NOEC then NOECrep = 52.9 mg/kg dws is the relevant endpoint to use in the risk assessment.

2.9.5 Summary of effects on soil nitrogen transformation

For cymoxanil, three studies investigating the effects on nitrogen transformation are available. No studies are available with the formulation Cymoxanil 45 WG.

The endpoints related to the effects on soil nitrogen transformation (please see Volume 3 (AS), Section B.9.5) are presented in the following table.

CTF and SFP

Table 90: Summary of data on the effects of Cymoxanil and its metabolites to soil nitrogen transformation

Test material	Time scale	Endpoint	Data point/ Author, year
Cymoxanil	28 days	Up to and including 1.60 mg /kg soil dw <25% deviation from control by the study end	CA 8.5-01 Feil, N., 2008
Cymoxanil	28 days	Up to and including **1.63 mg /kg soil dw** <25% deviation from control by the study end	CA 8.5-02 Feil, N., 2009
Cymoxanil	28 days	Up to and including 1.60 mg /kg soil dw <25% deviation from control by the study end	CA 8.5-03 Kölzer, U., 2003

Agria

Table 91: Summary of data on the effects of Cymoxanil and its metabolites to soil nitrogen transformation

Data Point	Endpoint	Substance	Exposure System	Results	Reference
CA 8.5/01	N-mineralisation	Cymoxanil	28 d, aerobic soil type	Effect on Nitrate formation rate < 25 % at 1.6 mg/kg soil dw	EFSA, 2008
CA 8.5/01	C-mineralisation	Cymoxanil	28 d, aerobic soil type	Effect on CO_2 formation rate < 25 % at 1.6 mg/kg soil dw	EFSA, 2008
	N-mineralisation	Propamocarb-HCL	28 d, aerobic soil type	Effect on Nitrate formation rate < 25 % at 28.9 kg/ha, ≡ 38.5 mg/kg soil dw	EFSA, 2006
	C-mineralisation	Propamocarb-HCL	28 d, aerobic soil type	Effect on CO_2 formation rate < 25 % at 28.9 kg/ha, ≡ 38.5 mg/kg soil dw	EFSA, 2006
CP 10.5/01	N-mineralisation	Cymoxanil 50 g/L + Propamocarb 400 g/L hydrochloride SC	28 d, aerobic soil type	Effect on Nitrate formation rate < 25 % at 29.1 mg f.p./kg soil dw (equivelant to: 10.61 mg propamocarb HCL /kg soil dw, 1.33 mg Cymoxanil /kg sil dw)	Hammesfahr, U., 2013, IBACON Report No. 81591080
CP10.5/01	C-mineralisation	Cymoxanil 50 g/L + Propamocarb 400 g/L hydrochloride SC	28 d, aerobic soil type	Effect on CO_2 formation rate < 25 % at 29.1 mg f.p./kg soil dw (equivelant to: 10.61 mg propamocarb HCL /kg soil dw, 1.33 mg cymoxanil/kg soil dw)	Hammesfahr, U., 2013, IBACON Report No. 81591080

2.9.6 Summary of effects on terrestrial non-target higher plants

For non-target plants the risk is assessed based on exposure by drift in the off-field environment, and the risk is best addressed using data for the relevant formulation. Therefore, the endpoints from the most recent valid studies with Cymoxanil 45 WG are applied in the risk assessment.

Table 92: Summary of data on the toxicity of Cymoxanil to non-target plants

Test species	**Test design**	**Test substance**	**Endpoint**	**Data point Author, year**
Allium cepa (Liliaceae), *Triticum aestivum* (Poaceae), *Beta vulgaris* (Chenopodiaceae), *Cucumis sativus* (Curcurbitacae), *Glycine max* (Fabaceae), *Brassica napus* (Brassicaceae)	Seedling emergence - Glasshouse	BCP308F	**ER_{50} > 248 g a.s./ha**	CP 10.6.2-03 Dickinson R.A., 2014a
Beta vulgaris (Chenopodiaceae), *Glycine max* (Fabaceae), *Helianthus annuus* (Compositeae), *Cucumis sativus* (Curcurbitacae), *Lolium perenne* (Poaceae), *Avena sativa* (Poaceae), *Allium cepa* (Liliaceae)	Vegetative vigour (visible adverse effects) - Glasshouse	Cymoxanil 45 WG	**ER_{50} > 240 g a.s./ha**	CP 10.6.2-01 Bützler, R. & Meinerling, M. 2008
Allium cepa (Liliaceae), *Triticum aestivum* (Poaceae), *Beta vulgaris* (Chenopodiaceae), *Cucumis sativus* (Curcurbitacae), *Glycine max* (Fabaceae), *Brassica napus* (Brassicaceae)	Vegetative vigour - Glasshouse	BCP308F	ER_{50} > 248 g a.s./ha	CP 10.6.2-02 Dickinson R.A., 2014b

When more than one endpoint is available for a substance for the same study type, the endpoint in **bold** is the one used in the risk assessment

Agria

Table 93: Summary of data on the toxicity of Cymoxanil and Propamocarb-HCL to non-target plants

Data Point	**Species**	**Substance**	**Exposure System**	**Results**	**Reference**
CA 8.6.2/01	*Allium cepa*	Cymoxanil 50 WP (Cymoxanil formulation)	21 d Vegetative vigour	ER_{50} >240 g a.s /ha	EFSA, 2008.. (Annex IIA, point 8.6, Annex IIIA, point 10.8)
	8 dicot + 3 monocot species tested	Previcur N (Propamocarb-HCL	21 d Vegetative	ER_{50} = 9180 g a.s /ha	EFSA, 2006. (Annex IIA,

Data Point	Species	Substance	Exposure System	Results	Reference
		formulation)	vigour (Tier 1 screening data)		point 8.6, Annex IIIA, point 10.8)
	Monocotyledons and dicotyledons (10 different taxa)	Previcur N (Propamocarb-HCL formulation)	21 d Seedling emergence (Tier II dose response)	Cucumber seedling emergence was significantly lower than the control at 27.54 and 82.62 kg a.s/ha (% effect ranged from 16% to +2%). No effect was found in wheat	EFSA, 2006. (Annex IIA, point 8.6, Annex IIIA, point 10.8)

2.9.7 Summary of effects on other terrestrial organisms (flora and fauna)

No specific information was submitted.

2.9.8 Summary of effects on biological methods for sewage treatment

Table 94: Summary of data on the effects of Cymoxanil on biological methods for sewage treatment

Test item	Organisms	Time scale	Endpoint	Data point/Author, year
Cymoxanil tech.	Activated sewage sludge	3 hours	EC_{50} > 32 mg/L	CA. 8.8-01., Feil, N., 2008b
			EC_{50} = 70.3 mg/L	CA. 8.8-02., Feil, N., 2009b
			EC_{50} = **19.4** mg/L	CA. 8.8-03., Desmares-Koopmans M. J.E., 2001

2.9.9 Summary of product exposure and risk assessment

Birds

The risk assessment for effects on birds has been performed according to the latest EFSA Guidance Document on Risk Assessment for Birds and Mammals (2009).

CTF

The risk assessment included in Volume 3 (PPP) section B.9.2.1 indicated an acceptable acute and long-term risk to birds.

Table 95: Risk assessment to birds for Potatoes at 150 g a.s./ha, 5 applications

Growth stage	Indicator or focal species	Time scale	DDD (mg/kg bw per day)	TER	Trigger
Screening Step (Birds)					
All	Small omnivorous bird	Acute	50.02	11	10
All	Small omnivorous bird	Long-term	14.42	**1.03**	5
Tier 1 (Birds)					
BBCH 10-39	Small omnivorous bird "lark"	Long-term	2.43	6.13	5
BBCH >40	Small omnivorous bird "lark"	Long-term	0.73	20.41	5
BBCH 10-19	Small insectivorous bird "wagtail"	Long-term	2.52	5.91	5
BBCH >20	Small insectivorous bird "wagtail"	Long-term	2.16	6.90	5
Risk from bioaccumulation and food chain behaviour					
Not relevant as L_{og} Pow <3					
Risk from consumption of contaminated water					

Scenarios	**Indicator or focal species**	**Time scale**	**PEC_{dw}xDWR**	**TER**	**Trigger**
Puddle scenario	Birds	acute	0.391	1401	10
Puddle scenario	Birds	Long-term	0.391	38	5

Table 96: Risk assessment to birds for Grapes at 120 g a.s./ha, 5 applications

Growth stage	**Indicator or focal species**	**Time scale**	**DDD (mg/kg bw per day)**	**TER**	**Trigger**
Screening Step (Birds)					
All	Small omnivorous bird	Acute	21.73	25	10
All	Small omnivorous bird	Long-term	5.94	**2.51**	5
Tier 1 (Birds)					
BBCH 10-19	Small insectivorous bird "redstart"	Long-term	1.76	8.47	5
BBCH >20	Small insectivorous bird "redstart"	Long-term	1.51	9.87	5
BBCH 10-19	Small granivorous bird "finch"	Long-term	1.05	14.2	5
BBCH 20-39	Small granivorous bird "finch"	Long-term	0.87	17.13	5
BBCH >40	Small granivorous bird "finch"	Long-term	0.52	28.65	5
Ripening	Frugivorous bird "Thrush/starling"	Long-term	2.20	6.77	5
BBCH 10-19	Small omnivorous bird "lark"	Long-term	0.99	15.1	5
BBCH 20-39	Small omnivorous bird "lark"	Long-term	0.82	18.2	5
BBCH >40	Small omnivorous bird "lark"	Long-term	0.50	29.8	5
Risk from bioaccumulation and food chain behaviour					
Not relevant as L_{og} Pow <3					
Risk from consumption of contaminated water					

Scenarios	**Indicator or focal species**	**Time scale**	**PEC_{dw}xDWR**	**TER**	**Trigger**
Puddle scenario	Birds	acute	0.313	1751	10
Puddle scenario	Birds	Long-term	0.313	48	5

Table 97: Risk assessment to birds for Tomatoes at 150 g a.s./ha, 5 applications

Growth stage	Indicator or focal species	Time scale	DDD (mg/kg bw per day)	TER	Trigger
Screening Step (Birds)					
All	Small omnivorous bird	Acute	45.26	12	10
All	Small omnivorous bird	Long-term	12.36	**1.21**	5
Tier 1 (Birds)					
BBCH 71-89	Frugivorous crow	Long-term	6.11	**2.44**	5
BBCH 10-49	Small granivorous bird ("finch")	Long-term	2.18	6.83	5
BBCH >50	Small granivorous bird ("finch")	Long-term	0.65	23	5
BBCH 10-49	Small omnivorous bird "lark"	Long-term	2.08	7.16	5
BBCH >50	Small omnivorous bird "lark"	Long-term	0.63	23.65	5
BBCH 71-89	Frugivorous starling	Long-term	3.95	**3.77**	5
BBCH 10-19	Small insectivorous bird ("wagtail")	Long-term	2.16	6.89	5
BBCH >20	Small insectivorous bird ("wagtail")	Long-term	1.85	8.1	5
Higher tier (Birds): Refinement of the risks for frugivorous birds was performed by recalculating the MAF_m x TWA factor using a moving time window approach based on the available residue DT_{50} data and a default TWA period of 21 days. The residue DT_{50} data was determined from trials performed in tomatoes. DT_{50} = 1 days, MAF x F_{twa} = 0.21.					
BBCH 71-89	Frugivorous crow	Long-term	1.01	14.75	5
BBCH 71-89	Frugivorous starling	Long-term	0.65	22.92	5
Risk from bioaccumulation and food chain behaviour					
Not relevant as L_{og} Pow <3					
Risk from consumption of contaminated water					

Scenarios	Indicator or focal species	Time scale	PEC_{dw}xDWR	TER	Trigger
Puddle scenario	Birds	acute	0.391	1401	10
Puddle scenario	Birds	Long-term	0.391	38	5

Agria

The risk assessment included in Volume 3 (PPP) section B.9.2.1 indicated an acceptable acute and long-term risk to birds.

Table 98: Risk assessment to birds for Potatoes at 125 g a.s./ha, 6 applications

Growth stage	Indicator or focal species	Time scale	DDD (mg/kg bw per day)	TER	Trigger
Screening Step (Birds)					
All	Small omnivorous bird	Acute	37.72	14.5	10
All	Small omnivorous bird	Long-term	10.73	**1.39**	5
Tier 1 (Birds)					
Potatoes, BBCH 10-39	Small omnivorous bird (Lark)	Long-term	1.81	8.23	5
Potatoes, BBCH ≥20	Small insectivorous bird (Wagtail)	Long-term	1.61	9.27	5
Potatoes, BBCH ≥40	Small omnivorous bird (Lark)	Long-term	0.55	27.26	5
Risk from bioaccumulation and food chain behaviour[*indicate when not relevant* i.e *if Log kow≤3]*					
Not relevant as the Log kow for Cymoxanil is ≤3					
Risk from consumption of contaminated water					

Scenarios	Indicator or focal species	Time scale	PEC_{dw}xDWR	TER	Trigger
Leaf scenario	Birds	Not relevant			
Puddle scenario, Screening step					
Application rate (125 g a.s./ha)/relevant endpoint <50 (koc<500 L/kg), TER calculation not needed					

SFP

The acute risk of cymoxanil to birds is acceptable following the intended uses of Dauphin 45 in grapes, potato and tomato.
The long-term risk of cymoxanil to birds is acceptable following the intended uses of Dauphin 45 in grapes, potato. However, long-term risk of cymoxanil to frugivorous birds at BBCH 71-89 in tomatoes is not acceptable.
The risk to birds through drinking water, secondary poisoning is considered acceptable.

Table 99: Risk assessment to birds for Potatoes at 120 g a.s./ha, 8 applications (SFP)

Growth stage	Indicator or focal species	Time scale	DDD (mg/kg bw per day)	TER	Trigger
Screening Step (Birds)					
All	Small omnivorous bird	Acute	36.2	15	10
All	Small omnivorous bird	Long-term	10.3	**1.45**	5
Tier 1 (Birds)					
BBCH 10-39	Small omnivorous bird "lark"	Long-term	1.73	8.6	5
BBCH >40	Small omnivorous bird "lark"	Long-term	0.52	29	5
BBCH >20	Small insectivorous bird "wagtail"	Long-term	1.54	9.7	5
Risk from bioaccumulation and food chain behaviour					
Not relevant as L_{og} Pow <3					
Risk from consumption of contaminated water					
Scenarios	**Indicator or focal species**	**Time scale**	**PEC_{dw}xDWR**	**TER**	**Trigger**
Puddle scenario, Screening step					
Application rate (120 g a.s./ha)/relevant endpoint <50 (koc<500 L/kg), TER calculation not needed					

Table 100: Risk assessment to birds for Grapes at 120 g a.s./ha, 4 applications (SFP)

Growth stage	Indicator or focal species	Time scale	DDD (mg/kg bw per day)	TER	Trigger
Screening Step (Birds)					
All	Small omnivorous bird	Acute	36.2	25	10
All	Small omnivorous bird	Long-term	5.94	**2.51**	5
Tier 1 (Birds)					
BBCH 10-19	Small insectivorous bird "redstart"	Long-term	1.76	8.47	5
BBCH >20	Small insectivorous bird "redstart"	Long-term	1.51	9.87	5
BBCH 10-19	Small granivorous bird "finch"	Long-term	1.05	14.2	5
BBCH 20-39	Small granivorous bird "finch"	Long-term	0.87	17.13	5
BBCH >40	Small granivorous bird "finch"	Long-term	0.52	28.65	5
Ripening	Frugivorous bird "Thrush/starling"	Long-term	2.20	6.77	5

Growth stage	Indicator or focal species	Time scale	DDD (mg/kg bw per day)	TER	Trigger
BBCH 10-19	Small omnivorous bird "lark"	Long-term	0.99	15.1	5
BBCH 20-39	Small omnivorous bird "lark"	Long-term	0.82	18.2	5
BBCH >40	Small omnivorous bird "lark"	Long-term	0.50	29.8	5
Risk from bioaccumulation and food chain behaviour					
Not relevant as L_{og} Pow <3					
Risk from consumption of contaminated water					
Puddle scenario, Screening step Application rate (120 g a.s./ha)/relevant endpoint <50 (koc<500 L/kg), TER calculation not needed					

Table 101: Risk assessment to birds for Tomatoes at 120 g a.s./ha, 5 applications

Growth stage	Indicator or focal species	Time scale	DDD (mg/kg bw per day)	TER	Trigger
Screening Step (Birds)					
All	Small omnivorous bird	Acute	36.2	15	10
All	Small omnivorous bird	Long-term	9.89	**1.51**	5
Tier 1 (Birds)					
BBCH 71-89	Frugivorous crow	Long-term	4.88	**3.05**	5
BBCH 10-49	Small granivorous bird ("finch")	Long-term	1.74	8.56	5
BBCH >50	Small granivorous bird ("finch")	Long-term	0.52	28.6	5
BBCH 10-49	Small omnivorous bird "lark"	Long-term	1.66	8.97	5
BBCH >50	Small omnivorous bird "lark"	Long-term	0.50	29.8	5
BBCH 71-89	Frugivorous starling	Long-term	3.16	**4.72**	5
BBCH 10-19	Small insectivorous bird ("wagtail")	Long-term	1.72	8.66	5
BBCH >20	Small insectivorous bird ("wagtail")	Long-term	1.48	10.1	5
Risk from bioaccumulation and food chain behaviour					
Not relevant as L_{og} Pow <3					
Risk from consumption of contaminated water					
Puddle scenario, Screening step Application rate (120 g a.s./ha)/relevant endpoint <50 (koc<500 L/kg), TER calculation not needed					

Mammals

The risk assessment for effects on birds has been performed according to the latest EFSA Guidance Document on Risk Assessment for Birds and Mammals (2009).

CTF

The acute risk of cymoxanil to mammals is acceptable following the intended uses of Cymoxanil 45 WG in grapes, potato and tomato.
The long-term risk of cymoxanil to mammals is acceptable following the intended uses of Cymoxanil 45 WG in grapes, potato. However, long-term risk of cymoxanil to voles at BBCH 10-49 in tomatoes is not acceptable.
The risk to mammals through drinking water, secondary poisoning can also be considered acceptable.

Table 102: Risk assessment to mammals for Potatoes at 150 g a.s./ha, 5 applications (CTF)

Growth stage	Indicator or focal species	Time scale	DDD (mg/kg bw per day)	TER	Trigger
Screening Step (Mammals)					
All	Small herbivorous mammal	Acute	37.30	13	10
All	Small herbivorous mammal	Long-term	10.75	**0.93**	5
Tier 1 (Mammals)					
BBCH 10-19	Insectivorous shrew	Long-term	0.93	11	5
BBCH >20	Insectivorous shrew	Long-term	0.42	24	5
BBCH >40	Herbivorous vole	Long-term	4.83	**2.1**	5
BBCH 10-40	Herbivorous lagomorph	Long-term	3.18	**3.14**	5
BBCH >40	Herbivorous lagomorph	Long-term	0.96	10	5
BBCH 10-39	Omnivorous mouse	Long-term	1.74	6	5
BBCH >40	Omnivorous mouse	Long-term	0.51	20	5
Higher tier (Mammals): Refinement of the risks was performed by recalculating the MAF_m x TWA factor based on the available residue DT_{50} data and a default TWA period of 21 days. The residue DT_{50} data was determined from trials performed in peas and wheat. The use of these crops as surrogates of monocotyledons and dicotyledens was considered appropriate given that the growth stage and period of application were relevant for contaminated weeds eaten by mammals					
BBCH >40	Herbivorous vole	Long-term	1.01	10	5
BBCH 10-40	Herbivorous lagomorph	Long-term	0.66	15	5
Risk from bioaccumulation and food chain behaviour					
Not relevant as L_{og} Pow <3					
Risk from consumption of contaminated water					
Scenarios	**Indicator or focal species**	**Time scale**	**PEC_{dw}xDWR**	**TER**	**Trigger**
Puddle scenario	Mammals	acute	0.204	2377	10
Puddle scenario	Mammals	Long-term	0.204	49	5

Table 103: Risk assessment to mammals for Grapes at 120 g a.s./ha, 5 applications (CTF)

Growth stage	**Indicator or focal species**	**Time scale**	**DDD (mg/kg bw per day)**	**TER**	**Trigger**
Screening Step (Mammals)					
All	Small herbivorous mammal	Acute	31.10	15.6	10
All	Small herbivorous mammal	Long-term	11.04	**0.91**	5
Tier 1 (Mammals)					
BBCH 10-19	Herbivorous lagomorph	Long-term	1.02	10	5
BBCH 20-39	Herbivorous lagomorph	Long-term	0.84	12	5
BBCH >40	Herbivorous lagomorph	Long-term	0.50	20	5
BBCH 10-19	Insectivorous shrew	Long-term	0.64	16	5
BBCH >20	Insectivorous shrew	Long-term	0.29	34.5	5
BBCH 10-19	Herbivorous vole	Long-term	6.62	**1.5**	5
BBCH 20-39	Herbivorous vole	Long-term	5.51	**1.81**	5
BBCH >40	Herbivorous vole	Long-term	3.31	**3.02**	5
BBCH 10-19	Omnivorous mouse	Long-term	0.72	14	5
BBCH 20-39	Omnivorous mouse	Long-term	0.60	17	5
BBCH >40	Omnivorous mouse	Long-term	0.35	28.6	5
Higher tier (Mammals): Refinement of the risks was performed by recalculating the MAF_m x TWA factor based on the available residue DT_{50} data and a default TWA period of 21 days. The residue DT_{50} data was determined from trials performed in peas and wheat. The use of these crops as surrogates of monocotyledons and dicotyledons was considered appropriate given that the growth stage and period of application were relevant for contaminated weeds eaten by mammals					
BBCH 10-19	Herbivorous vole	Long-term	1.09	9.2	5
BBCH 20-39	Herbivorous vole	Long-term	0.91	11	5
BBCH >40	Herbivorous vole	Long-term	0.55	18.2	5

Growth stage	Indicator or focal species	Time scale	DDD (mg/kg bw per day)	TER	Trigger
Risk from bioaccumulation and food chain behaviour					
Not relevant as L_{og} Pow <3					
Risk from consumption of contaminated water					
Scenarios	**Indicator or focal species**	**Time scale**	**PEC_{dw}xDWR**	**TER**	**Trigger**
Puddle scenario	Mammals	acute	0.163	2975	10
Puddle scenario	Mammals	Long-term	0.163	61	5

Table 104: Risk assessment to mammals for Tomatoes at 150 g a.s./ha, 5 applications (CTF)

Growth stage	Indicator or focal species	Time scale	DDD (mg/kg bw per day)	TER	Trigger
Screening Step (Mammals)					
All	Small herbivorous mammal	Acute	38.87	12.5	10
All	Small herbivorous mammal	Long-term	13.79	**0.72**	5
Tier 1 (Mammals)					
BBCH 71-89	Frugivorous rat	Long-term	4.81	**2.1**	5
BBCH 10-19	Insectivorous shrew	Long-term	0.80	12.5	5
BBCH >20	Insectivorous shrew	Long-term	0.36	28	5
BBCH 10-49	Herbivorous vole	Long-term	13.79	**0.72**	5
BBCH >50	Herbivorous vole	Long-term	4.14	**2.4**	5
BBCH 10-49	Omnivorous mouse	Long-term	1.49	6.7	5
BBCH >50	Omnivorous mouse	Long-term	0.44	23	5
Higher tier (Mammals): Refinement of the risks was performed by recalculating the MAFm x TWA factor based on the available residue DT_{50} data and a default TWA period of 21 days. The residue DT_{50} data was determined from trials performed in peas and wheat. The use of these crops as surrogates of monocotyledons and dicotyledens was considered appropriate given that the growth stage and period of application were relevant for contaminated weeds eaten by mammals					
BBCH 71-89	Frugivorous rat	Long-term	0.79	13	5
BBCH 10-49	Herbivorous vole	Long-term	**2.28**	**4.4**	5
BBCH >50	Herbivorous vole	Long-term	0.68	15	5
Risk from bioaccumulation and food chain behaviour					
Not relevant as L_{og} Pow <3					
Risk from consumption of contaminated water					
Scenarios	**Indicator or focal species**	**Time scale**	**PEC_{dw}xDWR**	**TER**	**Trigger**
Puddle scenario	Mammals	acute	0.204	2377	10
Puddle scenario	Mammals	Long-term	0.204	49	5

Agria

The acute risk to mammals after exposure to cymoxanil following the intended uses of Rival Duo in potato is acceptable at Tier 1, except for the 'small herbivorous mammal (vole)' scenario and 'large herbivorous mammal (lagomorph)' scenario for the representative formulation.

The long-term risk to mammals was acceptable at Tier 1 for all scenarios, except for the 'small herbivorous mammal (vole)' scenario and for the 'large herbivorous mammal (lagomorph)' scenario for active substance cymoxanil.

The risk to mammals through drinking water, secondary poisoning can also be considered acceptable.

Table 105: First-tier assessment of the acute risk for mammals due to use of Cymoxanil 50 g/L+ Propamocarb hydrochloride 400 g/L SC (Rival Duo) in potatoes.

Intended use	**Potatoes**
Product	Cymoxanil 50 g/L + Propamocarb hydrochloride 400 g/L (Rival Duo)
Application rate (g/ha)	2742.5 × 6 at 7-day interval
Acute toxicity (mg/kg bw)	>1115
TER criterion	10

Crop scenario **Growth stage**	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90}** **(mg/kg bw/d)**	**TER_a**
Potatoes, BBCH >20	Small insectivorous mammal (shrew)	5.4	1.9	28.1	>39.7
Potatoes, BBCH >40	**Small herbivorous mammal (vole)**	40.9	1.9	213.1	**>5.23**
Potatoes, BBCH 10-40	**Large herbivorous mammal (lagomorph)**	35.1	1.9	182.9	**>6.1**
Potatoes, BBCH >40	Large herbivorous mammal (lagomorph)	10.5	1.9	54.7	>20.4
Potatoes, BBCH 10-39	Small omnivorous mammal (mouse)	17.2	1.9	89.6	>12.4
Potatoes, BBCH >40	Small omnivorous mammal (mouse)	5.2	1.9	27.1	>41.1

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in **bold** fall below the relevant trigger.

Table 106: Risk assessment to mammals for Potatoes at 125 g a.s./ha, 6 applications

Growth stage	Indicator or focal species	Time scale	DDD (mg/kg bw per day)	TER	Trigger
Screening Step (Mammals)					
All	Small herbivorous mammal	Acute	28.12	17.2	10
All	Small herbivorous mammal	Long-term	8	**1.25**	5
Tier 1 (Mammals)					
Potatoes, BBCH >20	Small insectivorous mammal (Shrew)	Long-term	0.31	32	5
Potatoes, BBCH >40	Small herbivorous mammal (Vole)	Long-term	3.59	**2.78**	5
Potatoes, BBCH 10-40	Large herbivorous mammal (*Lagomorph*)	Long-term	2.37	**4.22**	5
Potatoes, BBCH >40	Large herbivorous mammal (Lagomorph)	Long-term	0.71	14	5
Potatoes, BBCH 10-39	Small omnivorous mammal (Mouse)	Long-term	1.29	8	5
Potatoes, BBCH >40	Small omnivorous mammal (Mouse)	Long-term	0.38	26	5

Risk from bioaccumulation and food chain behaviour[*indicate when not relevant* i.e *if Log kow≤3]*

Not relevant as the Log kow for Cymoxanil is ≤3

Risk from consumption of contaminated water

Scenarios	**Indicator or focal species**	**Time scale**	**PEC_{dw}xDWR**	**TER**	**Trigger**
Leaf scenario	Birds	Not relevant			

Puddle scenario, Screening step

Application rate (125 g a.s./ha)/relevant endpoint <50 (koc<500 L/kg), TER calculation not needed

SFP

The acute risk of cymoxanil to mammals is acceptable following the intended uses of Dauphin 45 in grapes, potato and tomato.
The long-term risk of cymoxanil to herbivorous and frugivorous mammals is unacceptable following the intended uses of Dauphin 45 in **grapes, potato and tomato.**
The risk to mammals through drinking water, secondary poisoning is considered acceptable.

Table 107: Risk assessment to mammals for Potatoes at 120 g a.s./ha, 8 applications (SFP)

Growth stage	Indicator or focal species	Time scale	DDD (mg/kg bw per day)	TER	Trigger
Screening Step (Mammals)					
All	Small herbivorous mammal	Acute	27	18	10
All	Small herbivorous mammal	Long-term	7.7	**1.3**	5
Tier 1 (Mammals)					
BBCH >20	Insectivorous shrew	Long-term	0.3	33	5
BBCH >40	Herbivorous vole	Long-term	3.45	**2.89**	5
BBCH 10-40	Herbivorous lagomorph	Long-term	2.27	**4.41**	5
BBCH >40	Herbivorous lagomorph	Long-term	0.68	15	5
BBCH 10-39	Omnivorous mouse	Long-term	1.24	8.1	5
BBCH >40	Omnivorous mouse	Long-term	0.36	28	5
Risk from bioaccumulation and food chain behaviour					
Not relevant as L_{og} Pow <3					
Risk from consumption of contaminated water					
Scenarios	**Indicator or focal species**	**Time scale**	**PEC_{dw}xDWR**	**TER**	**Trigger**
Puddle scenario, Screening step Application rate (120 g a.s./ha)/relevant endpoint <50 (koc<500 L/kg), TER calculation not needed					

Table 108: Risk assessment to mammals for Grapes at 120 g a.s./ha, 4 applications (SFP)

Growth stage	**Indicator or focal species**	**Time scale**	**DDD (mg/kg bw per day)**	**TER**	**Trigger**
Screening Step (Mammals)					
All	Small herbivorous mammal	Acute	29.5	16.4	10
All	Small herbivorous mammal	Long-term	10.12	**0.99**	5
Tier 1 (Mammals)					
BBCH 10-19	Herbivorous lagomorph	Long-term	0.94	11	5
BBCH 20-39	Herbivorous lagomorph	Long-term	0.77	13	5
BBCH >40	Herbivorous lagomorph	Long-term	0.46	22	5
BBCH 10-19	Insectivorous shrew	Long-term	0.59	17	5
BBCH >20	Insectivorous shrew	Long-term	0.27	37	5
BBCH 10-19	Herbivorous vole	Long-term	6.07	**1.65**	5
BBCH 20-39	Herbivorous vole	Long-term	5.05	**1.98**	5
BBCH >40	Herbivorous vole	Long-term	3.04	**3.29**	5
BBCH 10-19	Omnivorous mouse	Long-term	0.66	15	5
BBCH 20-39	Omnivorous mouse	Long-term	0.54	19	5
BBCH >40	Omnivorous mouse	Long-term	0.32	31	5
Risk from bioaccumulation and food chain behaviour					
Not relevant as L_{og} Pow <3					
Risk from consumption of contaminated water					

Puddle scenario, Screening step Application rate (120 g a.s./ha)/relevant endpoint <50 (koc<500 L/kg), TER calculation not needed

Table 109: Risk assessment to mammals for Tomatoes at 120 g a.s./ha, 5 applications (SFP)

Growth stage	Indicator or focal species	Time scale	DDD (mg/kg bw per day)	TER	Trigger
Screening Step (Mammals)					
All	Small herbivorous mammal	Acute	31.1	15.6	10
All	Small herbivorous mammal	Long-term	11.04	**0.9**	5
Tier 1 (Mammals)					
BBCH 71-89	Frugivorous rat	Long-term	3.85	**2.6**	5
BBCH 10-19	Insectivorous shrew	Long-term	0.64	16	5
BBCH >20	Insectivorous shrew	Long-term	0.29	34	5
BBCH 10-49	Herbivorous vole	Long-term	11.04	**0.9**	5
BBCH >50	Herbivorous vole	Long-term	3.31	**3**	5
BBCH 10-49	Omnivorous mouse	Long-term	1.2	8.3	5
BBCH >50	Omnivorous mouse	Long-term	0.35	28.6	5
Risk from bioaccumulation and food chain behaviour					
Not relevant as L_{og} Pow <3					
Risk from consumption of contaminated water					
Puddle scenario, Screening step Application rate (120 g a.s./ha)/relevant endpoint <50 (koc<500 L/kg), TER calculation not needed					

Aquatic organisms

The risk assessments for aquatic organisms (fish, aquatic invertebrates, algae and aquatic plants) were conducted in accordance to the new EFSA Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters (2013).

PEC_{sw} and PEC_{sed} values for cymoxanil and its metabolites were calculated at Step 1 and Step 2 simultaneously using the Steps 1-2 in FOCUS calculator (v. 3.2) with crop types according to the proposed GAP.

CTF

The risk to fish and aquatic invertebrates from intended uses of the plant protection product Cymoxanil 45 WG is considered acceptable after FOCUS Step 2 PECsw values were shown to be lower than the respective RAC_{sw} values.

No risk assessment to algae from cymoxanil was performed as no valid toxicity endpoints are available.

Table 110: FOCUSsw step 1-2 – PEC/RAC ratio for cymoxanil – grapes at 120 g a.s./ha [x 5 application]

Scenario	PEC global max (µg L)	fish acute	fish chronic	Aquatic invertebrates	Aquatic invertebrates prolonged	Algae	Higher plant
		Oncorhynchus mykiss	*Oncorhynchus mykiss*	*Daphnia magna*	*Daphnia magna*	*Indicate species*	*Indicate species*
		LC_{50}	NOEC	EC_{50}	EC_{10}	EC_{50}	EC_{50}
		29000 µg/L	44 µg/L	27000 µg/L	61.9 µg/L	x.xx µg/L	x.xx µg/L
RAC		290	4.4	270	6.19		

FOCUS Step 1								
	42.193	0.14	**9.6**	0.16	**6.8**			
FOCUS Step 2								
North Europe	3.2112		0.73		0.52			
South Europe	3.2112		0.73		0.52			
Trigger**		100	10	100	10	10	10	

*[Only scenarios where the trigger is not met at FOCUSsw step 1-2 should be included in step 3.]

**[If the Trigger value has been adjusted during the risk assessment, it should always be clear on what basis the risk assessment has been performed, i.e. what the AF value is and for which organism and endpoint it refers.]

Table 111: FOCUSsw step 1-2 – PEC/RAC ratio for cymoxanil metabolites– grapes at 120 g a.s./ha [x 5 applications]

Test organism	Metabolite	Tier 1-RAC (µg/L)	Max PEC_{sw} (µg/L)	PEC/RAC
Oncorhynchus mykiss	IN-T4226	530	35.3382	0.067
	IN-U3204	970	20.7907	0.02
	IN-KQ960	239.23	59.4568	0.25
	IN-R3274	880	27.0176	0.031
	IN-W3595	1000	57.5802	0.06
	IN-KP533	1000	42.059	0.042
	AS999	830	59.8735	0.072
	IN-JX915-003[a]	29	27.0219	0.93
	IN-R3273-006[a]	29	1.9716[b]	0.07
Daphnia magna	IN-T4226	370	35.3382	0.095
	IN-U3204	1000	20.7907	0.021
	IN-KQ960	8	2.0377[b]	0.25
	IN-KQ960	90[c]	59.4568	0.66
	IN-R3274	1000	27.0176	0.027
	IN-W3595	1000	57.5802	0.057
	IN-KP533	27	0.861[b]	0.032
	AS999	905	59.8735	0.066
	IN-JX915-003[a]	27	1.6891[b]	0.062
	IN-R3273-006[a]	27	1.9716[b]	0.073
Raphidocelis subcapitata	IN-T4226	7610	35.3382	0.005
	IN-U3204	3640	20.7907	0.006
	IN-KQ960	10000	59.4568	0.059
	IN-R3274	3800	27.0176	0.01
	AS999	7120	59.8735	0.008
	IN-JX915-003	93.1	27.0219	0.29
	IN-R3273-006	11600	69.0386	0.006
Anabaena flos-aquae	IN-W3595	10990	57.5802	0.005

Values in **bold** are above the trigger value of 1 and hence further consideration is needed

[a] Toxicity of metabolites are assumed to be 10 times more toxic than cymoxanil

[b] FOCUS Step 2 PEC values

Table 112: FOCUSsw step 1-2 – PEC/RAC ratio for cymoxanil – potatoes at 150 g a.s./ha [x 5 application]

Scenario	PEC global max (µg L)	fish acute	fish chronic	Aquatic invertebrates	Aquatic invertebrates prolonged	Algae	Higher plant
		Oncorhynchus mykiss	*Oncorhynchus mykiss*	*Daphnia magna*	*Daphnia magna*	*Indicate species*	*Indicate species*
		LC_{50}	NOEC	EC_{50}	EC_{10}	EC_{50}	EC_{50}
		29000 µg/L	44 µg/L	27000 µg/L	61.9 µg/L	x.xx µg/L	x.xx µg/L
RAC		290	4.4	270	6.19		
FOCUS Step 1							
	50.1067	0.17	**11.39**	0.18	**8.1**		
FOCUS Step 2							
North Europe	1.3795		0.313		0.22		
South Europe	1.3795		0.313		0.22		
Trigger**		100	10	100	10	10	10

*[Only scenarios where the trigger is not met at FOCUSsw step 1-2 should be included in step 3.]

**[If the Trigger value has been adjusted during the risk assessment, it should always be clear on what basis the risk assessment has been performed, i.e. what the AF value is and for which organism and endpoint it refers.]

Table 113: FOCUSsw step 1-2 – PEC/RAC ratio for cymoxanil metabolites– potatoes at 150 g a.s./ha [x 5 applications]

Test organism	Metabolite	Tier 1-RAC (µg/L)	Max PEC_{SW} (µg/L)	PEC/RAC
Oncorhynchus mykiss	IN-T4226	530	43.0395	0.08
	IN-U3204	970	25.3192	0.03
	IN-KQ960	239.23	72.2735	0.25
	IN-R3274	880	32.6315	0.302
	IN-W3595	1000	69.881	0.07
	IN-KP533	1000	50.2755	0.05
	AS999	830	71.1915	0.086
	IN-JX915-003[a]	29	0.7256[b]	0.025
	IN-R3273-006[a]	29	1.4391[b]	0.049
Daphnia magna	IN-T4226	370	43.0395	0.116
	IN-U3204	1000	25.3192	0.025
	IN-KQ960	8	1.1426[b]	0.14
	IN-KQ960	90[c]	72.2735	0.80
	IN-R3274	1000	32.6315	0.033
	IN-W3595	1000	69.881	0.070
	IN-KP533	27	0.353[b]	0.013
	AS999	905	71.1915	0.079
	IN-JX915-003[a]	27	0.7256[b]	0.027
	IN-R3273-006[a]	27	1.4391[b]	0.053
Raphidocelis subcapitata	IN-T4226	7610	43.0395	0.0056
	IN-U3204	3640	25.3192	0.0069
	IN-KQ960	10000	72.2735	0.0072
	IN-R3274	3800	32.6315	0.0085

Test organism	Metabolite	Tier 1-RAC (µg/L)	Max PEC_{sw} (µg/L)	PEC/RAC
	AS999	7120	71.1915	0.0099
	IN-JX915-003	93.1	0.7256 [b]	0.0078
	IN-R3273-006	11600	82.2704	0.0071
Anabaena flos-aquae	IN-W3595	10990	69.881	0.0063

Values in **bold** are above the trigger value of 1 and hence further consideration is needed
[a] Toxicity of metabolites are assumed to be 10 times more toxic than cymoxanil
[b] FOCUS Step 2 PEC values
[c] chronic endpoint EC_{10} (21d)

Table 114: FOCUSsw step 1-2 – PEC/RAC ratio for cymoxanil – tomatoes at 150 g a.s./ha [x 5 application]

Scenario	PEC global max (µg L)	fish acute	fish chronic	Aquatic invertebrates	Aquatic invertebrates prolonged	Algae	Higher plant
		Oncorhynchus mykiss	*Oncorhynchus mykiss*	*Daphnia magna*	*Daphnia magna*	*Indicate species*	*Indicate species*
		LC_{50}	NOEC	EC_{50}	EC_{10}	EC_{50}	EC_{50}
		29000 µg/L	44 µg/L	27000 µg/L	61.9 µg/L	x.xx µg/L	x.xx µg/L
RAC		290	4.4	270	6.19		
FOCUS Step 1							
	50.1067	0.17	**11.39**	0.18	**8.1**		
FOCUS Step 2							
North Europe	1.3795		0.313		0.22		
South Europe	1.3795		0.313		0.22		
Trigger**		100	10	100	10	10	10

*[Only scenarios where the trigger is not met at FOCUSsw step 1-2 should be included in step 3.]
**[If the Trigger value has been adjusted during the risk assessment, it should always be clear on what basis the risk assessment has been performed, i.e. what the AF value is and for which organism and endpoint it refers.]

Table 115: FOCUSsw step 1-2 – PEC/RAC ratio for cymoxanil metabolites– tomatoes at 150 g a.s./ha [x 5 applications]

Test organism	Metabolite	Tier 1-RAC (µg/L)	Max PEC_{sw} (µg/L)	PEC/RAC
Oncorhynchus mykiss	IN-T4226	530	43.0395	0.08
	IN-U3204	970	25.3192	0.03
	IN-KQ960	239.23	72.2735	0.25
	IN-R3274	880	32.6315	0.302
	IN-W3595	1000	69.881	0.07
	IN-KP533	1000	50.2755	0.05
	AS999	830	71.1915	0.086
	IN-JX915-003[a]	29	0.7256[b]	0.025
	IN-R3273-006[a]	29	1.1509[b]	0.039
Daphnia magna	IN-T4226	370	43.0395	0.116

Test organism	Metabolite	Tier 1-RAC (µg/L)	Max PEC_{SW} (µg/L)	PEC/RAC
	IN-U3204	1000	25.3192	0.025
	IN-KQ960	8	1.0509 [b]	0.13
	IN-KQ960	90[c]	72.2735	0.80
	IN-R3274	1000	32.6315	0.033
	IN-W3595	1000	69.881	0.070
	IN-KP533	27	0.291 [b]	0.011
	AS999	905	71.1915	0.079
	IN-JX915-003[a]	27	0.7256 [b]	0.027
	IN-R3273-006[a]	27	1.1509 [b]	0.043
Raphidocelis subcapitata	IN-T4226	7610	43.0395	0.009
	IN-U3204	3640	25.3192	0.007
	IN-KQ960	10000	72.2735	0.007
	IN-R3274	3800	32.6315	0.008
	AS999	7120	71.1915	0.008
	IN-JX915-003	93.1	32.3916	0.35
	IN-R3273-006	11600	82.2704	0.007
Anabaena flos-aquae	IN-W3595	10990	69.881	0.006

Values in **bold** are above the trigger value of 1 and hence further consideration is needed
[a] Toxicity of metabolites are assumed to be 10 times more toxic than cymoxanil
[b] FOCUS Step 2 PEC values
[c] chronic endpoint EC_{10} (21d)

Agria

The risk to fish and aquatic invertebrates from intended uses of the plant protection product Rival Duo is considered acceptable after FOCUS Step 2 PECsw values were shown to be lower than the respective RAC_{sw} values.

No risk assessment to algae from cymoxanil was performed as no valid toxicity endpoints are available.

Table 116: FOCUSsw step 1-2 – PEC/RAC ratio for cymoxanil – potatoes at 125 g a.s./ha [x 6 application]

Scenario	PEC global max (µg L)	fish acute	fish chronic	Aquatic invertebrates	Aquatic invertebrates prolonged	Algae	Higher plant
		Oncorhynchus mykiss	*Oncorhynchus mykiss*	*Daphnia magna*	*Daphnia magna*	*Indicate species*	*Indicate species*
		LC_{50}	NOEC	EC_{50}	EC_{10}	EC_{50}	EC_{50}
		29000 µg/L	44 µg/L	27000 µg/L	61.9 µg/L	x.xx µg/L	x.xx µg/L
RAC		290	4.4	270	6.19		
FOCUS Step 1							
	40.92	0.34	**9.3**	0.15	**6.6**		
FOCUS Step 2							
North Europe	1.1496		0.261		0.186		
South Europe	1.1496		0.261		0.186		
Trigger**		100	10	100	10	10	10

*[Only scenarios where the trigger is not met at FOCUSsw step 1-2 should be included in step 3.]

**[If the Trigger value has been adjusted during the risk assessment, it should always be clear on what basis the risk assessment has been performed, i.e. what the AF value is and for which organism and endpoint it refers.]

Table 117: FOCUSsw step 1-2 – PEC/RAC ratio for cymoxanil metabolites– potatoes at 125 g a.s./ha [x 6 applications]

Test organism	Metabolite	Tier 1-RAC (µg/L)	Max PEC_{SW} (µg/L)	PEC/RAC
Oncorhynchus mykiss	IN-T4226	530	25.98	0.05
	IN-U3204	970	20.13	0.02
	IN-KQ960	239.23	55.68	0.23
	IN-W3595	1000	61.56	0.06
Daphnia magna	IN-T4226	370	25.98	0.07
	IN-U3204	1000	20.13	0.02
	IN-KQ960	8	1.7280[b]	0.22
	IN-KQ960	90[c]	55.68	0.62
	IN-R3273 [a]	27	2.0142	0.075
	IN-W3595	1000	61.56	0.061
	(M5) AS999 [a]	27	4.36	0.16
	IN-JX915[a]	27	1.1573 [b]	0.043
	IN-KP533 [a]	27	1.4209 [b]	0.053

Values in **bold** are above the trigger value of 1 and hence further consideration is needed
[a] Toxicity of metabolites are assumed to be 10 times more toxic than cymoxanil
[b] FOCUS Step 2 PEC values
[c] chronic endpoint EC_{10} (21d)

Table 118: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for Rival Duo(Cymoxanil 50 g/L + Propamocarb hydrochloride 400 g/L SC)for each organism group based on FOCUS spray drift calculator (application to potatoes).

Group		**Invertebrate acute**	**Algae**
Test species		***Daphnia magna***	***P. subcapitata***
Endpoint		EC_{50}	E_rC_{50}/E_yC_{50}
(µg/L)		100000	39780
AF		100	10
RAC (µg/L)		1000	3978
FOCUS Scenario	**PEC_{gl-max} (µg/L)**	**PEC/RAC Ratio**	
Step 2			
Ditch	87.41	0.09	0.021
Pond	3.491	0.004	0.001
Stream	68.08	0.07	0.02

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold

SFP

The risk to fish and aquatic invertebrates from intended uses of the plant protection product Dauphin 45 is considered acceptable after FOCUS Step 2 PECsw values were shown to be lower than the respective RAC_{sw} values.

No risk assessment to algae from cymoxanil was performed as no valid toxicity endpoints are available.

Table 119: FOCUSsw step 1-2 – PEC/RAC ratio for cymoxanil – grapes at 120 g a.s./ha [x 4 application]

Scenario	PEC global max (µg L)	fish acute	fish chronic	Aquatic invertebrates	Aquatic invertebrates prolonged	Algae	Higher plant
		Oncorhynchus mykiss	*Oncorhynchus mykiss*	*Daphnia magna*	*Daphnia magna*	*Indicate species*	*Indicate species*
		LC_{50}	NOEC	EC_{50}	EC_{10}	EC_{50}	EC_{50}
		29000 µg/L	44 µg/L	27000 µg/L	61.9 µg/L	x.xx µg/L	x.xx µg/L
RAC		290	4.4	270	6.19		
FOCUS Step 1							
	41.014	0.14	**9.32**	0.15	**6.63**		
FOCUS Step 2							
South Europe	3.211		0.73		0.52		
Trigger**		100	10	100	10	10	10

*[Only scenarios where the trigger is not met at FOCUSsw step 1-2 should be included in step 3.]

**[If the Trigger value has been adjusted during the risk assessment, it should always be clear on what basis the risk assessment has been performed, i.e. what the AF value is and for which organism and endpoint it refers.]

Table 120: FOCUSsw step 1-2 – PEC/RAC ratio for cymoxanil metabolites– grapes at 120 g a.s./ha [x 4 applications]

Test organism	Metabolite	Tier 1-RAC (µg/L)	Max PEC_{SW} (µg/L)	PEC/RAC
Oncorhynchus mykiss	IN-T4226	530	16.46	0.031
	IN-U3204	970	19.84	0.02
	IN-KQ960	239.23	36.95	0.15
	IN-R3273	1000	53.40	0.053
	IN-W3595	1000	40.96	0.041
	IN-KP533	1000	39.16	0.039
	AS999 (M5)	830	9.78	0.012
Daphnia magna	IN-T4226	370	16.46	0.044
	IN-U3204	1000	19.84	0.02
	IN-KQ960	8	1.99[b]	0.25
	IN-KQ960	90[c]	36.95	0.41
	IN-W3595	1000	40.96	0.041
Anabaena flos-aquae	IN-W3595	10990	40.96	0.004

Values in **bold** are above the trigger value of 1 and hence further consideration is needed

[a] Toxicity of metabolites are assumed to be 10 times more toxic than cymoxanil
[b] FOCUS Step 2 PEC values
[c] chronic endpoint EC_{10} (21d)

Table 121: FOCUSsw step 1-2 – PEC/RAC ratio for cymoxanil – potato at 120 g a.s./ha [x 8 application]

Scenario	**PEC global max (µg L)**	**fish acute**	**fish chronic**	**Aquatic invertebrates**	**Aquatic invertebrates prolonged**	**Algae**	**Higher plant**
		Oncorhynchus mykiss	*Oncorhynchus mykiss*	*Daphnia magna*	*Daphnia magna*	*Indicate species*	*Indicate species*
		LC_{50}	NOEC	EC_{50}	EC_{10}	EC_{50}	EC_{50}
		29000 µg/L	44 µg/L	27000 µg/L	61.9 µg/L	x.xx µg/L	x.xx µg/L
RAC		290	4.4	270	6.19		
FOCUS Step 1							
	38.906	0.13	**8.84**	0.14	**6.28**		
FOCUS Step 2							
South Europe	1.104		0.25		0.18		
Trigger**		100	10	100	10	10	10

*[Only scenarios where the trigger is not met at FOCUSsw step 1-2 should be included in step 3.]
**[If the Trigger value has been adjusted during the risk assessment, it should always be clear on what basis the risk assessment has been performed, i.e. what the AF value is and for which organism and endpoint it refers.]

Table 122: FOCUSsw step 1-2 – PEC/RAC ratio for cymoxanil metabolites– potato at 120 g a.s./ha [x 8 applications]

Test organism	**Metabolite**	**Tier 1-RAC (µg/L)**	**Max PEC_{SW} (µg/L)**	**PEC/RAC**
Oncorhynchus mykiss	IN-T4226	530	31.47	0.06
	IN-U3204	970	19.32	0.02
	IN-KQ960	239.23	71.27	0.3
	IN-R3273	1000	101.60	0.1
	IN-W3595	1000	78.39	0.078
	IN-KP533	1000	74.79	0.075
	AS999 (M5)	830	9.30	0.011
Daphnia magna	IN-T4226	370	31.47	0.085
	IN-U3204	1000	19.32	0.02
	IN-KQ960	8	1.08[b]	0.135
	IN-KQ960	90[c]	71.27	0.79
	IN-W3595	1000	78.39	0.078
Anabaena flos-aquae	IN-W3595	10990	78.39	0.007

Values in **bold** are above the trigger value of 1 and hence further consideration is needed
Toxicity of metabolites are assumed to be 10 times more toxic than cymoxanil
[b] FOCUS Step 2 PEC values
[c] chronic endpoint EC_{10} (21d)

Table 123: FOCUSsw step 1-2 – PEC/RAC ratio for cymoxanil – tomato at 120 g a.s./ha [x 5 application]

Scenario	**PEC global**	**fish acute**	**fish chronic**	**Aquatic invertebrates**	**Aquatic invertebrates**	**Algae**	**Higher plant**

	max (µg L)				**prolonged**		
		Oncorhynchus mykiss	*Oncorhynchus mykiss*	*Daphnia magna*	*Daphnia magna*	*Indicate species*	*Indicate species*
		LC_{50}	NOEC	EC_{50}	EC_{10}	EC_{50}	EC_{50}
		29000 µg/L	44 µg/L	27000 µg/L	61.9 µg/L	x.xx µg/L	x.xx µg/L
RAC		290	4.4	270	6.19		
FOCUS Step 1							
	38.906	0.13	**8.84**	0.14	**6.28**		
FOCUS Step 2							
South Europe	1.145		0.26		0.18		
Trigger**		100	10	100	10	10	10

*[Only scenarios where the trigger is not met at FOCUSsw step 1-2 should be included in step 3.]

**[If the Trigger value has been adjusted during the risk assessment, it should always be clear on what basis the risk assessment has been performed, i.e. what the AF value is and for which organism and endpoint it refers.]

Table 124: FOCUSsw step 1-2 – PEC/RAC ratio for cymoxanil metabolites– tomato at 120 g a.s./ha [x 5 applications]

Test organism	**Metabolite**	**Tier 1-RAC (µg/L)**	**Max PEC_{SW} (µg/L)**	**PEC/RAC**
Oncorhynchus mykiss	IN-T4226	530	19.67	0.04
	IN-U3204	970	19.32	0.02
	IN-KQ960	239.23	44.33	0.2
	IN-R3273	1000	63.53	0.06
	IN-W3595	1000	48.99	0.05
	IN-KP533	1000	46.74	0.05
	AS999 (M5)	830	9.30	0.011
Daphnia magna	IN-T4226	370	19.67	0.053
	IN-U3204	1000	19.32	0.02
	IN-KQ960	8	2.19[b]	0.27
	IN-KQ960	90[c]	44.33	0.5
	IN-W3595	1000	48.99	0.05
Anabaena flos-aquae	IN-W3595	10990	48.99	0.004

Values in **bold** are above the trigger value of 1 and hence further consideration is needed

[a] Toxicity of metabolites are assumed to be 10 times more toxic than cymoxanil

[b] FOCUS Step 2 PEC values

[c] chronic endpoint EC_{10} (21d)

Non-target arthropods

Risk assessment for bees

CTF

The risk assessment for bees has been conducted according to EFSA bee guidance document including acute assessment of adult honey bees and honey bee larva, long-term honey bee risk and risk to bumble bees. The risk to solitary bees was not addressed due to lack of data.

The acute risk to adult honeybees and bumblebees from the active substance cymoxanil is considered acceptable for the proposed uses in grapevines, potato and tomato.

The chronic risk to adult honeybees is considered acceptable for the proposed use of Cymoxanil in grapevines and also in potatoes and tomatoes when bees are foraging on the weeds in the field when refined TWA based on the available DT_{50} data and nurse bee SV values for the application in grapes are used.
Also the risk to honeybee larvae is unacceptable for the proposed use of Cymoxanil in grapevines (BBCH 10-19), potatoes and tomatoes (BBCH 10-39) when bees are foraging on the weeds in the field.
A higher tier assessment could not be performed as no suitable data were available.
The risk from consumption of contaminated water is acceptable.

Table 125: Risk assessment to bees and bumblebees for Potato, Tomato 150 g a.s./ha, 5 applications – Screening step

Species	Test substance	Risk quotient	HQ/ETR	Trigger
Apis mellifera L.	cymoxanil	HQcontact	1.5	>42
Bombus terrestris L.	cymoxanil	HQcontact	1.5	>7
Apis mellifera L.	cymoxanil	ETRacute adult oral	0.01	>0.2
Bombus terrestris L.	cymoxanil	ETRacute adult oral	0.02	>0.036
Apis mellifera L.	cymoxanil	ETRchronic adult oral	**0.234**	>0.03
Apis mellifera L.	cymoxanil	ETRlarvae	**2.74**	>0.2

Table 126: Risk assessment for Potato, Tomato 150 g a.s./ha, 5 applications – 1st tier assessment – chronic adult bees

Species	Test substance	Risk quotient	Scenario	BBCH	ETR	Trigger
Apis mellifera L.	cymoxanil	ETRchronic adult oral	Risks from foraging on the treated crop	10 - 69	0.02	>0.03
				≥ 70	0.000	>0.03
			Risks from foraging on the weeds	10 - 39	**0.064**	>0.03
				40 - 69	0.02	>0.03
				≥ 70	0.02	>0.03
			Risks from foraging on the field margins	10 - 69	0.0006	>0.03
				≥ 70	0.0006	>0.03
			Risks from foraging on the adjacent crop	10 - 69	0.0004	>0.03
				≥ 70	0.0004	>0.03

Table 127: Risk assessment for Potato, Tomato 150 g a.s./ha, 5 applications – higher tier assessment – chronic adult bees (TWA refined using DT50 data)

Species	Test substance	Risk quotient	Scenario	BBCH	ETR	Trigger
Apis mellifera L.	cymoxanil	ETRchronic adult oral	Risks from foraging on the weeds	10 - 39	0.029	>0.03

Table 128: Risk assessment for Potato, Tomato 150 g a.s./ha, 5 applications – 1st tier assessment – chronic honey bee larvae

Species	Test substance	Risk quotient	Scenario	BBCH	ETR	Trigger
Apis mellifera L.	cymoxanil	ETRlarvae	Risks from foraging on the treated crop	10 - 69	0.008	>0.2
				≥ 70	0.000	>0.2
			Risks from foraging on the weeds	10-39	**0.98**	>0.2
				40 - 69	**0.29**	>0.2
				≥ 70	**0.29**	>0.2
			Risks from foraging on the field margins	10 - 69	0.009	>0.2
				≥ 70	0.009	>0.2
			Risks from foraging on the adjacent crop	10 - 69	0.006	>0.2
				≥ 70	0.006	>0.2

Table 129: Risk assessment for Potato, Tomato 150 g a.s./ha, 5 applications – higher tier assessment – chronic honey bee larvae (TWA refined using DT50 data)

Species	Test substance	Risk quotient	Scenario	BBCH	ETR	Trigger
Apis mellifera L.	cymoxanil	ETRlarvae	Risks from foraging on the weeds	10 - 39	**0.45**	>0.2
				40 - 69	0.14	>0.2
				≥ 70	0.14	>0.2

Table 130: Risk assessment for Grape, 120 g a.s./ha, 5 applications – Screening step

Species	Test substance	Risk quotient	HQ/ETR	Trigger
Apis mellifera L.	cymoxanil	HQcontact	1.2	>85
Bombus terrestris L.	cymoxanil	HQcontact	1.2	>14
Apis mellifera L.	cymoxanil	ETRacute adult oral	0.01	>0.2

Bombus terrestris L.	cymoxanil	ETRacute adult oral	0.02	>0.036
Apis mellifera L.	cymoxanil	ETRchronic adult oral	**0.261**	>0.03
Apis mellifera L.	cymoxanil	ETRlarvae	**0.27**	>0.2

Table 131: Risk assessment for Grape, 120 g a.s./ha, 5 applications – 1st tier assessment – chronic adult bees

Species	Test substance	Risk quotient	Scenario	BBCH	ETR	Trigger
Apis mellifera L.	cymoxanil	ETRchronic adult oral	Risks from foraging on the treated crop	10 - 69	**0.145**	>0.03
				≥ 70	0.000	>0.03
			Risks from foraging on the weeds	10 - 19	**0.03**	>0.03
				20 - 39	0.02	>0.03
				40 - 69	0.015	>0.03
				≥ 70	0.015	>0.03
			Risks from foraging on the field margins	10 - 19	0.0005	>0.03
				20 - 69	0.001	>0.03
				≥ 70	0.001	>0.03
			Risks from foraging on the adjacent crop	10 - 19	0.0005	>0.03
				20 - 69	0.0015	>0.03
				≥ 70	0.0015	>0.03

Table 132: Risk assessment for Grape, 120 g a.s./ha, 5 applications – higher tier assessment – chronic adult bees (SV refined using nectar attractiveness data and TWA refined using DT50 data)

Species	Test substance	Risk quotient	Scenario	BBCH	ETR	Trigger
Apis mellifera L.	cymoxanil	ETRchronic adult oral	Risks from foraging on the treated crop	10 - 69	0.0005	>0.03
Apis mellifera L.	cymoxanil	ETRchronic adult oral	Risks from foraging on the weeds	10 - 19	0.014	>0.03

Table 133: Risk assessment for Grape, 120 g a.s./ha, 5 applications – 1st tier assessment – chronic honey bee larvae

Species	Test substance	Risk quotient	Scenario	BBCH	ETR	Trigger

Apis mellifera L.	cymoxanil	ETRlarvae	Risks from foraging on the treated crop	10 - 69	**2.19**	>0.2
				≥ 70	0.000	>0.2
			Risks from foraging on the weeds	10 - 19	**0.47**	>0.2
				20 - 39	**0.39**	>0.2
				40 - 69	**0.24**	>0.2
				≥ 70	**0.24**	>0.2
			Risks from foraging on the field margins	10 - 19	0.007	>0.2
				20 - 69	0.005	>0.2
				≥ 70	0.005	>0.2
			Risks from foraging on the adjacent crop	10 - 19	0.007	>0.2
				20 - 69	0.02	>0.2
				≥ 70	0.02	>0.2

Table 134: Risk assessment for Grape, 120 g a.s./ha, 5 applications – higher tier assessment – chronic honey bee larvae (SV refined using nectar attractiveness data and TWA refined using DT50 data)

Species	Test substance	Risk quotient	Scenario	BBCH	ETR	Trigger
Apis mellifera L.	cymoxanil	ETRlarvae	Risks from foraging on the treated crop	10 - 69	0.003	>0.2
			Risks from foraging on the weeds	10 - 19	**0.22**	>0.2
				20 - 39	0.18	>0.2
				40 - 69	0.11	>0.2
				≥ 70	0.11	>0.2

Table 135: Assessment of risk from exposure to contaminated water

Life stage	ETR	Trigger
Guttation water (1st tier)		
Acute Adult	0.10	0.2
Chronic Adult	**0.98**	0.03
Chronic larvae	**258**	0.2
Guttation water (Higher tier): Refinement of the chronic risk from exposure to guttation water was performed by taking the $PEC_{soil, porewater}$ as a refined approximation of the concentration in guttation fluid		
Chronic Adult	4.5×10^{-6}	0.03
Chronic larvae	9×10^{-4}	0.2
Surface water (1st tier)		
Acute Adult	6.7×10^{-6}	0.2
Chronic Adult	1.2×10^{-4}	0.03
Chronic larvae	0.023	0.2

Puddle water (1st tier)		
Acute Adult	6.7×10^{-7}	0.2
Chronic Adult	1.2×10^{-5}	0.03
Chronic larvae	0.0023	0.2

SFP

The risk assessment for bees has been conducted according to EFSA bee guidance document including acute assessment of adult honey bees and honey bee larva, long-term honey bee risk and honey bee larva.

The acute risk from the active substance cymoxanil is considered acceptable for the proposed uses in potato, grapevines and tomato, however the chronic risk to adult honeybees and the risk to honeybee larvae are not acceptable in the treated crop in grapevines and in potato and tomato when bees are foraging on weeds in the field. A higher tier assessment could not be performed as no suitable data were available. The risk from consumption of guttation water was acceptable for acute risk, however not acceptable for chronic risk.

Table 136: Risk assessment for Potato, Tomato, grapes 120 g a.s./ha, 4-8 applications – Screening step

Species	Test substance	Risk quotient	HQ/ETR	Trigger
Apis mellifera L.	cymoxanil	HQcontact	1.2	>42
Apis mellifera L.	cymoxanil	ETRacute adult oral	0.015	>0.2
Apis mellifera L.	cymoxanil	ETRchronic adult oral	**0.26**	>0.03
Apis mellifera L.	cymoxanil	ETRlarvae	**2.2**	>0.2

Table 137: Risk assessment for Potato 120 g a.s./ha, 8 applications – 1st tier assessment – chronic adult bees

Species	Test substance	Risk quotient	Scenario	BBCH	ETR	Trigger
Apis mellifera L.	cymoxanil	ETRchronic adult oral	Risks from foraging on the treated crop	10 - 39	0.02	>0.03
				40-69	0.02	>0.03
				≥ 70	0.00	>0.03
			Risks from foraging on the weeds	10 - 39	**0.05**	>0.03
				40 - 69	0.02	>0.03
				≥ 70	0.011	>0.03
			Risks from foraging on the field margins	10 - 39	0.00	>0.03
				40 - 69	0.00	>0.03
				≥ 70	0.00	>0.03
			Risks from foraging on the adjacent crop	10 - 39	0.00	>0.03
				40 - 69	0.00	>0.03
				≥ 70	0.00	>0.03

Table 138: Potato 120 g a.s./ha, 8 applications – 1st tier assessment – chronic honey bee larvae

Species	Test substance	Risk quotient	Scenario	BBCH	ETR	Trigger
Apis mellifera L.	cymoxanil	ETRlarvae	Risks from foraging on the treated crop	10 - 39	0.06	>0.2
				40-69	0.06	>0.2
				≥ 70	0.00	>0.2
			Risks from foraging on the weeds	10 - 39	**0.93**	>0.2
				40 - 69	**0.28**	>0.2
				≥ 70	**0.28**	>0.2
			Risks from foraging on the field margins	10 - 39	0.01	>0.2
				40 - 69	0.01	>0.2
				≥ 70	0.01	>0.2
			Risks from foraging on the adjacent crop	10 - 39	0.01	>0.2
				40 - 69	0.01	>0.2
				≥ 70	0.01	>0.2

Table 139: Risk assessment for Totato 120 g a.s./ha, 5 applications – 1st tier assessment – chronic adult bees

Species	Test substance	Risk quotient	Scenario	BBCH	ETR	Trigger
Apis mellifera L.	cymoxanil	ETRchronic adult oral	Risks from foraging on the treated crop	10 - 49	0.02	>0.03
				50-69	0.02	>0.03
				≥ 70	0.00	>0.03
			Risks from foraging on the weeds	10 - 49	**0.05**	>0.03
				50 - 69	0.02	>0.03
				≥ 70	0.02	>0.03
			Risks from foraging on the field margins	10 - 49	0.00	>0.03
				50 - 69	0.00	>0.03
				≥ 70	0.00	>0.03
			Risks from foraging on the adjacent crop	10 - 49	0.00	>0.03
				50 - 69	0.00	>0.03
				≥ 70	0.00	>0.03

Table 140: Risk assessment for Tomato 120 g a.s./ha, 5 applications – 1st tier assessment – chronic honey bee larvae

Species	Test substance	Risk quotient	Scenario	BBCH	ETR	Trigger

Apis mellifera L.	cymoxanil	ETRlarvae	Risks from foraging on the treated crop	10 - 49	0.06	>0.2
				50-69	0.06	>0.2
				≥ 70	0.00	>0.2
			Risks from foraging on the weeds	10 - 49	**0.93**	>0.2
				50 - 69	**0.28**	>0.2
				≥ 70	**0.28**	>0.2
			Risks from foraging on the field margins	10 - 49	0.01	>0.2
				50 - 69	0.01	>0.2
				≥ 70	0.01	>0.2
			Risks from foraging on the adjacent crop	10 - 49	0.01	>0.2
				50 - 69	0.01	>0.2
				≥ 70	0.01	>0.2

Table 141: Risk assessment for Grapes 120 g a.s./ha, 4 applications – 1st tier assessment – chronic adult bees

Species	Test substance	Risk quotient	Scenario	BBCH	ETR	Trigger
Apis mellifera L.	cymoxanil	ETRchronic adult oral	Risks from foraging on the treated crop	10 - 19	**0.15**	>0.03
				20 - 39	**0.15**	>0.03
				40 - 69	**0.15**	>0.03
				≥ 70	0,00	>0.03
			Risks from foraging on the weeds	10 - 19	**0.03**	>0.03
				20 - 39	0.03	>0.03
				40 - 69	0.02	>0.03
				≥ 70	0.02	>0.03
			Risks from foraging on the field margins	10 - 19	0.00	>0.03
				20 - 39	0.00	>0.03
				40 - 69	0.00	>0.03
				≥ 70	0.00	>0.03
			Risks from foraging on the adjacent crop	10 - 19	0.00	>0.03
				20 - 39	0.00	>0.03
				40 - 69	0.00	>0.03
				≥ 70	0.00	>0.03

Table 142: Risk assessment for Grapes 120 g a.s./ha, 4 applications – 1st tier assessment – chronic honey bee larvae

Species	Test substance	Risk quotient	Scenario	BBCH	ETR	Trigger
Apis mellifera L.	cymoxanil	ETRlarvae	Risks from foraging on the treated crop	10 - 19	**2.58**	>0.2
				20 - 39	**2.58**	>0.2
				40 - 69	**2.58**	>0.2
				≥ 70	0.00	>0.2
			Risks from foraging on the weeds	10 - 19	**0.56**	>0.2
				20 - 39	**0.47**	>0.2
				40 - 69	**0.28**	>0.2
				≥ 70	**0.28**	>0.2
			Risks from foraging on the field margins	10 - 19	0.01	>0.2
				20 - 39	0.03	>0.2
				40 - 69	0.03	>0.2
				≥ 70	0.03	>0.2
			Risks from foraging on the adjacent crop	10 - 19	0.01	>0.2
				20 - 39	0.01	>0.2
				40 - 69	0.03	>0.2
				≥ 70	0.03	>0.2

Table 143: Assessment of risk from exposure to contaminated water

Life stage	ETR	Trigger
Guttation water (1st tier)		
Acute Adult	0.12	0.2
Chronic Adult	**1.13**	0.03

Agria

The evaluation of the risk for bees was performed in accordance with the recommendations of the "Guidance Document on Terrestrial Ecotoxicology", as provided by the Commission Services (SANCO/10329/2002 rev.2 (final), October 17, 2002) and EPPO 2010.

No risk assessment to bees was provided according to the EFSA Bee Guidance Document

Table 144: Risk assessment to bees for – potatoes at 125 g cymoxanil/ha, 6 application

Species	Test substance	Risk quotient	HQ/ETR	Trigger
Apis mellifera	cymoxanil	HQoral	<1.45	≤ 50
Apis mellifera	cymoxanil	HQcontact	<1.25	≤ 50
Apis mellifera	Propamocarb Hydrochloride 400 g/L + Cymoxanil 50 g/L SC	HQoral	<11.37	≤ 50

Apis mellifera	Propamocarb Hydrochloride 400 g/L + Cymoxanil 50 g/L SC	HQcontact	<11.37	≤ 50
Apis mellifera	cymoxanil	ETRadultoral	>5.83	1*
Apis mellifera	cymoxanil	ETRlarval	3.3	1*

*The risk assessment is based upon the EPPO 2010

Risk assessment for arthropods

CTF

The in-field risk and off-field risk of cymoxanil to non-target terrestrial arthropods based on laboratory studies and higher tier studies is acceptable for the intended uses of Cymoxanil 45 WG in grapevines, potatoes and tomatoes.

Table 145: Risk assessment to non-target arthropods for potato, 150 g a.s./ha, 5 applications

Test substance	Species	Effect (LR_{50} g/ha)	HQ in-field	HQ off-field[1]	Trigger
cymoxanil	*Typhlodromus pyri*	480	0.94	0.016	2
cymoxanil	*Aphidius rhopalosiphi*	960	0.47	0.008	2

[1] based on Ganzelmeier drift data at 1 m distance

Table 146: Risk assessment to non-target arthropods for grape, 120 g a.s./ha, 5 applications

Test substance	Species	Effect (LR_{50} g/ha)	HQ in-field	HQ off-field[1]	Trigger
cymoxanil	*Typhlodromus pyri*	480	0.75	0.049	2
cymoxanil	*Aphidius rhopalosiphi*	960	0.38	0.025	2

[1] based on Ganzelmeier drift data at 3 m distance

Table 147: Risk assessment to non-target arthropods for tomato, 150 g a.s./ha, 5 applications

Test substance	Species	Effect (LR_{50} g/ha)	HQ in-field	HQ off-field[1]	Trigger
cymoxanil	*Typhlodromus pyri*	480	0.94	0.062	2
cymoxanil	*Aphidius rhopalosiphi*	960	0.47	0.031	2

[1] based on Ganzelmeier drift data at 3 m distance

Table 148: Risk assessment to non-target arthropods for potato, 150 g a.s./ha, 5 applications based on extended lab test or aged residue tests

Species	ER_{50} (g/ha)	In-field rate	Off-field rate[1]
Typhlodromus pyri	> 394.12	450	39.38
Chrysoperla carnea	> 480	450	39.38
Poecilus cupreus	> 480	450	39.38

[1]indicate distance assumed to calculate the drift rate and if 3D or 2D.

Table 149: Risk assessment to non-target arthropods for grape, 120 g a.s./ha, 5 applications based on extended lab test or aged residue tests

Species	ER_{50} (g/ha)	In-field rate	Off-field rate[1]
Typhlodromus pyri	> 394.12	360	118.6
Chrysoperla carnea	> 480	360	118.6
Poecilus cupreus	> 480	360	118.6

[1]indicate distance assumed to calculate the drift rate and if 3D or 2D.

Table 150: Risk assessment to non-target arthropods for tomato, 150 g a.s./ha, 5 applications based on extended lab test or aged residue tests

Species	ER_{50} (g/ha)	In-field rate	Off-field rate[1]
Typhlodromus pyri	> 394.12	450	148.3
Chrysoperla carnea	> 480	450	148.3
Poecilus cupreus	> 480	450	148.3

[1]indicate distance assumed to calculate the drift rate and if 3D or 2D.

Field studies
Effects of Cymbal 45 WG on Predatory Mites (Acari, Phytoseiidae) under Field Conditions in Vine (4 Applications) No unacceptable effects on predatory mite populations (Acari: Phytoseiidae) mobile stages or the eggs were observed if Cymbal 45 WG is applied 4 times at an application rate of 400 g product/ha in grapevine (equivalent to 180 g of Cymoxanil/ha).

Agria

The in-field risk to non-target terrestrial arthropods based on laboratory studies and extend laboratory studies is not acceptable for the intended uses of Cymoxanil 50 g/L + Propamocarb hydrochloride 400 g/L SC in potatoes.
The off-field risk to non-target terrestrial arthropods based on laboratory studies and extend studies is acceptable for the intended uses of Cymoxanil 50 g/L + Propamocarb hydrochloride

Table 151: Risk assessment to non-target arthropods for potato, 2.5 L prod./ha, 125 g cymoxanil/ha, 6 applications

Test substance	Species	Effect (LR_{50} L prod./ha)	HQ in-field	HQ off-field (1 m)	Trigger

Test substance	Species	Effect (LR_{50} L prod./ha)	HQ in-field	HQ off-field (1 m)	Trigger
Propamocarb Hydrochloride 400 g/L + Cymoxanil 50 g/L SC	*Typhlodromus pyri*	>4.375	1.83	0.03	2
Propamocarb Hydrochloride 400 g/L + Cymoxanil 50 g/L SC	*Aphidius rhopalosiphi*	0.924	**8.66**	0.14	2

[2] for preparations indicate whether dose is expressed in units of a.s. or preparation
[3] indicate if positive percentages relate to adverse effects or not

Table 152: Risk assessment to non-target arthropods for Potato, 2.5 L prod./ha, 125 g cymoxanil/ha, 6 applications based on extended lab test or aged residue tests

Species	ER_{50} (L/ha)	In-field rate	Off-field rate[1]
Typhlodromus pyri	> 2.5	**8**	0.656
Aphidius rhopalosiphi	> 2.5	**8**	0.656

[1]indicate distance assumed to calculate the drift rate and if 3D or 2D.

SFP

The in-field and off-field HQ values for non-target arthropods were below the Annex VI trigger value of 2 for *Aphidius rhopalosiphi* and *Typhlodromus pyri*. Therefore, FDJ03 poses an acceptable in-field and off-field risk following application in accordance with the proposed uses.

Table 153: Risk assessment to non-target arthropods for potato, 120 g a.s./ha, 8 applications

Test substance	Species	Effect (LR_{50} g/ha)	HQ in-field	HQ off-field[1]	Trigger
cymoxanil	*Typhlodromus pyri*	240	1	0.015	2
cymoxanil	*Aphidius rhopalosiphi*	240	1	0.015	2

[1] based on Ganzelmeier drift data at 1 m distance

Table 154: Risk assessment to non-target arthropods for grape, 120 g a.s./ha, 4 applications

Test substance	Species	Effect (LR_{50} g/ha)	HQ in-field	HQ off-field[1]	Trigger
cymoxanil	*Typhlodromus pyri*	240	1.01	0.052	2
cymoxanil	*Aphidius rhopalosiphi*	240	1.01	0.052	2

[1] based on Ganzelmeier drift data at 3 m distance

Table 155: Risk assessment to non-target arthropods for tomato, 120 g a.s./ha, 5 applications

Test substance	Species	Effect (LR_{50} g/ha)	HQ in-field	HQ off-field[1]	Trigger
cymoxanil	*Typhlodromus pyri*	240	0.86	0.056	2
cymoxanil	*Aphidius rhopalosiphi*	240	0.86	0.056	2

[1] based on Ganzelmeier drift data at 3 m distance

Risk assessment for earthworms and other soil macroorganisms

CTF

The long-term risk from exposure to cymoxanil is acceptable for earthworms and for both *Folsomia candida* and *Hypoaspis aculeifer* following the intended uses of Cymoxanil 45 WG in grapevines, potato and tomato.

Table 156: Risk assessment for worst case use of Cymoxanil 45 WG in grapevines, potato and tomato as spray application.

Test organism	Test substance	Time scale	Soil PEC_{max}	TER	Trigger
Earthworms					
Eisenia fetida	cymoxanil	Chronic	0.4531	28	5
Eisenia fetida	IN-T4226	Chronic	0.0149	>1000	5
Eisenia fetida	IN-U3204	Chronic	0.0808	>1000	5
Eisenia fetida	IN-KQ960	Chronic	0.0496	>1000	5
Eisenia fetida	IN-R3273	Chronic	-*	-	5
Eisenia fetida	IN-W3595	Chronic	0.0287	>1000	5
Eisenia fetida	IN-JX915	Chronic	0.0198	>1000	5
Other soil macroorganisms					
Folsomia candida	cymoxanil	Chronic	0.4531	117	5
Hypoaspis aculeifer	cymoxanil	Chronic	0.4531	616	5

*No PEC_{soil} available as not a relevant metabolite in soil

Agria

Table 157: Risk assessment for worst case use of of Cymoxanil 50 g/L + Propamocarb 400 g/L hydrochloride SC in potatoes as spray application.

Test organism	Test substance	Time scale	Soil PEC_{max}	TER	Trigger
Earthworms					
Eisenia fetida	cymoxanil	Chronic	0.1016	126	5
Eisenia fetida	IN-U3204*	Chronic	0.0988	13	5
Eisenia fetida	IN-KQ960*	Chronic	0.0275	46	5

Test organism	Test substance	Time scale	Soil PEC_{max}	TER	Trigger
Eisenia fetida	IN-W3595*	Chronic	0.0261	49	5
Eisenia fetida	IN-JX915*	Chronic	0.0436	29	5
Eisenia fetida	Cymoxanil 50 g/L + Propamocarb 400 g/L hydrochloride SC	Chronic	8.776	29.28	5
Other soil macroorganisms					
Folsomia candida	cymoxanil	Chronic	0.1016	521	5
Folsomia candida	Cymoxanil 50 g/L + Propamocarb 400 g/L hydrochloride SC	Chronic	8.776	57	5
Hypoaspis aculeifer	cymoxanil	Chronic	0.1016	2747	5
Hypoaspis aculeifer	Cymoxanil 50 g/L + Propamocarb 400 g/L hydrochloride SC	Chronic	8.776	> 113.9	5

*Where measured data for metabolites is not available, the endpoint has been assumed to be 10x lower than that of the parent molecule

SFP

Table 158: Risk assessment for worst case use of FDJO3 in grapevines, potato and tomato as spray application.

Test organism	Test substance	Time scale	Soil PEC_{max}	TER	Trigger
Earthworms					
Eisenia fetida	cymoxanil	Chronic	0.184	69.56	5
Eisenia fetida	IN-U3204	Chronic	No data available		5
Eisenia fetida	IN-KQ960	Chronic			5
Eisenia fetida	IN-W3595	Chronic			5
Eisenia fetida	IN-JX915	Chronic			5
Other soil macroorganisms					
Folsomia candida	cymoxanil	Chronic	No data available		5
Hypoaspis aculeifer	cymoxanil	Chronic			5

Table 159: Risk assessment for soil nitrogen transformation

Nitrogen transformation	cymoxanil	28 d, aerobic soil type	<25% effect at day 28 at 1.63 mg a.s./kg d.w.soil

Nitrogen transformation	Cymoxanil 50 g/L + Propamocarb 400 g/L hydrochloride SC	28 d, aerobic soil type	<25% effect at at 29.1 mg f.p./kg soil dw (equivelant to: 10.61 mg propamocarb HCL /kg soil dw, 1.33 mg Cymoxanil /kg sil dw)

CTF

The risk of cymoxanil is acceptable for soil non-target micro-organisms following the intended uses of Cymoxanil 45 WG in grapevines, potato and tomato.

Table 160: Summary of the soil nitrogen transformation endpoints for the active substance cymoxanil and risk assessment based on max PECsoil values, for the intended uses in grapevines, potato and tomato.

Test substance	Endpoint (mg/kg soil dw)	Crop	Maximum PEC_{SOIL} (mg/kg soil d.w.)	Acceptable risk
Cymoxanil	1.63	Grapevines	0.2146	yes
		Potato	0.4531	yes
		Tomato	0.2299	yes

Agria

The risk of cymoxanil is acceptable for soil non-target micro-organisms following the intended uses of Cymoxanil 50 g/L + Propamocarb HC 400 g/L SC in potato.

Table 161: Assessment of the risk for effects on soil micro-organisms due to the use of Propamocarb 400 g/L hydrochloride + cymoxanil 50 g/L SC in potatoes.

Intended use	Potatoes		
N-mineralisation			
Product/active substance	**Max. conc. with effects ≤ 25 % (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**TER_{lt} (criterion TER ≥ 1)**
Cymoxanil	1.6 (at 28 d)	0.1016	15.7
Propamocarb-HCL	38.5 (at 28 d)	8.664	3.34
Propamocarb 400 g/L hydrochloride + cymoxanil 50 g/L SC	29.1 (at 28 d)	8.776	3.32
C-mineralisation			
Product/active substance	**Max. conc. with effects ≤ 25 % (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**TER_{lt} (criterion TER ≥ 1)**
Cymoxanil	1.6 (at 28 d)	0.1016	15.7
Propamocarb-HCL	38.5 (at 28 d)	8.664	3.34
Propamocarb 400 g/L hydrochloride + cymoxanil 50 g/L SC	29.1 (at 28 d)	8.776	3.32

SFP

The risk of cymoxanil is acceptable for soil non-target micro-organisms following the intended uses of FDJ03 in grapevines, potato and tomato.

Table 162: Summary of the soil nitrogen transformation endpoints for the active substance cymoxanil and risk assessment based on max PECsoil values, for the intended uses in grapevines, potato and tomato.

Test substance	Endpoint (mg/kg soil dw)	Crop	PEC_{SOIL} (mg/kg soil d.w.)	Acceptable risk
Cymoxanil	1.63	Grapevines	0.1839	yes
		Potato	0.1640	yes
		Tomato	0.1640	yes

Risk assessment for non-target plants

CTF

The risk of cymoxanil to non-target terrestrial plants following the intended use of Cymoxanil 45 WG in grapevines, potato and tomato is acceptable.

Table 163: Risk assessment to non-target plants for use of Cymoxanil 45 WG in grapevines, potato and tomato as spray application

Most sensitive species	Test substance	ER_{50} (g/ha) vegetative vigour	ER_{50} (g/ha) emergence	Exposure[1] (g/ha)	TER vegetative vigour	TER emergence	Trigger
Potato							
Allium cepa	Cymoxanil 45% WG, BCP308F	>240 [a.s.]	>248 g a.s /ha	2.625	91.4	94.5	5
Grape							
Allium cepa	Cymoxanil 45% WG, BCP308F	>240 [a.s.]	>248 g a.s /ha	7.908	30.3	31.4	5
Tomato							
Allium cepa	Cymoxanil 45% WG, BCP308F	>240 [a.s.]	>248 g a.s /ha	9.885	24.3	25.1	5
Extended laboratory studies: No data submitted Semi-field and field test: No data submitted							

[1] based on Ganzelmeier drift data at 1 m distance

Agria

The risk of cymoxanil to non-target terrestrial plants following the intended use of Cymoxanil 50 g/L + Propamocarb 400 g/L hydrochloride SC in potato is acceptable.

Table 164: Risk assessment to non-target plants for use of Cymoxanil 50 g/L + Propamocarb 400 g/L hydrochloride SC in potato

Species	Test substance	ER_{50} (g/ha)[2] vegetative vigour	ER_{50} (g/ha)[2] emergence	Exposure[1] (g/ha)[2]	TER	Trigger
Allium cepa	Cymoxanil 50 WP	>240 g a.s /ha	-	2.05	117	5
Extended laboratory studies: No data submitted Semi-field and field test: No data submitted						

SFP

The risk of cymoxanil to non-target terrestrial plants following the intended use of FDJO3 in grapevines, potato and tomato is acceptable.

Table 165: Risk assessment to non-target plants for use of FDJO3 in grapevines, potato and tomato

Species	Test substance	ER_{50} (g/ha)[2] vegetative vigour	ER_{50} (g/ha)[2] emergence	Exposure[1] (g/ha)[2]	TER	Trigger
Potato						
Allium cepa	Cymoxanil 50 WP	>240 g a.s /ha	-	1.82	132	5
Grape						
Allium cepa	Cymoxanil 50 WP	>240 g a.s /ha	-	8.05	30	5
Tomato						
Allium cepa	Cymoxanil 50 WP	>240 g a.s /ha	-	7.91	30	5
Extended laboratory studies: No data submitted Semi-field and field test: No data submitted						

2.10 ENDOCRINE DISRUPTING PROPERTIES

RMS assessment: An assessment of the endocrine disrupting properties of the active substance cymoxanil in line with the EFSA/ECHA guidance for the identification of endocrine disruptors (2018) has been conducted.

1. Gather all relevant information

Regarding the mammalian toxicology area, data have been collected from all repeated dose toxicity studies in mammals (evaluated by the Rapporteur Member State) available in the RAR as well as *in vitro* mechanistic ToxCast data from the US EPA CompTox Chemicals Dashboard (http://comptox.epa.gov/dashboard).

The available literature review for humans was performed in line with the recommendations of the ECHA/EFSA Guidance (2018).

The available literature review for non-target organisms was performed in line with the recommendations of the ECHA/EFSA Guidance (2018). No relevant studies for this assessment were retrieved.

For non-target organisms in the DRAR for cymoxanil, two reproductive toxicity study on birds (OECD TG 206) and four fish early life stage toxicity tests (OECD TG 210) were available.

Data were gathered in the updated version of Excel template (2019-02-20) provided as Appendix E to the EFSA/ECHA guidance (2018) and it is prepared by the RMS as *EDGD Appendix to Cymoxanil RAR Volume 1*. The table part related to the *in vitro* mechanistic ToxCast data provided by the applicant served as a basis for the RMS assessment, while the table part related to all repeated dose toxicity studies in mammals was prepared by the RMS itself to take into account RMS assessment of the studies assessed in the RAR.

According to this template each study was given a unique identification number (Study ID Matrix) that is important for its identification in the data-matrix and Lines of Evidence (LoE) spreadsheets of the Excel.

A summary of all studies considered for the mammalian toxicology, including the Study ID Matrix is outlined in Table 1.

Table 1: Outline of dataset considered for mammalian toxicology assessment

Type of toxicity	**Study type**		**Study ID Matrix**
Repeated dose toxicity studies in mammals	Repeated dose 28-day oral (feeding) toxicity study in rat		1
	Repeated dose 28-day oral (feeding) toxicity study in mice		2
	Repeated dose 90-day oral (feeding) toxicity study in rat		3
	Repeated dose 90-day oral (feeding) toxicity study in rat		4
	Repeated dose 90-day oral (feeding) toxicity study in mice		5
	Repeated dose 90-day oral (feeding) toxicity study in dog		6
	Repeated dose 90-day oral (feeding) toxicity study in dog		7
	Repeated dose 1-year oral (feeding) toxicity study in dog		8
	Repeated dose 1-year oral (feeding) toxicity study in dog		9
	Repeated dose 28-day dermal toxicity study in rat		10
	Combined chronic toxicity/carcinogenicity oral (feeding) study in rat		11
	Combined chronic toxicity/carcinogenicity oral (feeding) study in rat		12
	Carcinogenicity oral (feeding) study in mouse		13
	Carcinogenicity oral (feeding) study in mouse		14
	Two-generation reproduction oral (feeding) toxicity test in rat		15
	Two-generation reproduction oral (feeding) toxicity test in rat		16
	One generation reproduction toxicity (feeding) study in rat		17
	Prenatal developmental toxicity oral (gavage) study in rat		18
	Prenatal developmental toxicity oral (gavage) study in rat		19
	Prenatal developmental toxicity oral (gavage) study in rabbit		20
	Prenatal developmental toxicity oral (gavage) study in rabbit		21
	Prenatal developmental toxicity oral (gavage) study in rabbit		22
	Prenatal developmental toxicity oral (gavage) study in rabbit		23
	Developmental neurotoxicity oral (gavage) study in rat		24
***In vitro* mechanistic**	**ToxCast ER prediction model/agonistic**		
	InVitroToxCast Androgen	ATG_AR_TRANS_up	31
	InVitroToxCast Androgen	NVS_NR_hAR	32
	InVitroToxCast Androgen	OT_AR_ARELUC_AG_1440	33
	InVitroToxCast Androgen	OT_AR_ARSRC1_0480	34
	InVitroToxCast Androgen	OT_AR_ARSRC1_0960	35
	InVitroToxCast Androgen	Tox21_AR_BLA_Agonist_ratio	36
	InVitroToxCast Androgen	Tox21_AR_BLA_Antagonist_ratio	37
	InVitroToxCast Androgen	Tox21_AR_LUC_MDAKB2_Agonist	38
	InVitroToxCast Androgen	Tox21_AR_LUC_MDAKB2_Antagonist	39
	InVitroToxCast Estrogen	ACEA_T47D_80 h	40
	InVitroToxCast Estrogen	ATG_ERE_CIS_up	41
	InVitroToxCast Estrogen	ATG_ERa_TRANS_up	42

	InVitroToxCast Estrogen	NVS_NR_hER	43
	InVitroToxCast Estrogen	NVS_NR_mERa	44
	InVitroToxCast Estrogen	OT_ER_ERaERa_0480	45
	InVitroToxCast Estrogen	OT_ER_ERaERa_1440	46
	InVitroToxCast Estrogen	OT_ER_ERaERb_048	47
	InVitroToxCast Estrogen	OT_ER_ERaERb_1440	48
	InVitroToxCast Estrogen	OT_ER_ERbERb_0480	49
	InVitroToxCast Estrogen	OT_ER_ERbERb_1440	50
	InVitroToxCast Estrogen	OT_ERa_EREGFP_0120	51
	InVitroToxCast Estrogen	OT_ERa_EREGFP_0480	52
	InVitroToxCast Estrogen	Tox21_ERa_BLA_Agonist_ratio	53
	InVitroToxCast Estrogen	Tox21_ERa_BLA_Antagonist_ratio	54
	InVitroToxCast Estrogen	Tox21_ERa_LUC_BG1_Agonist	55
	InVitroToxCast Estrogen	Tox21_ERa_LUC_BG1_Antagonist	56
	InVitroToxCast Thyroid	ATG_THRa1_TRANS_up	57
	InVitroToxCast Thyroid	Tox21_TR_LUC_GH3_Agonist	58
	InVitroToxCast Thyroid	Tox21_TR_LUC_GH3_Antagonist	59
	InVitroToxCast Steroidogenesis	CEETOX_H295R_11DCORT_dn	60
	InVitroToxCast Steroidogenesis	CEETOX_H295R_ANDR_dn	61
	InVitroToxCast Steroidogenesis	CEETOX_H295R_CORTISOL_dn	62
	InVitroToxCast Steroidogenesis	CEETOX_H295R_DOC_dn	63
	InVitroToxCast Steroidogenesis	CEETOX_H295R_ESTRADIOL_dn	64
	InVitroToxCast Steroidogenesis	CEETOX_H295R_ESTRONE_dn	65
	InVitroToxCast Steroidogenesis	CEETOX_H295R_OHPREG_dn	66
	InVitroToxCast Steroidogenesis	CEETOX_H295R_OHPREG_up	67
	InVitroToxCast Steroidogenesis	CEETOX_H295R_OHPROG_dn	68
	InVitroToxCast Steroidogenesis	CEETOX_H295R_PROG_dn	69
	InVitroToxCast Steroidogenesis	CEETOX_H295R_PROG_up	70
	InVitroToxCast Steroidogenesis	CEETOX_H295R_TESTO_dn	71
	InVitroToxCast Steroidogenesis	TOX21_Aromatase_Inhibition	72
	InVitroToxCast Estrogen	ToxCast ER prediction model	73
	InVitroToxCast Androgen	ToxCast AR prediction model	74

2. ED assessment for humans

2.1. ED assessment for T-modality

2.1.1. Have T-mediated parameters been sufficiently investigated?

	Sufficiently investigated
T-mediated parameters	Yes, based on availability of the following studies in which thyroid adversity is addressed: - OECD TG 407 (1995) (ID:1)*#, (ID:2)*# - OECD TG 408 (1981) (ID:3)*, (ID:4)*, (ID:5)* - OECD TG 409 (1981) (ID:6), (ID:7)

	- OECD TG 452 (1981) (ID:8), (ID:9) - OECD TG 453 (1981) (ID:11)*, (ID:12)* - OECD TG 451 (1981) (ID:13)*, (ID:14)* - OECD TG 416 (1983) (ID:15)*#, (ID:16)*# - OECD TG 415 (1983) (ID:17)*#

* Thyroid weight was not measured in the following studies: OECD TG 407 (ID:1 and ID:2), OECD TG 408 (ID:3, ID:4 and ID:5), OECD TG 453 (ID:11 and ID:12), OECD TG 451 (1981) (ID:13 and ID:14), OECD TG 416 (1983) (ID:15 and ID:16) and OECD TG 415 (ID:17)

[#] Thyroid histopathology was not assessed in the following studies: OECD TG 407 (ID:1 and ID:2), OECD TG 416 (1983) (ID:15 and ID:16) and OECD TG 453 (1981) ID:12

2.1.2. Lines of evidence for adverse effects and endocrine activity related to T-modality

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
57	In vitro mechanistic	Thyroid receptor	Human	24	Hours		1000	µM	No effect	ToxCast assay: No TR-mediated activity	No evidence of T-mediated activity *in vitro*.	No evidence of T-mediated activity *in vitro*.	T
58			rat	28	Hours		83,8	µM	No effect				
59			rat	28	Hours		1000	µM	No effect				
3	EATS-mediated	Thyroid histopathology	Rat	90	Days	Oral		mg/kg bw/day	No effect	No effects in thyroid histopathology in dogs, rat and mice up to the top doses	No effects on thyroid weight and in thyroid histopathology in dogs and no effects in rat and mice thyroid histopathology up to the top doses	No T-mediated adversity	
3			Rat	90	Days	Oral		mg/kg bw/day	No effect				
4			Rat	90	Days	Oral		mg/kg bw/day	No effect				
5			Mouse	90	Days	Oral		mg/kg bw/day	No effect				
6			Dog	92/93	Days	Oral		mg/kg bw/day	No effect				
6			Dog	92/93	Days	Oral		mg/kg bw/day	No effect				
7			Dog	90	Days	Oral		mg/kg bw/day	No effect				
7			Dog	90	Days	Oral		mg/kg bw/day	No effect				
8			Dog	52	Weeks	Oral		mg/kg bw/day	No effect				
9			Dog	52	Weeks	Oral		mg/kg bw/day	No effect				
6		Thyroid weight	Dog	92/93	Days	Oral		mg/kg bw/day	No effect	No effects on thyroid weight in dogs up to the top doses			
6			Dog	92/93	Days	Oral		mg/kg bw/day	No effect				
7			Dog	90	Days	Oral		mg/kg bw/day	No effect				
7			Dog	90	Days	Oral		mg/kg bw/day	No effect				
8			Dog	52	Weeks	Oral		mg/kg bw/day	No effect				
9			Dog	52	Weeks	Oral		mg/kg bw/day	No effect				
2	Sensitive to, but not diagnostic of, EATS	Adrenals weight	Mouse	28	Days	Oral	179.1	mg/kg bw/day	Decrease	The absolute weight of adrenals (12.5%) was statistical significantly reduced in females. No histopathology was performed; therefore, the adversity of this finding cannot be assessed.	Equivocal effect on absolute adrenals weight of females in carcinogenicity study in mouse and in 28-day mouse toxicity study.		
13			Mouse	18	Months	Oral	298	mg/kg bw/day	Decrease	Statistically significant reduction (>14%) of absolute adrenals weight in females at the two high dose groups was not associated with any microscopic findings, therefore, it was considered as non-adverse.			
24			Rat	16	Days	Oral		mg/kg bw/day	No effect		No effects on auditory startle and brain		

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
24		Brain morphometric (quantitative) evaluation	Rat	16	Days	Oral		mg/kg bw/day	No effect	Relative weights of brain (37.5%) were higher while the absolute weights of brain was lower (6%); no histopathology was performed. Dose above MTD	morphometric (quantitative) in rat		
1		Brain weight	Rat	28	Days	Oral	260.0	mg/kg bw/day	Increase	Relative weights of brain (37.5%) were higher while the absolute weights of brain was lower (6%); no histopathology was performed, therefore, the adversity of this finding cannot be assessed. Dose above MTD	Equivocal effect on rat brain weight		
16		Fertility (mammals)	Rat	21	Weeks	Oral	103.0	mg/kg bw/day	Increase	The statistically significant increases in fertility index for the F1 generation parents (highest dose group) are resulting from the poor reproductive performance of the control group. The effect is not considered treatment relevant.	Some males were infertile and the female fertility index was reduced.		
17			Rat	15	Weeks	Oral	226.3	mg/kg bw/day	Change	At necropsy 5 parent males in the 226.2 mg/kg bw/day group had bilateral small, flaccid testes, rendering them infertile. However, microscopic examination of testes was not performed.			
17			Rat	16	Weeks	Oral	235.2	mg/kg bw/day	Decrease	Statistically significantly reduced the female fertility index (%).			
24		Functional observation battery	Rat	16	Days	Oral		mg/kg bw/day	No effect		No effect on functional observation battery in rats.		
16		Gestation length	Rat	21	Weeks	Oral	103.0	mg/kg bw/day	Decrease	The shorter gestation length of the F1 female rats is resulting from the unusually high mean gestation length value for the control group. The effect is not considered treatment relevant.	Equivocal effect on gestation length of the F1 female rats		
24		Learning and memory in offspring	Rat	16	Days	Oral		mg/kg bw/day	No effect		No effect on learning and memory in rat offspring		
15		Litter size	Rat	24	Weeks	Oral	116.3	mg/kg bw/day	Decrease	For the F1 generation parents, there were a statistically significant decrease in mean litter size in the high dose only (94.0 mg/kg bw/day for males and 116.3 mg/kg bw/day for females).	Decreased litter size, pup weight, viability index in rat		
17			Rat	16	Weeks	Oral	235.2	mg/kg bw/day	Decrease	Statistically significantly reduced mean litter size at high dose level (235.2 mg/kg bw/day). The statistically significantly lower mean litter size was likely a consequence of the lower number of corpora lutea, an increased percentage of pre and post-implantation losses and a reduced mean number of implantations of the dams.			
24		Litter viability	Rat	16	Days	Oral	100	mg/kg bw/day	Decrease	Reduced viability index (number of live pups on day 5 post-partum/number of live born pups on day 1 post-partum)			
16		Litter/pup weight	Rat	21	Weeks	Oral	32.1	mg/kg bw/day	Decrease	Concerning pup weight (both sexes combined), statistically significant reductions (reduction 13-18%) were evident at the mid dose level of 32.1 mg/kg bw/day for the F2B generation and also statistically significant reductions (11-40%) at 97.9 mg/kg bw/day (all generations).			
17			Rat	16	Weeks	Oral	68.4	mg/kg bw/day	Decrease	Statistically significantly decreased (>11%) body weight (both sexes combined) for pups were reported from second week of lactation at 68.4 mg/kg bw/day (combined), statistically			

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
										significant reduction (19.6 – 31.2%) of body weight was observed from LD 7 at 127.7 mg/kg bw/day (combined) and decreased (15.1 – 46.7%) body weight for pups were reported from LD 4 at 235.2 mg/kg bw/day (combined).			
24		Motor activity	Rat	16	Days	Oral		mg/kg bw/day	No effect		No effect on rat motor activity		
15		Number of implantations, corpora lutea	Rat	24	Weeks	Oral	116.3	mg/kg bw/day	Decrease	The reproductive data for the F0 treatment groups did not show any statistically significant differences attributed to treatment of cymoxanil up to 1350 ppm. For the F1 generation parents, there were a statistically significant reduced in mean number of corpora lutea and mean number of implantations in the high dose only (94.0 mg/kg bw/day for males and 116.3 mg/kg bw/day for females). Although these findings were outside the range of the HCD declared, due to limited information provided by the Applicant the relevance of the HCD was not evaluated by the RMS. It should be noted that at the high dose female parents of the F1 some maternal effects (reduced initial body weight, reduced body weight gain during gestation and reduced food consumption during all phases) were observed.	Reduced in mean number of mean number of implantations and corpora lutea in rats in one multigenerational (out of two) and in one one-generation reproductive toxicity studies.		
17			Rat	16	Weeks	Oral	235.2	mg/kg bw/day	Decrease	Statistically significantly reduced numbers of corpora lutea.			
17			Rat	16	Weeks	Oral	235.2	mg/kg bw/day	Decrease	Statistically significantly reduced mean number of implantations			
18			Rat	10	Days	Oral		mg/kg bw/day	No effect		No effects on mean number of implantations in rabbits and in rats [one developmental (out of two) and in one developmental neurotoxicity studies].		
20			Rabbit	13	Days	Oral		mg/kg bw/day	No effect				
21			Rabbit	13	Days	Oral		mg/kg bw/day	No effect				
22			Rabbit	13	Days	Oral		mg/kg bw/day	No effect				
23			Rabbit	13	Days	Oral		mg/kg bw/day	No effect				
24			Rat	16	Days	Oral		mg/kg bw/day	No effect				
15		Number of live births	Rat	24	Weeks	Oral	116.3	mg/kg bw/day	Decrease	For the F1 generation parents, there was a statistically significant decrease in the percentage of live pups born in the high dose only (94.0 mg/kg bw/day for males and 116.3 mg/kg bw/day for females).	Decreased the number of live pups born or number of male viable foetuses per litter in rats in ID:15 and ID:18		
18			Rat	10	Days	Oral	75	mg/kg bw/day	Decrease	There was a statistically significant decrease in the number of male viable foetuses per litter from 75 mg/kg bw/day dose. In the high dose group there was a statistically significant reduction in the total number of viable foetuses per litter.			
24			Rat	16	Days	Oral		mg/kg bw/day	No effect				

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15		Post implantation loss	Rat	24	Weeks	Oral	116.3	mg/kg bw/day	Increase	For the F1 generation parents, there was a statistically significant increase in percentage of post-implantation loss in the high dose only (94.0 mg/kg bw/day for males and 116.3 mg/kg bw/day for females).	Increased of post-implantation loss and pre-implantation loss in rats.		
17			Rat	16	Weeks	Oral	235.2	mg/kg bw/day	Increase	Statistically significantly increased post-implantation loss.			
18			Rat	10	Days	Oral	150	mg/kg bw/day	Increase	In the high dose group there was a statistically significant increase in the total number of resorptions/litter and an increase in the number of early resorptions/litter			
19			Rat	10	Days	Oral	120	mg/kg bw/day	Increase	In the high dose group there was a non-statistically significant but marked increase in the number of late resorptions and post-implantation loss.			
17			Rat	16	Weeks	Oral	235.2	mg/kg bw/day	Increase	Statistically significantly increased pre-implantation loss.			
18		Presence of anomalies (external, visceral, skeletal	Rat	10	Days	Oral	25	mg/kg bw/day	Increase	Increased incidences of treatment related skeletal variations (mean percent of affected foetuses per litter and number of foetuses affected) as well as incidences of total variations (mean percent of affected foetuses per litter) were observed from 25 mg/kg bw/day dose level. The skeletal variations included partially ossified skull, partially ossified/unossified sternebra, partially ossified vertebra, wavy ribs, unossified hyoid and partially ossified pelvis and these variations indicated retarded/ skeletal development/delay ossification.	Effect on foetal development producing also malformations in rats ((two developmental toxicity studies) and rabbits (three out of four developmental toxicity studies)		
18			Rat	10	Days	Oral	150	mg/kg bw/day	Increase	The variations, i.e. partially ossified pelvis, partially ossified sternebra, unossified sternebra and wavy ribs, at the highest dose group (150 mg/kg bw/day) tested are considered to be treatment related and of toxicological relevance.			
18			Rat	10	Days	Oral	25	mg/kg bw/day	Increase	The percentage of affected foetuses per litter with any malformations (external visceral or skeletal) as well as the total number of foetuses affected was significantly increased from 25 mg/kg bw/day.			
18			Rat	10	Days	Oral	75	mg/kg bw/day	Increase	Increased incidences of malformations such as hemi vertebra were reported from 75 mg/kg bw/day and such malformations as exencephalic head and fused ribs at 150 mg/kg bw/day. These findings were above the range of historical control values declared, however, the relevance of the HCD was not evaluated by the RMS. Although incidences of these malformations observed were low, treatment-relation with respect to these findings cannot be excluded. Cleft sternebrae occurred at the upper two dose levels and the incidence was within the HCD. These effects were observed in the presence of maternal toxicity evident as statistically significant reduced maternal body weight, body			

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										weight gain and food consumption.			
19			Rat	10	Days	Oral	30	mg/kg bw/day	Increase	Concerning foetal skeletal alterations, incidences for minor anomalies (dumb-bell shaped thoracic vertebra 6/13) were shown to be statistically significantly increased and above the historical control data declared even at the lowest dose tested (30 mg/kg bw/day). These alterations are demonstrating an impact of the test material to the development of foetuses.			
19			Rat	10	Days	Oral	60	mg/kg bw/day	Increase	Several skeletal variations comprising incidences of delayed ossification (cervical vertebra: 7/7 and supraoccipital) and some related minor anomalies (hypoplasia of sternum: sternebra no. 1/2 and rudimentary 14th rib) were statistically significantly increased and were above the range of HCD from 60 mg/kg bw/day.			
19			Rat	10	Days	Oral		mg/kg bw/day	No effect	There were no visceral alterations (variants, minor anomalies and major malformations) that could be attributed to treatment with cymoxanil. There were no major skeletal malformations.			
20			Rabbit	13	Days	Oral		mg/kg bw/day	No effect	There were no meaningful intergroup differences in the incidences of either major malformations or minor abnormalities/variantions in either viscera or skeleton.			
21			Rabbit	13	Days	Oral	32	mg/kg bw/day	Increase	For the skeletal malformations, there were increases in the incidence of a vertebral and/or rib alterations, including hemivertebra, absent or fused vertebrae, misaligned vertebral centra/arches, fused/absent ribs, and various degrees of resulting scoliosis at the high dose of 32 mg/kg bw/day. Although these findings were not statistically significant, the percentage of foetuses with these malformations in the high dose group was above the HCD range submitted. However, the historical control data provided does not meet all requirements and is quite limited.			
21			Rabbit	13	Days	Oral	16	mg/kg bw/day	Increase	Although the finding "vertebral and other changes between upper cervical and mid-thoracic regions" was also not statistically significant, the percentage of foetuses affected in the mid and high dose groups was clearly above the HCD range declared based on results presented in the summary. These all skeletal alterations include malformations and other alterations that were classified as falling into a "borderline area" between malformation and anomaly allocating them into the category malformation. Diagnostic criteria for malformations and variations were not available in the study summary. The glossary of terminology under development by the International Federation of Teratology Societies was not considered by the Applicant. Since			

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										malformations and variations should be reported separately according to the Regulation (EU) No 283/2013, skeletal abnormality as "vertebral and other changes between upper cervical and mid-thoracic regions" is not considered as relevant.			
21			Rabbit	13	Days	Oral		mg/kg bw/day	No effect	Concerning skeletal variations, no effects were reported separately in the summary.			
22			Rabbit	13	Days	Oral		mg/kg bw/day	Increase	Visceral malformation such as *hydrocephaly* was found in two foetuses of the highest dose group (32 mg/kg bw/day); the increased number of foetuses affected was without statistical significance but clearly above the range of historical control data. In addition, incidences of foetuses with cleft palates were found in the highest dose tested, the increased number of foetuses affected showed statistical significance and was above the range of historical control. These two visceral malformations (cleft palate and hydrocephaly) occurring in the highest dose group tested were found in two foetuses (i.e. each foetus with hydrocephaly and cleft palate) from dams that lost weight during the dosing period (i.e. 0.44 and 0.16 kg during 6-18 days) and showed anorexia (5 and 2 days during 12-19 days of gestation) indicating maternal toxicity. Hence, these visceral malformations (cleft palate and hydrocephaly) are considered to be of toxicological relevance and treatment related. Skeletal malformations like fused/asymmetric sternebra were shown in the two mid dose groups without statistical significance and dose relationship; furthermore, the increased incidences were within the range of historical control. Vertebra and/or rib alterations (malformed and absent vertebra, fused vertebra, hemivertebra, branched and fused ribs) were shown to be dose related increased; the increase of incidences for the two highest dose groups were of statistical significance but within the range of historical control.			
22			Rabbit	13	Days	Oral		mg/kg bw/day	No effect	The number and incidence of foetuses with variations (external, visceral and skeletal variations; variations due to retarded development) was considered to be comparable with the concurrent control and revealed no statistical significance.			
23			Rabbit	13	Days	Oral	25	mg/kg bw/day	Increase	Visceral variants such as slight renal pelvis dilation were statistically significantly increased for the high dose (25 mg/kg bw/day) foetuses showing a clear dose relationship. The incidence of dilation of heart ventricles was statistically significantly increased in the high dose animals and was above the historical control data. As dilation of heart ventricles must be classified as a			

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										structural change that could impair foetal survival, development or function, this alteration should be indicated as major visceral malformations rather than minor anomalies.			
23			Rabbit	13	Days	Oral	25	mg/kg bw/day	Increase	Regarding the skeletal alterations, the incidences of skeletal variants as incomplete/poor ossification of fore limb (middle phalange: 1/5) as well as skeletal minor anomalies as accessory floating rib no. 13 were also shown to be relevant at maternal toxic dose levels (25 mg/kg bw/day).			
18		Pup development	Rat	10	Days	Oral	150	mg/kg bw/day	Change	In the high dose group there was a statistically significant reduction by 16.7% in the mean foetal body weight	Reduced mean rat foetal body weight and increased number of pups found dead on Days 2-5/9-12, reduced lactation indexes and number of surviving pups/litter in rats		
19			Rat	10	Days	Oral	120	mg/kg bw/day	Change	In the high dose group there was statistically significant reduction by 11% in the mean male foetal body weight.			
24			Rat	16	Days	Oral	100	mg/kg bw/day	Decrease	In pups of the highest dose (100 mg/kg bw/day) group, following treatment-related statistically significant changes were observed: statistically significantly increased number of pups (%) found dead or presumed cannibalized ((13.2% and 2.3% on Days 2-5/9-12, respectively), reduced lactation indexes (number of live pups on day 12 post-partum/number of live pups on day 5 post-partum; number of live pups on day 22 post-partum (weaning)/number of live pups in subsets 2 – 4 on day 12 post-partum), lower number of surviving pups/litter (from Day 5 post-partum to Day 22 post-partum) and live litter size (Days 12/18/22 post-partum).			
13	Target organ toxicity	Bone marrow histopathology	Mouse	18	Months	Oral	582	mg/kg bw/day	Change	Increased incidence of congestion in bone marrow of female mouse at 582 mg/kg bw/day .	Increased incidence of congestion in bone marrow in thes Carcinogenicity study in female mouse at MTD	Sufficient evidence for blood (anaemia) and thymus toxicity in dogs following 90 days and 1 year exposure. Sufficient evidence for eyes toxicity in dogs following 1 year exposure. Sufficient evidence for systemic toxicity (clinical signs, decreased BW/BW gain and food consumption).	
2		Eyes histopathology	Mouse	28	Days	Oral	624.4	mg/kg bw/day	Change	An incidental condition of cataract in both the eyes was observed in one male. MTD dose	Adverse effects on eyes (cataract/lenticular degeneration/retinal atrophy) in dogs (90 days and 1 year studies) and rats (combined chronic toxicity/carcinogenicity study).		
8			Dog	52	Weeks	Oral	5.7	mg/kg bw/day	Change	Bilateral cataract (slight, grade 1) in one male. Since such finding is uncommon in young dogs (< 2 years), a relationship to treatment cannot be excluded. No information whether retina was included in histopathological examination of eyes.			
8			Dog	52	Weeks	Oral	3.1	mg/kg bw/day	Change	Unilateral cataract (slight, grade 1) in one female at 3.1 mg/kg bw /day. Since such finding is uncommon in young dogs (< 2 years), a relationship to treatment cannot be excluded. No information whether retina was included in histopathological examination of eyes.			
9			Dog	52	Weeks	Oral	5.6	mg/kg bw/day	Change	Lenticular degeneration of a slight degree was recorded in both eyes of one dog; since this finding may occur in untreated Beagle dogs at a very low incidence, a relationship to treatment cannot be excluded in this case.			

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11			Rat	23	Months	Oral	30.3	mg/kg bw/day	Change	At study termination, the ocular examinations showed that retinal (photoreceptor cell) atrophy incidence was statistical significantly increased in both sexes at 700 and 2000 ppm (30.3 and 90.1 mg/kg bw/day for males): from 22% to 76.1/96.3% in males and from 60% to 90.4/98.2% in females, respectively. The incidence and severity of retinal atrophy in both males and females behaved in a dose respose manner in 700 ppm and 2000 ppm rats. The atrophy occurred either bilaterally or unilaterally and ranged in severity from single discrete foci to complete loss of photoreceptor and outer nuclear layers. The 1-year interim sacrifice revealed statistically significant increases in the incidence of retinal atrophy in the 32.8 mg/kg bw/day males and 134 mg/kg bw/day males and females: 1/9, 1/10, 1/10, 4/10* and 9/10* in males groups and 3/10, 3/10, 5/10, 3/10 and 9/10* in female groups. This lesion was atypical in 1-year control rats and therefore, the RMS considers that a treatment related effect of cymoxanil to male retina at two high doses the first year of treatment (equal to 32.8 and 97.4 mg/kg bw/day, respectively) could not be excluded. 30.3 mg/kg bw/day is MTD for males and 126 mg/kg bw/day is MTD for females.			
12			Rat	24	Months	Oral		mg/kg bw/day	No effect				
14		Heart histopathology	Mouse	18	Months	Oral		mg/kg bw/day	Change	A statistically significant increase in the incidence of heart myocardial degeneration was seen in all treatment male groups found dead and sacrificed moribund (0/18 [0%], 5/22* [22.7%], 4/18* [22.2%], 8/12* [66.7%], 8/24* [33.3%]). This statistically significant increase was not dose related and it was not replicated in the males reaching terminal sacrifice. The total incidence of heart myocardial degeneration over the course of the study was also seen in all treatment male groups, however, values did not attain statistical significance. Based on the HCD provided, the incidences of heart myocardial degeneration in dead and moribund as well as terminally sacrificed male mice are not so rare case: six studies, overall 97 males - from 0% to 56% and overall 203 males - from 0% to 50%, respectively.	Increase in the incidence of heart myocardial degeneration in all treatment male groups found dead and sacrificed moribund in mouse carcinogenicity study		
1		Heart weight	Rat	28	Days	Oral	260.0	mg/kg bw/day	Increase	Relative weights of heart (15.9%) was higher; no histopathology was performed. Dose above MTD			
5		Kidney histopathology	Mouse	90	Days	Oral		mg/kg bw/day	No effect		Not consistent effect (decrease/increase) on kidney weight in short-term rat and mice studies		
1		Kidney weight	Rat	28	Days	Oral	143.5	mg/kg bw/day	Increase	Statistically significant increase in relative kidneys (11.8%, dose related); no histopathology			

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										was performed. MTD dose	were of limited toxicological relevance.		
2			Mouse	28	Days	Oral	303.4	mg/kg bw/day	Decrease	Statistically significant increase in relative kidneys (11.8%, dose related); no histopathology was performed. MTD dose			
2			Mouse	28	Days	Oral	679.3	mg/kg bw/day	Decrease	The absolute weight of kidneys was statistical significantly lower (21.5%); however, the toxicological relevance of these changes were considered unclear, as the relative weight of kidney did not show statistically significant changes and no histopathology was performed in this study. MTD dose.			
4			Rat	90	Days	Oral	174.3	mg/kg bw/day	Increase	Statistically significant increase (7.8-17.3%) in relative kidney weight was observed at ≥ 85.1 mg/kg bw . Although this increase was dose related, relative kidney weight was higher than 15% of the control at 174.3 mg/kg bw only. No histopathological effects were seen in male kidney at high dose, except slight higher amount of hyaline casts (control 1/10, high dose 3/10).			
5			Mouse	90	Days	Oral		mg/kg bw/day	No effect				
5		Liver histopathology	Mouse	90	Days	Oral	256.6	mg/kg bw/day	Change	Vacuolar changes (minimal to mild/moderate) of liver cells have been observed in all treated animals with highest incidences in the high dose groups. No statistical analysis has been performed with respect to histopathological changes. The aetiology of this change was uncertain. The macroscopic examination provided no information on damage to liver, there was no effect on liver enzymes in this study, there was the quite small size of the difference in numbers of affected animals compared to concurrent controls and vacuolar changes of liver cells were not reproducible in other toxicity studies on cymoxanil. Therefore, vacuolar changes of liver cells were disregarded in this study.	Histopathological changes in the liver of female rats and liver of mice ((Combined chronic toxicity/carcinogenicity studies) were accompanied by increased liver weight of female mice. Effects were observed below the MTD dose.		
5			Mouse	90	Days	Oral	302.5	mg/kg bw/day	Change	Vacuolar changes (minimal to mild/moderate) of liver cells have been observed in all treated animals with highest incidences in the high dose groups. No statistical analysis has been performed with respect to histopathological changes. The aetiology of this change was uncertain. The macroscopic examination provided no information on damage to liver, there was no effect on liver enzymes in this study, there was the quite small size of the difference in numbers of affected animals compared to concurrent controls and vacuolar changes of liver cells were not reproducible in other toxicity studies on cymoxanil. Therefore, vacuolar changes of liver cells were disregarded in this study.			

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7			Dog	90	Days	Oral		mg/kg bw/day	No effect				
11			Rat	23	Months	Oral	38.4	mg/kg bw/day	Change	At study termination, substance related and adverse histopathological changes (inflammation and/or polyarteritis) were reported in the liver of female rats at 38.4 and 126 mg/kg bw/day. At 126 mg/kg bw/day the MTD was reached for females.			
12			Rat	24	Months	Oral		mg/kg bw/day	Change	The higher incidence of liver metastatic adenocarcinoma (MM) was observed in the high dose females of dead and moribund animals (D&M).It is noteworthy that these were not primary liver tumours in D&M females. In all these animals the uterine adenocarcinoma was metastatic to several organs including the liver. The primary liver tumour (hepatocellular carcinoma) was observed only in a single high dose terminally sacrificed female. For combined subgroup animals (i.e. animals found dead and moribund plus animals sacrificed at study termination), the following incidences of neoplasms were found to be increased with dose but revealed no statistically significance: liver adenocarcinoma (MM) in females. If a weight of the evidence approach is used, this very slight increase in female rats is not test substance related.			
13			Mouse	18	Months	Oral	42	mg/kg bw/day	Change	Statistically significant treatment-related histological findings in liver (centrilobular apoptotic hepatocytes, macrophages containing a pigment, granuloma, diffuse centrilobular hypertrophy) in males of three dose groups (42.0, 216 and 446 mg/kg bw/day) were not accompanied by liver weight changes. Effects were observed below the MTD dose.			
1		Liver weight	Rat	28	Days	Oral	143.5	mg/kg bw/day	Increase	Statistically significant increase in relative liver (12.7%); no histopathology was performed. MTD dose			
4			Rat	90	Days	Oral	174.3	mg/kg bw/day	Increase	A statistically significant increase (15.7%) in relative liver weight. No histopathological changes were seen at high dose. MTD dose			
5			Mouse	90	Days	Oral		mg/kg bw/day	No effect				
5			Mouse	90	Days	Oral	302.5	mg/kg bw/day	Increase	Statistically significant increase (11.2%) in relative liver weight			
7			Dog	90	Days	Oral	9.9	mg/kg bw/day	Increase	Relative liver weight was statistically significantly increased (>20%) at 9.9 mg/kg bw to 15.5 mg/kg bw doses with no histopathological correlation.			
13			Mouse	18	Months	Oral	298	mg/kg bw/day	Increase	Absolute and relative liver weight showed a statistically significant increase at the two high dose groups (10.3/18.1% and 13.0/26.8%, respectively) of females and this was accompanied by statistically significant			

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										treatment-related histological findings in liver			
11		Lymph nodes histopathology	Rat	23	Months	Oral	126	mg/kg bw/day	Change	Polyarteritis and cystic atrophy were observed in the mesenteric lymph node	Histopathological changes in the mesenteric lymph nodes (polyarteritis/haemorrhage) in female rats and male mice ((Combined chronic toxicity/carcinogenicity studies) at top dose.		
14			Mouse	18	Months	Oral	178.3	mg/kg bw/day	Change	A significant increase in the incidence of discolouration of the mesenteric lymph nodes in the highest dose males found dead and sacrificed moribund. This dicolouration of mesenteric lymph nodes was a red discolouration resulting from microscopically identified haemorrhage (3/18 [17%], 7/22 [32%], 1/18 [6%], 4/12 [33%], 11/24* [46%]). It should be noted that this gross finding as well as histopathological finding (haemorrhage) were not replicated in the animals reaching terminal sacrifice. The total incidence of haemorrhage in mesenteric lymph nodes over the course of the study was seen in all treatment male groups, however, values did not attain statistical significance. Based on the HCD provided, the incidence of haemorrhage in mesenteric lymph nodes in dead and moribund sacrificed male mice was below the value obtained in this study: six studies, from 0% to 40%, overall 97 males, overall 21 cases.			
11		Lung histopathology	Rat	23	Months	Oral	90.1	mg/kg bw/day	Change	The incidence of the granulomatous inflammation and haemorage in the lung was elevated	Histopathological changes in the lung (inflammation/haemorage/bronchopneumonia) in Combined chronic toxicity/carcinogenicity rat studies		
11			Rat	23	Months	Oral	38.4	mg/kg bw/day	Change	In the lung, at the terminal sacrifice, the incidence of polyarteritis was statistical significantly increased at 38.4, 126 mg/kg bw/day. In addition, at 126 mg/kg bw/day females had significant increases in the incidence of histocytosis, alveolar inflammation, type II cell hyperplasia, alveolar wall squamous metaplasia and fibrosis/inflammation. Electron microscopy suggests test substance-induced phospholipidose. The incidence of the granulomatous inflammation was elevated in the females at 126 mg/kg bw/day. 126 mg/kg bw/day is MTD.			
12		Lung histopathology	Rat	24	Months	Oral	58.8	mg/kg bw/day	Change	Regarding histopathological examination of animals terminally sacrificed including animals found dead and sacrificed moribund, statistically significant increases in the following non-neoplastic incidences were seen: suppurative bronchopneumonia in lungs of the highest dose group			
12			Rat	24	Months	Oral	75.8	mg/kg bw/day	Change	Regarding histopathological examination of animals terminally sacrificed including animals found dead and sacrificed moribund, statistically significant increases in the following non-neoplastic incidences were seen: suppurative bronchopneumonia in lungs of the highest dose group			

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11		Pancreas histopathology	Rat	23	Months	Oral	126	mg/kg bw/day	Change	Inflammation and/or polyarteritis were observed in the pancreas/stomach/small intestine/large intestine/urinary bladder was documented at 126 mg/kg bw/day. MTD dose.	Histopathological changes in the pancreas (Inflammation/polyarteritis/acinar cell necrosis) in female rats and mice in Combined chronic toxicity/carcinogenicity studies at or above MTD dose.		
13			Mouse	18	Months	Oral	582	mg/kg bw/day	Change	Based on pathological evaluation of 5 females which were sacrificed on or before test day 35, pancreatic acinar cell necrosis was considered an adverse effect at 582 mg/kg bw/day.			
11		Peripheral nerve histopathology	Rat	23	Months	Oral	38.4	mg/kg bw/day	Change	A statistically significant compound related increase in incidence of axon/myelin degeneration of the sciatic nerve occurred in females at 38.4 and 126 mg/kg bw/day , without any other signs of peripheral neuropathy.	Increase in incidence of axon/myelin degeneration of the sciatic nerve in female rats in combined chronic toxicity/carcinogenicity study below MTD.		
12		Small and large intestines histopathology	Rat	24	Months	Oral	23.5	mg/kg bw/day	Change	Regarding histopathological examination of animals terminally sacrificed including animals found dead and sacrificed moribund, statistically significant increases in the following non-neoplastic incidences were seen: lymphoid hyperplasia in rectum of males of the mid and high dose group (23.5 and 58.8 mg/kg bw/day, respectively).	Histopathological non-neoplastic changes in the small and large intestines of rats and mice ((Combined chronic toxicity/carcinogenicity studies).		
12			Rat	24	Months	Oral	75.8	mg/kg bw/day	Change	Regarding histopathological examination of animals terminally sacrificed including animals found dead and sacrificed moribund, statistically significant increases in the following non-neoplastic incidences were seen: lymphoid hyperplasia in colon in females of the highest dose group (75.8 mg/kg bw/day)			
13			Mouse	18	Months	Oral	216	mg/kg bw/day	Change	Increased incidence of cystic enteropathy in jejunum from 216 mg/kg bw/day			
13			Mouse	18	Months	Oral	58.1	mg/kg bw/day	Change	Increased incidence of cystic enteropathy in duodenum from 58.1 mg/kg bw/day. Increased incidence of cystic enteropathy in jejunum from 298 mg/kg bw/day.			
13		Spleen histopathology	Mouse	18	Months	Oral	582	mg/kg bw/day	Change	Increased incidence of diffuse atrophy in spleen of female mice above MTD.	Increased incidence of diffuse atrophy in spleen of female mice in carcinogenicity study above MTD.		
1		Spleen weight	Rat	28	Dats	Oral	260.0	mg/kg bw/day	Increase	Relative weights of spleen (49.4%) was higher; no histopathology was performed. Dose above MTD			
13		Stomach histopathology	Mouse	18	Months	Oral	58.1	mg/kg bw/day	Change	Increased incidence of hyperplastic gastropathy in stomach of female mouse from 58.1 mg/kg bw/day.	Increased incidence of hyperplastic gastropathy in stomach of female mouse in carcinogenicity study below MTD.		
6		Thymus histopathology	Dog	92/93	Days	Oral		mg/kg bw/day	No effect	No changes, one animal from highest dose group was investigated only. Weight of thymus was not investigated.	Increase in thymus atrophy of male and female dogs in a 90 day study and in male dogs in a 1 year study.		
6			Dog	92/93	Days	Oral		mg/kg bw/day	No effect	No changes, one animal from highest dose group was investigated only. Weight of thymus was not investigated.			

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
7			Dog	90	Days	Oral	9.7	mg/kg bw/day	Change	A dose dependent increase in lymphoid atrophy of male thymus with increasing severity was reported from 9.7 mg/kg bw/day [2/4 (mild and moderate)] to 14.2 mg/kg bw/day [3/4 (2 moderate and 1 severe)]			
7			Dog	90	Days	Oral	9.9	mg/kg bw/day	Change	A dose dependent increase in lymphoid atrophy was reported from 9.9 mg/kg bw/day [2/4 (minimal and moderate)] to 15.5 mg/kg bw/day [4/4 (2 minimal and 2 moderate)].there were changes in other lymphoid tissues (e.g. of bene marrow and lymph nodes) of female at the highest dose (equal to 15.5 mg/kg bw/day), whereas these tissues were not examined at other doses. The following histopathological findings of lymphoid tissues were noted in one emaciated female: lymphoid atrophy in thymus (moderate), lymphoid atrophy in mesenteric lymph nodes (mild), atrophy in bone marrow (severe) and in sternum marrow (severe). No such histopathological findings of lymphoid tissues were noted in one emaciated male with lymphoid atrophy in the thymus (severe) at high dose. There were not such histopathological findings of lymphoid tissues in both control groups.			
8			Dog	52	Weeks	Oral		mg/kg bw/day	No effect	No toxicologically significant changes.			
9			Dog	52	Weeks	Oral	1.3	mg/kg bw/day	Change	Microscopically, thymic lymphoid atrophy - involution was observed in three groups (1.3, 2.8, 5.6 mg/kg bw/day) of treated males but not in the control group (0/4; 2/4; 3/4 and 3/4, respectively). According to the grading system the severity of thymus atrophy from minimal to severe was reported: in Group 2 (2, 3), Group 3 (2, 2, 3) and Group 4 (1, 2, 4), where grade 1 - minimal, 2 - slight, 3 - moderate and 4 - severe. Furthermore, these microscopic observations correlated with thymus weights findings in male groups. Given that a dose-response relationship in absolute and relative thymus weights of males was observed, these thymus weights findings correlated with the microscopic observations (thymic lymphoid atrophy – involution) and the MTD have not been clear reached at the highest dose, these findings at least in males may indicate that a decrease of thymus weight in males of the high dose (5.6 mg/kg bw/day) group might be a substance-specific effect but not a result of generalised high-dose stress response.			
9			Dog	52	Weeks	Oral		mg/kg bw/day	No effect	Females were less affected than males with respect to thymus weight and thymus histopathology. Microscopically, thymic lymphoid atrophy - involution was observed in all groups of females including control (0, 0.8, 1.4, 2.9 mg/kg bw; 4/4; 2/4; 2/4; and 3/4,			

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
										respectively) and these microscopic findings likely to be attributable to normal age-associated thymic involution. According to the grading system the severity of thymus atrophy from minimal to moderate was reported.			
13			Mouse	18	Months	Oral		mg/kg bw/day	Change	No toxicologically significant changes.			
13			Mouse	18	Months	Oral	582	mg/kg bw/day	Change	Increased incidence of thymus atrophy at 582 mg/kg bw/day .			
7		Thymus weight	Dog	90	Days	Oral	9.7	mg/kg bw/day	Decrease	Reduction in absolute (>52%) and relative (>30%) thymus weight at ≥ 9.7 mg/kg bw was non-statistically significant as well as not clear dose-dependent			
7			Dog	90	Days	Oral	5.2	mg/kg bw/day	Decrease	Clear dose-dependent and statistically significant reduction in relative thymus weight from 5.2 mg/kg bw dose (42%, 51.9% and 55.6%, respectively), whereas there was not significant loss of body weight gain as well as lymphoid atrophy in the thymus in females at 5.2 mg/kg bw dose. Dose-dependent and statistically significant reduction in absolute (>56%) and relative (>50%) thymus weight at ≥ 9.9 mg/kg bw dose with histological evidence of lymphoid atrophy in the thymus was observed. Females affected were 2/4 (minimal and moderate) at 9.9 mg/kg bw/day, and 4/4 (2 minimal and 2 moderate) females at 14.2 mg/kg bw/day indicating a dose relationship.			
9			Dog	52	Weeks	Oral	5.6	mg/kg bw/day	Decrease	Statistically significant decrease of absolute thymus weight (52.7 %*). Dose-dependent decrease in mean absolute and relative thymus weights of all three male groups treated (equal to 1.3, 2.8 and 5.6 mg/kg bw) was observed (23.2-57.7% and 24.2-44.4%, respectively), however, the values did not attain statistical significance except the value mentioned above.			
9			Dog	52	Weeks	Oral		mg/kg bw/day	No effect				
1	Systemic toxicity	Body weight	Rat	28	Days	Oral	143.5	mg/kg bw/day	Decrease		Sufficient evidence for blood (anaemia) and thymus toxicity in dogs following 90 days and 1 year exposure. Sufficient evidence for eyes toxicity in dogs following 1 year exposure. Sufficient evidence for systemic toxicity (clinical signs, decreased BW/BW gain and food consumption).		
1			Rat	28	Days	Oral	287.8	mg/kg bw/day	Decrease				
2			Mouse	28	Days	Oral	303.4	mg/kg bw/day	Decrease				
2			Mouse	28	Days	Oral	679.3	mg/kg bw/day	Decrease				
3			Rat	90	Days	Oral	224	mg/kg bw/day	Decrease				
3			Rat	90	Days	Oral	333	mg/kg bw/day	Decrease				
4			Rat	90	Days	Oral	174.3	mg/kg bw/day	Decrease				

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
4			Rat	90	Days	Oral	187.7	mg/kg bw/day	Decrease				
5			Mouse	90	Days	Oral	256.6	mg/kg bw/day	Decrease				
5			Mouse	90	Days	Oral	302.5	mg/kg bw/day	Decrease				
6			Dog	92/93	Days	Oral	10.56	mg/kg bw/day	Decrease				
6			Dog	92/93	Days	Oral	5.27	mg/kg bw/day	Decrease				
7			Dog	90	Days	Oral	9.7	mg/kg bw/day	Decrease				
7			Dog	90	Days	Oral	9.9	mg/kg bw/day	Decrease				
8			Dog	52	Weeks	Oral		mg/kg bw/day	No effect				
8			Dog	52	Weeks	Oral		mg/kg bw/day	No effect				
9			Dog	52	Weeks	Oral	5.6	mg/kg bw/day	Decrease				
9			Dog	52	Weeks	Oral		mg/kg bw/day	No effect				
10			Rat	28	Days	Dermal		mg/kg bw/day	No effect				
11			Rat	23	Months	Oral	30.3	mg/kg bw/day	Decrease				
11			Rat	23	Months	Oral	126	mg/kg bw/day	Decrease				
12			Rat	24	Months	Oral	58.8	mg/kg bw/day	Decrease				
12			Rat	24	Months	Oral		mg/kg bw/day	No effect				
13			Mouse	18	Months	Oral	216	mg/kg bw/day	Decrease				
13			Mouse	18	Months	Oral	298	mg/kg bw/day	Decrease				
14			Mouse	18	Months	Oral	178.3	mg/kg bw/day	Change				
14			Mouse	18	Months	Oral	92.4	mg/kg bw/day	Change				
15			Rat	14-18	Weeks	Oral	94.0	mg/kg bw/day	Decrease				
15			Rat	24	Weeks	Oral	116.3	mg/kg bw/day	Decrease				
15			Rat	24	Weeks	Oral	31.6	mg/kg bw/day	Decrease				
16			Rat	16/32	Weeks	Oral	97.9	mg/kg bw/day	Decrease				
16			Rat	21	Weeks	Oral	103.0	mg/kg bw/day	Decrease				
17			Rat	16	Weeks	Oral	127.7	mg/kg bw/day	Decrease				

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
18			Rat	10	Days	Oral	25	mg/kg bw/day	Decrease				
19			Rat	10	Days	Oral	120	mg/kg bw/day	Decrease				
20			Rabbit	13	Days	Oral		mg/kg bw/day	No effect				
21			Rabbit	13	Days	Oral	32	mg/kg bw/day	Decrease				
22			Rabbit	13	Days	Oral		mg/kg bw/day	No effect				
23			Rabbit	13	Days	Oral	25	mg/kg bw/day	Decrease				
24			Rat	16	Days	Oral	50	mg/kg bw/day	Decrease				
24			Rat	16	Days	Oral		mg/kg bw/day	No effect				
4		Clinical chemistry and haematology	Rat	90	Days	Oral	85.1	mg/kg bw/day	Change		Adverse effect on haematology (reductions in haemoglobin) in dogs in the 90-days and 1-year studies (ID:6, ID:7 and ID:8).		
5			Mouse	90	Days	Oral	256.6	mg/kg bw/day	Change				
5			Mouse	90	Days	Oral	302.5	mg/kg bw/day	Change				
6			Dog	92/93	Days	Oral	5.13	mg/kg bw/day	Change				
6			Dog	92/93	Days	Oral	10.56	mg/kg bw/day	Change				
6			Dog	92/93	Days	Oral	10.51	mg/kg bw/day	Change				
6			Dog	92/93	Days	Oral	10.51	mg/kg bw/day	Change				
7			Dog	90	Days	Oral	14.2	mg/kg bw/day	Change				
7			Dog	90	Days	Oral	9.9	mg/kg bw/day	Change				
7			Dog	90	Days	Oral	5.2	mg/kg bw/day	Change				
8			Dog	52	Weeks	Oral	5.7	mg/kg bw/day	Change				
8			Dog	52	Weeks	Oral	5.7	mg/kg bw/day	Change				
8			Dog	52	Weeks	Oral		mg/kg bw/day	No effect				
9			Dog	52	Weeks	Oral		mg/kg bw/day	No effect				
9			Dog	52	Weeks	Oral	5.6	mg/kg bw/day	Change				
9			Dog	52	Weeks	Oral		mg/kg bw/day	No effect				
11			Rat	23	Months	Oral	90.1	mg/kg bw/day	Change				
11			Rat	23	Months	Oral		mg/kg bw/day	No effect				

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
11			Rat	23	Months	Oral		mg/kg bw/day	No effect				
12			Rat	24	Months	Oral		mg/kg bw/day	No effect				
12			Rat	24	Months	Oral		mg/kg bw/day	No effect				
13			Mouse	18	Months	Oral	216	mg/kg bw/day	Change				
13			Mouse	18	Months	Oral		mg/kg bw/day	No effect				
14			Mouse	18	Months	Oral		mg/kg bw/day	No effect				
14			Mouse	18	Months	Oral		mg/kg bw/day	No effect				
1		Clinical signs	Rat	28	Days	Oral	260.0	mg/kg bw/day	Change		Clinical signs in rats (weak, hyperreactivity, end of tail missing, sore and alopecia), mouse (weak, dull, pallor and stained fur), dogs (diarrhoea and weakness) and rabbits (anorexia/reduced faecal) were observed in short and long term studies.		
1			Rat	28	Days	Oral	415.9	mg/kg bw/day	Change				
2			Mouse	28	Days	Oral	624.4	mg/kg bw/day	Change				
6			Dog	92/93	Days	Oral	5.13	mg/kg bw/day	Induction				
6			Dog	92/93	Days	Oral	5.27	mg/kg bw/day	Induction				
7			Dog	90	Days	Oral	9.7	mg/kg bw/day	Induction				
7			Dog	90	Days	Oral	9.9	mg/kg bw/day	Induction				
11			Rat	23	Months	Oral	30.3	mg/kg bw/day	Induction				
12			Rat	24	Months	Oral		mg/kg bw/day	Change				
13			Mouse	18	Months	Oral	446	mg/kg bw/day	Induction				
13			Mouse	18	Months	Oral	582	mg/kg bw/day	Induction				
14			Mouse	18	Months	Oral		mg/kg bw/day	No effect				
14			Mouse	18	Months	Oral		mg/kg bw/day	No effect				
15			Rat	14-18	Weeks	Oral		mg/kg bw/day	No effect				
15			Rat	24	Weeks	Oral	116.3	mg/kg bw/day	No effect				
16			Rat	16/32	Weeks	Oral	97.9	mg/kg bw/day	Induction				
16			Rat	21	Weeks	Oral	103.0	mg/kg bw/day	Induction				
16			Rat	21	Weeks	Oral	97.9	mg/kg bw/day	Induction				
18			Rat	10	Days	Oral	150	mg/kg bw/day	Induction				

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
21			Rabbit	13	Days	Oral	16	mg/kg bw/day	Induction				
22			Rabbit	13	Days	Oral		mg/kg bw/day	No effect				
24			Rat	16	Days	Oral	100	mg/kg bw/day	Induction				
1		Food consumption	Rat	28	Days	Oral	143.5	mg/kg bw/day	Decrease		Decreased food consumption was usually consistent with decreased body weight/ body weight gain in rat, mouse, dog and rabbit in 28-day, 90-day, chronic, multigenerational reproductive toxicity, prenatal developmental and developmental neurotoxicity studies.		
1			Rat	28	Days	Oral	287.8	mg/kg bw/day	Decrease				
2			Mouse	28	Days	Oral	303.4	mg/kg bw/day	Decrease				
2			Mouse	28	Days	Oral	679.3	mg/kg bw/day	Decrease				
3			Rat	90	Days	Oral	224	mg/kg bw/day	Decrease				
3			Rat	90	Days	Oral	333	mg/kg bw/day	Decrease				
4			Rat	90	Days	Oral	174.3	mg/kg bw/day	Decrease				
4			Rat	90	Days	Oral	187.7	mg/kg bw/day	Decrease				
5			Mouse	90	Days	Oral	256.6	mg/kg bw/day	Decrease				
5			Mouse	90	Days	Oral		mg/kg bw/day	No effect				
6			Dog	92/93	Days	Oral	10.56	mg/kg bw/day	Decrease				
6			Dog	92/93	Days	Oral	5.27	mg/kg bw/day	Decrease				
7			Dog	90	Days	Oral	9.7	mg/kg bw/day	Decrease				
7			Dog	90	Days	Oral	15.5	mg/kg bw/day	Decrease				
11			Rat	23	Months	Oral		mg/kg bw/day	No effect				
12			Rat	24	Months	Oral		mg/kg bw/day	Decrease				
13			Mouse	18	Months	Oral		mg/kg bw/day	No effect				
13			Mouse	18	Months	Oral		mg/kg bw/day	No effect				
15			Rat	14-18	Weeks	Oral	94.0	mg/kg bw/day	Decrease				
15			Rat	24	Weeks	Oral	116.3	mg/kg bw/day	Decrease				
16			Rat	16/32	Weeks	Oral	97.9	mg/kg bw/day	Decrease				
16			Rat	21	Weeks	Oral	103.0	mg/kg bw/day	Decrease				
17			Rat	16	Weeks	Oral	127.7	mg/kg bw/day	Decrease				

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
18			Rat	10	Days	Oral	25	mg/kg bw/day	Decrease				
19			Rat	10	Days	Oral	120	mg/kg bw/day	Decrease				
23			Rabbit	13	Days	Oral	15	mg/kg bw/day	Decrease				
24			Rat	16	Days	Oral	50	mg/kg bw/day	Decrease				
6		Mortality	Dog	92/93	Days	Oral	10.51	mg/kg bw/day	Increase		There were no dose related trends in mortality in chronic studies; no evidence of mortality in adults in other studies.		
11			Rat	23	Months	Oral	0	mg/kg bw/day	Increase				
11			Rat	23	Months	Oral	0	mg/kg bw/day	Increase				
12			Rat	24	Months	Oral	0	mg/kg bw/day	Increase				
12			Rat	24	Months	Oral	0	mg/kg bw/day	Increase				
13			Mouse	18	Months	Oral		mg/kg bw/day	Increase				
13			Mouse	18	Months	Oral	582	mg/kg bw/day	Increase				
14			Mouse	18	Months	Oral		mg/kg bw/day	Increase				
14			Mouse	18	Months	Oral		mg/kg bw/day	Increase				
15			Rat	14-18	Weeks	Oral		mg/kg bw/day	No effect				
15			Rat	24	Weeks	Oral	116.3	mg/kg bw/day	No effect				
15			Rat	24	Weeks	Oral	94.0	mg/kg bw/day	Increase				
16			Rat	16/32	Weeks	Oral		mg/kg bw/day	No effect				
16			Rat	21	Weeks	Oral		mg/kg bw/day	No effect				
16			Rat	21	Weeks	Oral	32.1	mg/kg bw/day	Increase				
17			Rat	16	Weeks	Oral	235.2	mg/kg bw/day	Increase				
18			Rat	10	Days	Oral		mg/kg bw/day	No effect				
19			Rat	10	Days	Oral		mg/kg bw/day	No effect				
21			Rabbit	13	Days	Oral		mg/kg bw/day	No effect				
22			Rabbit	13	Days	Oral		mg/kg bw/day	No effect				
23			Rabbit	13	Days	Oral		mg/kg bw/day	No effect				

2.1.2.1. Assessment of the integrated lines of evidence and weight of evidence for T-mediated adversity and endocrine activity

Table 2: WoE for T-mediated adversity

- In rats, no effects in thyroid histopathology were observed in repeated dose 90-day oral toxicity studies (study ID:3 and ID:4) up to the top doses (174.3 - 333 mg/kg bw/day). Thyroid weight was not measured in these studies.
- No notable histopathological changes in the thyroid were observed in the rat combined chronic toxicity/carcinogenicity studies (study ID:11 and ID:12) up to the top doses (58.8 – 126 mg/kg bw/day). Thyroid weight was not measured in these studies.
- In mice, no effects in thyroid histopathology were observed in repeated dose 90-day oral toxicity study (study ID:5) up to the top doses (approximately 256.6 mg/kg bw/day). Thyroid weight was not measured in this study.
- No notable histopathological changes in the thyroid were observed in the mice carcinogenicity studies (study ID:13 and ID:14) up to the top doses (178.3 – 582 mg/kg bw/day). Thyroid weight was not measured in these studies.
- In dogs, no effects on thyroid weight and in thyroid histopathology were observed in repeated dose 90-day oral toxicity studies (study ID:6 and ID:7) up to the top doses (14.2 – 10.51 mg/kg bw/day) and 1-year studies (study ID:8 and ID:9) up to the top doses (approximately 5.7 mg/kg bw/day).

Table 3: WoE for T-mediated endocrine activity

- Cymoxanil was tested in 3 out of 4 relevant Thyroid related assays (as is indicated in the guidance) in the ToxCast database. Cymoxanil was inactive in all. No effect on TR (trans)activation from In Vitro ToxCast data (ID:57, ID:58 and ID:59) were observed.

2.1.3. Initial analysis of the evidence and identification of relevant scenario for the ED assessment of T-modality

Table 4: Selection of relevant scenario

Adversity based on T-mediated parameters	**Positive mechanistic OECD CF level 2/3 Test**	**Scenario**	**Next step of the assessment**	**Scenario selected** *(indicate with an "x" the scenario selected based on the assessed lines of evidence)*
No (sufficiently investigated)	Yes/No	1a	Conclude: ED criteria not met because there is not "**T-mediated**" adversity	X
Yes (sufficiently investigated)	Yes/No	1b	Perform MoA analysis	
No (not sufficiently investigated)	Yes	2a (i)	Perform MoA analysis (additional information may be needed for the analysis)	
No (not sufficiently investigated)	No (sufficiently investigated)	2a (ii)	Conclude: ED criteria not met because no **T-mediated endocrine activity** observed	
No (not sufficiently investigated)	No (not sufficiently investigated)	2a (iii)	Generate missing level 2 and 3 information. Alternatively, generate missing "EATS-mediated" parameters. Depending on the outcome move to corresponding scenario	
Yes (not sufficiently investigated)	Yes/No	2b	Perform MoA analysis	

2.1.4. MoA analysis for T-modality

The overall WoE suggests that T-mediated parameters have been sufficiently investigated and a pattern of T-mediated adversity was not observed. Therefore, the ED criteria are not met for this modality.

2.2. ED assessment for EAS-modalities

2.2.1. Have EAS-mediated parameters been sufficiently investigated?

	Sufficiently investigated
EAS-mediated parameters	**No**, based on the following studies: - OECD TG 416, test protocol according to latest version of January 2001* - OECD TG 443, test protocol according to latest version of October 2012* **No,** due to the lack of the following studies: - Level 2 and 3 assays (e.g. OECD 458, 456, 441) for determining EAS-mediated endocrine activity

* Note: 2 two-generation reproduction studies (ID:15 and ID:16) and reduced-size one-generation study (ID:17) included in the RAR, was conducted according to the OECD TG 416 (1983) and OECD TG 415 (1983), respectively, and several EAS-mediated parameters were not investigated.
The following EAS-mediated parameters were not investigated in these studies:

- Sperm parameters (sperm count, sperm motility and sperm morphology);
- Oestrus cyclicity;
- Sexual maturation: age and body weight at vaginal opening and preputial separation,
- Anogenital distance;
- Weight of the following organs: testes[#], seminal vesicles with coagulating gland, epididymis, prostate, uterus (with cervix), ovaries;
- Quantitative evaluation of primordial follicles of the ovaries for F1 females.

[#] Note: *weight of testes was investigated in one two-generation reproduction study: OECD TG 416 (ID:16)*

Additionally, developmental neurotoxicity study in rats was conducted according to OPPTS 870.6300 which complied generally with OECD 426 (2007), and in which some EAS-mediated parameters were investigated (e.g. age at vaginal opening and preputial separation).

2.2.2. Lines of evidence for adverse effects and endocrine activity related to EAS-modalities

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
31	In vitro mechanistic	Androgen receptor	Human	24	Hours		1000	µM	No effect	No evidence of A-mediated activity *in vitro*.	No evidence of EAS-mediated activity *in vitro*.	No evidence of EAS-mediated activity *in vitro*.	EAS
32			Human	20	Hours		1000	µM	No effect				
33			Chinese Hamster	24	Hours		1000	µM	No effect				
34			Human	8	Hours		1000	µM	No effect				
35			Human	16	Hours		1000	µM	No effect				
36			Human	24	Hours		1000	µM	No effect				
37			Human	24	Hours		1000	µM	No effect				
38			Human	24	Hours		1000	µM	No effect				
39			Human	24	Hours		1000	µM	No effect				
74							0		No effect				
40		Estrogen receptor	Human	80	Hours		1000	µM	No effect	No evidence of E-mediated activity *in vitro*.			
41			Human	24	Hours		46,7	µM	Change				
42			Human	24	Hours		1000	µM	No effect				
43			bovine	18	Hours		1000	µM	No effect				
44			Human	18	Hours		1000	µM	No effect				
45			Human	8	Hours		1000	µM	No effect				
46			Human	24	Hours		1000	µM	No effect				
47			Human	8	Hours		1000	µM	No effect				
48			Human	24	Hours		1000	µM	No effect				
49			Human	8	Hours		1000	µM	No effect				
50			Human	24	Hours		1000	µM	No effect				
51			Human	2	Hours		1000	µM	No effect				
52			Human	8	Hours		1000	µM	No effect				
53			Human	24	Hours		1000	µM	No effect				
54			Human	24	Hours		1000	µM	No effect				

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
55			Human	48	Hours		1000	µM	No effect				
56			Human	48	Hours		1000	µM	No effect				
73							0		No effect				
60		Steroidogenesis (genes/enzyme changes) (in vitro)	Human	48	Hours		1000	µM	No effect	No evidence of S-mediated activity *in vitro.*			
61			Human	48	Hours		1000	µM	No effect				
62			Human	48	Hours		1000	µM	No effect				
63			Human	48	Hours		1000	µM	No effect				
64			Human	48	Hours		1000	µM	No effect				
65			Human	48	Hours		1000	µM	No effect				
66			Human	48	Hours		1000	µM	No effect				
67			Human	48	Hours		1000	µM	No effect				
68			Human	48	Hours		1000	µM	No effect				
69			Human	48	Hours		1000	µM	No effect				
70			Human	48	Hours		1000	µM	No effect				
71			Human	48	Hours		1000	µM	No effect				
72			Human	24	Hours		1000	µM	No effect				
24	EATS-mediated	Age at balanopreputial separation	Rat	16	Days	Oral		mg/kg bw/day	No effect		No effect on age at balanopreputial separation and at vaginal opening	A convincing pattern of adversity, indicative of endocrine disruption, cannot be drawn.	
24		Age at Vaginal opening	Rat	16	Days	Oral		mg/kg bw/day	No effect				
15		Coagulating gland histopathology	Rat	24	Weeks	Oral		mg/kg bw/day	No effect		No effect on coagulating gland histopathology		
16		Coagulating gland histopathology	Rat	16/32	Weeks	Oral		mg/kg bw/day	No effect				
3		Epididymis histopathology	Rat	90	Days	Oral	224	mg/kg bw/day	Change	The following histopathological changes have been observed with respect to epididymides: cell debris (1 animal and 6 animals of the two highest dose groups), bilateral hypospermia (4 animals of the highest dose group) and multinucleated spermatids (one animal each of the two highest dose groups tested); statistical significance was shown for cell debris and hypospermia of the highest dose group. No weight of epididymides	Effects on epididymis histopathology in rats at MTD (90 day, 1 year interim sacrifice and combined chronic toxicity/carcinogenicity studies), in dogs most likely below the MTD (1 year study) and in mice		

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
										was assessed in the study. 224 mg/kg bw is MTD dose.	below the MTD (carcinogenicity study). **Effect related to treatment uncertain; human health relevance uncertain.**		
6			Dog	92/93	Days	Oral		mg/kg bw/day	No effect				
7			Dog	90	Days	Oral		mg/kg bw/day	No effect				
8			Dog	52	Weeks	Oral	5.7	mg/kg bw/day	Change	Histopathological examination revealed mild periarteritis (0/4, 0/4, 0/4 and 1/4) in the 5.7 mg/kg be group only.			
9			Dog	52	Weeks	Oral	5.6	mg/kg bw/day	Change	The histological findings in the epididymis were bilateral seminiferous cell debris (minimal) and unilateral atrophy (moderate) with aspermia of one animal of the high dose group each. The MTD have not been clear reached at the highest dose (5.6 mg/mg bw/day).			
11			Rat	23	Months	Oral	90.1	mg/kg bw/day	Change	At study termination increased bilateral oligospermia in epididymis at high dose (10/63, 8/62, 11/62, 8/56 and 23/62) did not attain statistical significance. At the one year interim sacrifice: multinucleated spermatids in epididymis had a statistically significantly increased occurrence in 97.4 mg/kg bw/day males (0/9, 0/10, 0/10, 0/10 and 3/10*). 30.3 mg/kg bw/day is MTD dose.			
11			Rat	12	Months	Oral	97.4	mg/kg bw/day	Change	At the one year interim sacrifice: multinucleated spermatids in epididymis had a statistically significantly increased occurrence in 97.4 mg/kg bw/day males (0/9, 0/10, 0/10, 0/10 and 3/10*). MTD dose			
12			Rat	24	Months	Oral	58.8?	mg/kg bw/day	Change	There was a clear increased combined incidence of epididymal oligospermia with aspermia (a low sperm count and the complete lack of semen) at the highest dose level (58.8 mg/kg bw/day); however, no statistical analysis has been performed. In all cases aspermia was exceptionally identified in terminal sacrificed males: this finding was not identified in animals found dead and sacrificed moribund. Due to methodological limitation (no histopathological examination was performed from all animals in all dose groups) the NOAEL cannot be set properly with respect to findings on male reproductive organs. 58.8 mg/kg bw/day is MTD dose.			
13			Mouse	18	Months	Oral	42	mg/kg bw/day	Change	Tubular dilatation, aggregate lymphoid and sperm cysts/cystic dilation of epididymis were statistically significantly increased in a dose-dependent manner from 42.0 mg/kg bw/day. From 216 mg/kg bw/day statistically significantly increased unilateral and bilateral oligospermia and sperm granuloma in epididymis were observed. All these changes are considered test substance related and adverse. 216 mg/kg bw/day is MTD dose.			

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
15			Rat	14-18	Weeks	Oral		mg/kg bw/day	No effect	Tubular dilatation, aggregate lymphoid and sperm cysts/cystic dilation of epididymis were statistically significantly increased in a dose-dependent manner from 42.0 mg/kg bw/day. From 216 mg/kg bw/day statistically significantly increased unilateral and bilateral oligospermia and sperm granuloma in epididymis were observed. All these changes are considered test substance related and adverse. 216 mg/kg bw/day is MTD dose.			
16			Rat	16/32	Weeks	Oral		mg/kg bw/day	No effect				
1		Epididymis weight	Rat	28	Days	Oral	260.0	mg/kg bw/day	Increase	Relative weights of epididymis (27.4%) were higher; no histopathology was performed. Dose above MTD	Limited effects on epididymis weights		
6			Dog	92/93	Days	Oral	10.56	mg/kg bw/day	Decrease	Statistically significant decrease of relative (24.1%) and absolute (48.4%) epididymis weight. MTD dose			
7			Dog	90	Days	Oral		mg/kg bw/day	No effect				
8			Dog	52	Weeks	Oral		mg/kg bw/day	No effect				
9			Dog	52	Weeks	Oral		mg/kg bw/day	No effect				
3		Ovary histopathology	Rat	90	Days	Oral		mg/kg bw/day	No effect		Limited evidence of ovary histopathology (ovaries follicular cysts) in the mice below the MTD in the carcinogenicity study (ID:14).		
4			Rat	90	Days	Oral		mg/kg bw/day	No effect				
5			Mouse	90	Days	Oral		mg/kg bw/day	No effect				
6			Dog	92/93	Days	Oral		mg/kg bw/day	No effect				
7			Dog	90	Days	Oral		mg/kg bw/day	No effect				
8			Dog	52	Weeks	Oral		mg/kg bw/day	No effect				
9			Dog	52	Weeks	Oral		mg/kg bw/day	No effect				
14			Mouse	18	Months	Oral	179.8	mg/kg bw/day	Change	A statistically significant increase in ovaries follicular cysts in the highest dose group was observed in females terminal sacrificed and combined fates (4 incidences from 50 females, 8%*). Based on the HCD provided, the incidences of ovaries follicular cysts in female mice terminal sacrificed were similar to the value obtained in this study: six studies, from 0% to 12%, overall 207 females, overall 7 incidences.			
15			Rat	24	Weeks	Oral	116.3	mg/kg bw/day	No effect				
16			Rat	21	Weeks	Oral		mg/kg bw/day	No effect				
1		Ovary weight	Rat	28	Days	Oral	415.9	mg/kg bw/day	Decrease	The absolute weight was lower (31.6%). Dose above MTD. No histopathology was performed.	Decreased absolute/relative ovary		

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
2			Mouse	28	Days	Oral	179.1	mg/kg bw/day	Decrease	The absolute weight of ovaries (15.2%) was statistical significantly reduced. The relative ovaries weight was statistical significantly lower (27.2%) at 329.9 mg/kg bw only. MTD dose is 679.3 mg/kg bw. No histopathology was performed.	weight in the rat and mice repeated dose 28-day studies (in rats at MTD, while in mice below MTD).		
4			Rat	90	Days	Oral		mg/kg bw/day	No effect				
5			Mouse	90	Days	Oral		mg/kg bw/day	No effect				
6			Dog	92/93	Days	Oral		mg/kg bw/day	No effect				
7			Dog	90	Days	Oral		mg/kg bw/day	No effect				
7		Prostate histopathology (with seminal vesicles and coagulating glands)	Dog	90	Days	Oral	14.2	mg/kg bw/day	Change	Prostate hypoplasia (1/4, 1/4 and 2/4) was reported in the 4.9 mg/kg bw/ day, 9.7 mg/kg bw/ day and 14.2 mg/kg bw/ day groups, respectively.	Limited evidence of prostate histopathology (prostate hypoplasia and benign adenoma) in the dogs at or below the MTD, respectively, in the repeated dose 90-day and 1-year studies (ID:7 and ID:8).		
8			Dog	52	Weeks	Oral	5.7	mg/kg bw/day	Change	Histopathological examination revealed prostate benign adenoma (0/4, 0/4, 0/4 and 1/4) in the 5.7 mg/kg bw/day group only.			
9			Dog	52	Weeks	Oral		mg/kg bw/day	No effect				
15			Rat	24	Weeks	Oral		mg/kg bw/day	No effect				
16			Rat	16/32	Weeks	Oral		mg/kg bw/day	No effect				
15		Seminal vesicles histopathology	Rat	24	Weeks	Oral		mg/kg bw/day	No effect		No effect on seminal vesicles histopathology		
16			Rat	16/32	Weeks	Oral		mg/kg bw/day	No effect				
3		Testis histopathology	Rat	90	Days	Oral	47.6	mg/kg bw/day	Change	The changes in testis weight were correlated with histopathological findings: increased bilateral elongate spermatid degeneration occurred at ≥ 47.6 mg/kg bw, the incidence and severity increasing with dosage. Increased elongate spermatid degeneration was observed in three animals at 47.6 mg/kg bw, five at 102 mg/kg bw and seven animals at 224 mg/kg bw dose: the increased incidence showed a clear dose-relationship and was statistically significant at the highest dose level. Furthermore, one male rat each from 102 mg/kg bw and 224 mg/kg bw dose group had multinucleated spermatids: despite of no statistical significance the finding supports a compound related effect to male reproductive organ. 224 mg/kg bw is MTD dose.	**Effects on histopathology of testis in rats (90 days rat study, ID:3) and in dogs (1-year dog study, ID:9) in the absent of general systemic toxicity (below the MTD). Human health relevance uncertain.**		
4			Rat	90	Days	Oral		mg/kg bw/day	No effect				
5			Mouse	90	Days	Oral		mg/kg bw/day	No effect				
6			Dog	92/93	Days	Oral	10.56	mg/kg bw/day	Change	Histopathology showed aspermatogenesis in the testes of 2 animals from 4 of the high dose group.			

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										For Male 1 moderate (grade 3) aspermatogenesis bilateral, no evidence of development beyond primary spermatocyte were reported; for Male 2 mild (grade 2) aspermatogenesis, minimal spermatid formation were reported (at the scheduled necropsy "small testes" were described too). MTD dose			
7			Dog	90	Days	Oral		mg/kg bw/day	No effect				
8			Dog	52	Weeks	Oral		mg/kg bw/day	No effect				
9			Dog	52	Weeks	Oral	2.8	mg/kg bw/day	Change	Apparent trend in the incidence of atrophy of testes, with two and three males (out of four) in 2.8 mg/kg bw/day and 5.6 mg/kg bw/day groups, respectively, showing from minimal to severe grade in the absence of such findings in control animals. Changes in testes of this study are outside the range of background pathology in Beagle dogs of this age (Michael J. Goedken et al., 2008) as well as based on the RMS selected 54 dogs over one year of age from background control data provided by the Applicant. The MTD have not been clear reached at the highest dose (5.6 mg/mg bw/day).			
11			Rat	23	Months	Oral	30.3	mg/kg bw/day	Change	Histopathological examination of testes revealed statistically significant and test compound-related increase of elongate spermatid degeneration at 30.3 and 90.1 mg/kg bw/day (7/63, 5/62, 4/62, 17/56* and 29/62*). Although this lesion could be found in control animals, the incidence and severity increased with increasing dietary concentration of the test compound, e.g. grades of lesions were 14 minimal, 3 mild at 30.3 mg/kg bw/day and 20 minimal, 4 mild and 5 moderate at 90.1 mg/kg bw/day . In addition to the elongate spermatid lesion there was a statistically significant increase in multinucleate spermatids in the 90.1 mg/kg bw/day male testes which was secondary to the elongate spermatid abnormality. Furthermore, these histopathological findings in testes were evident also in animals at the one year interim sacrifice: statistically significant increase of elongate spermatid degeneration at 32.8 mg/kg bw/day and 97.4 mg/kg bw/day [1/9, 0/10, 0/10, 4/10* and 5/10*] as well as multinucleated spermatids in testes in one 97.4 mg/kg bw/day male. MTD dose.			
11			Rat	12	Months	Oral	32.8	mg/kg bw/day	Change	Histopathological findings in testes were evident at the one year interim sacrifice: statistically significant increase of elongate spermatid degeneration at 32.8 mg/kg bw/day and 97.4 mg/kg bw/day [1/9, 0/10, 0/10, 4/10* and 5/10*] as well as multinucleated spermatids in testes in			

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
										one 97.4 mg/kg bw/day male. MTD dose			
12			Rat	24	Months	Oral	58.8?	mg/kg bw/day	Change	Histopathological examination of testes after 24 months with cymoxanil (animals terminally sacrificed including animals found dead and sacrificed moribund) revealed statistically significanty increased incidence of mild-to-moderate seminiferous tubule atrophy in the testis at 58.8 mg/kg bw/day. It was seen in both testes (bilaterally) in 3 of the 12 cases at 58.8 mg/kg bw/day . It should be noted that tissues of male reproductive organs of all rats from control and high dose groups, all the dead / moribund sacrificed rats and all gross lesions were examines for histopathological changes. Since male reproductive organs (testes and epididymis) are target tissues of cymoxanil and these tissues showed treatment-related changes in the high dose group, histopathological examinations had to be performed from all animals in all dose groups. Due to methodological limitation (no histopathological examination was performed from all animals in all dose groups) the NOAEL cannot be set properly with respect to findings on male reproductive organs. 58.8 mg/kg bw/day is MTD dose.			
13			Mouse	18	Months	Oral	446	mg/kg bw/day	Change	Statistical significantly increased incidence of tubular atrophy of testes. 216 mg/kg bw/day is MTD dose.			
15			Rat	24	Weeks	Oral		mg/kg bw/day	No effect				
16			Rat	16/32	Weeks	Oral		mg/kg bw/day	No effect				
1		Testis weight	Rat	28	Days	Oral	400.3	mg/kg bw/day	Increase	Relative weights of testes (24.5%) were higher; no histopathology was performed. Dose above MTD	Not consistent effect (decrease/increase) on relative/absolute testis weight: absolute (and in some cases relative) testis weight decreased in rat, dog and mouse at/above the MTD (ID:6, ID:7, ID:8, ID:13 and ID:16), relative testis weight increased in rat only below/at the MTD (ID:1, ID:3, ID:11 and ID:16). **Limited evidence of testis weight in rat, mouse and dog.**		
3			Rat	90	Days	Oral	47.6	mg/kg bw/day	Increase	Increases in mean relative testis weights at 47.6 mg/kg bw, 102 mg/kg bw and 224 mg/kg (9.9%, 17.7%* and 15.6%*, respectively), however, the increase at low-intermediate dose was non-statistically significant. These changes in testis weight were correlated with histopathological findings. 224 mg/kg bw is MTD dose.			
4			Rat	90	Days	Oral		mg/kg bw/day	No effect				
5			Mouse	90	Days	Oral		mg/kg bw/day	No effect				
6			Dog	92/93	Days	Oral	10.56	mg/kg bw/day	Decrease	Non-statistically significant decrease of relative (9.8%) and absolute (35.7%) testes weight of animals. These findings correlate with histopathological changes. MTD dose			
7			Dog	90	Days	Oral	9.7	mg/kg bw/day	Decrease	Statistically significant dose-dependent decrease of absolute testes weight from dose 9.7 mg/kg bw to 14.2 mg/kg bw (30.3% and 38.1%,			

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										respectively) was reported; however, no histopathological changes were observed in these groups.			
8			Dog	52	Weeks	Oral	5.7	mg/kg bw/day	Decrease	Non-statistically significant and non-dose-dependent decrease of absolute and relative testes weight (10.1% and 19.2%, respectively) was reported; however, no histopathological changes testes were observed in this group. Effects on testes and epididymides found in a 90 days study on dogs (at 250/500 ppm equal to 10.56 mg/kg bw) [*RAR B.6.3.2.3. Study 1, 1993*] could not be confirmed in this 1 year dog study due to low amount administered. Therefore, considering the top dose selection, not high enough dose level was selected.			
9			Dog	52	Weeks	Oral		mg/kg bw/day	No effect				
11			Rat	23	Months	Oral	90.1	mg/kg bw/day	Increase	Statistically significant increase (30.7%*) of relative testes weight in the high dose group was reported. Furthermore, these findings correlate with histopathological changes. 30.3 mg/kg bw/day MTD dose.			
12			Rat	24	Months	Oral		mg/kg bw/day	No effect				
13			Mouse	18	Months	Oral	446	mg/kg bw/day	Decrease	Absolute testes weight was statistically significant reduced (19.8%). Macroscopic examination showed increased incidences of small and "soft" testes at this dose. Furthermore, these findings correlate with histopathological changes. 216 mg/kg bw/day is MTD dose.			
16			Rat	16/32	Weeks	Oral	32.1	mg/kg bw/day	Increase	Mean relative testes weight of F0 parental males was statistically significantly greater than controls at 32.1 mg/kg bw/day and 97.9 mg/kg bw/day (10% and 19%, respectively). Since there were no histopathological correlates evident that would account for the increase in the F0 testes weight, these effects on testes were considered not adverse.			
16			Rat	16/32	Weeks	Oral	97.9	mg/kg bw/day	Decrease	The absolute weight of the testes was 12% lower than controls in F1 males at 97.9 mg/kg bw/day. Since there were no histopathological correlates evident that would account for the decrease in the F1 testes weight, these effects on testes were considered not adverse.			
3		Uterus histopathology (with cervix)	Rat	90	Days	Oral		mg/kg bw/day	No effect		Limited evidence of absolute and relative weight of uterus in dog in 90-day study (ID:7).		
4			Rat	90	Days	Oral		mg/kg bw/day	No effect				
5			Mouse	90	Days	Oral		mg/kg bw/day	No effect				
7			Dog	90	Days	Oral		mg/kg bw/day	No effect				
8			Dog	52	Weeks	Oral		mg/kg bw/day	No effect				

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9			Dog	52	Weeks	Oral		mg/kg bw/day	No effect				
12			Rat	24	Months	Oral		mg/kg bw/day	Change	Non-statistically significant slight increase was observed for uterus adenocarcinoma (M) in D&M females of the mid and high dose groups. The uterine adenocarcinoma was metastatic to several organs including the liver and stomach. For combined subgroup animals (i.e. animals found dead and moribund plus animals sacrificed at study termination), the following incidences of neoplasms were found to be increased with dose but revealed no statistically significance: uterus adenocarcinoma (M) and adenoma (B). If a weight of the evidence approach is used, this very slight increase in female rats is not test substance related.			
15			Rat	24	Weeks	Oral	116.3	mg/kg bw/day	No effect				
16			Rat	21	Weeks	Oral		mg/kg bw/day	No effect				
7		Uterus weight (with cervix)	Dog	90	Days	Oral	15.5	mg/kg bw/day	Decrease	Absolute and relative weight of uterus was statistically significantly decreased by 90.2% and 85.4%, respectively, at 15.5 mg/kg bw; however, no histopathological changes were observed in this goup.			
3		Vagina histopathology	Rat	90	Days	Oral		mg/kg bw/day	No effect		No effect on vagina histopathology		
2	Sensitive to, but not diagnostic of, EATS	Adrenals weight	Mouse	28	Days	Oral	179.1	mg/kg bw/day	Decrease	The absolute weight of adrenals (12.5%) was statistical significantly reduced in females. No histopathology was performed, therefore, the adversity of this finding cannot be assessed.	Equivocal effect on absolute adrenals weight of females in carcinogenicity study in mouse and in 28-day mouse toxicity study.		
13		Adrenals weight	Mouse	18	Months	Oral	298	mg/kg bw/day	Decrease	Statistically significant reduction (>14%) of absolute adrenals weight in females at the two high dose groups was not associated with any microscopic findings, therefore, it was considered as non-adverse.			
24		Auditory startle	Rat	16	Days	Oral		mg/kg bw/day	No effect		No effects on auditory startle and brain morphometric (quantitative) in rat		
24		Brain morphometric (quantitative) evaluation	Rat	16	Days	Oral		mg/kg bw/day	No effect				
1		Brain weight	Rat	28	Days	Oral	260.0	mg/kg bw/day	Increase	Relative weight of brain (37.5%) was higher while the absolute weights of brain was lower (6%); no histopathology was performed, therefore, the adversity of this finding cannot be assessed. Dose above MTD	Equivocal effect on rat brain weight		
16		Fertility (mammals)	Rat	21	Weeks	Oral	103.0	mg/kg bw/day	Increase	The statistically significant increases in fertility index for the F1 generation parents (highest dose group) are resulting from the poor reproductive performance of the control group. The effect is not considered treatment relevant.	Some males were infertile and the female fertility index was reduced.		
17		Fertility (mammals)	Rat	15	Weeks	Oral	226.3	mg/kg bw/day	Change	At necropsy 5 parent males in the 226.2 mg/kg bw/day group had bilateral small, flaccid testes,			

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										rendering them infertile. However, microscopic examination of testes was not performed.			
17		Fertility (mammals)	Rat	16	Weeks	Oral	235.2	mg/kg bw/day	Decrease	Statistically significantly reduced the female fertility index (%).			
24		Functional observation battery	Rat	16	Days	Oral		mg/kg bw/day	No effect		No effect on functional observation battery in rats.		
16		Gestation length	Rat	21	Weeks	Oral	103.0	mg/kg bw/day	Decrease	The shorter gestation length of the F1 female rats is resulting from the unusually high mean gestation length value for the control group. The effect is not considered treatment relevant.	Equivocal effect on gestation length of the F1 female rats		
24		Learning and memory in offspring	Rat	16	Days	Oral		mg/kg bw/day	No effect		No effect on learning and memory in rat offspring		
15		Litter size	Rat	24	Weeks	Oral	116.3	mg/kg bw/day	Decrease	For the F1 generation parents, there was a statistically significant decrease in mean litter size in the high dose only (94.0 mg/kg bw/day for males and 116.3 mg/kg bw/day for females).	Decreased litter size, pup weight, viability index in rat		
17		Litter size	Rat	16	Weeks	Oral	235.2	mg/kg bw/day	Decrease	Statistically significantly reduced mean litter size at high dose level (235.2 mg/kg bw/day). The statistically significantly lower mean litter size was likely a consequence of the lower number of corpora lutea, an increased percentage of pre and post-implantation losses and a reduced mean number of implantations of the dams.			
24		Litter viability	Rat	16	Days	Oral	100	mg/kg bw/day	Decrease	Reduced viability index (number of live pups on day 5 post-partum/number of live born pups on day 1 post-partum)			
16		Litter/pup weight	Rat	21	Weeks	Oral	32.1	mg/kg bw/day	Decrease	Concerning pup weight (both sexes combined), statistically significant reductions (reduction 13-18%) were evident at the mid dose level of 32.1 mg/kg bw/day for the F2B generation and also statistically significant reductions (11-40%) at 97.9 mg/kg bw/day (all generations).			
17		Litter/pup weight	Rat	16	Weeks	Oral	68.4	mg/kg bw/day	Decrease	Statistically significantly decreased (>11%) body weight (both sexes combined) for pups were reported from second week of lactation at 68.4 mg/kg bw/day (combined), statistically significant reduction (19.6 – 31.2%) of body weight was observed from LD 7 at 127.7 mg/kg bw/day (combined) and decreased (15.1 – 46.7%) body weight for pups were reported from LD 4 at 235.2 mg/kg bw/day (combined).			
24		Motor activity	Rat	16	Days	Oral		mg/kg bw/day	No effect		No effect on rat motor activity		
15		Number of implantations, corpora lutea	Rat	24	Weeks	Oral	116.3	mg/kg bw/day	Decrease	The reproductive data for the F0 treatment groups did not show any statistically significant differences attributed to treatment of cymoxanil up to 1350 ppm. For the F1 generation parents, there were a statistically significant reduced in mean number of corpora lutea and mean number of implantations in the high dose only (94.0 mg/kg bw/day for males and 116.3 mg/kg bw/day for females). Although these findings	Reduced in mean number of mean number of implantations and corpora lutea in rats in one multigenerational (out of two) and in one one-generation reproductive toxicity studies.		

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										were outside the range of the HCD declared, due to limited information provided by the Applicant the relevance of the HCD was not evaluated by the RMS. It should be noted that at the high dose female parents of the F1 some maternal effects (reduced initial body weight, reduced body weight gain during gestation and reduced food consumption during all phases) were observed.			
17			Rat	16	Weeks	Oral	235.2	mg/kg bw/day	Decrease	Statistically significantly reduced numbers of corpora lutea.			
17			Rat	16	Weeks	Oral	235.2	mg/kg bw/day	Decrease	Statistically significantly reduced mean number of implantations			
18			Rat	10	Days	Oral		mg/kg bw/day	No effect		No effects on mean number of implantations in rabbits and in rats [one developmental (out of two) and in one developmental neurotoxicity studies].		
20			Rabbit	13	Days	Oral		mg/kg bw/day	No effect				
21			Rabbit	13	Days	Oral		mg/kg bw/day	No effect				
22			Rabbit	13	Days	Oral		mg/kg bw/day	No effect				
23			Rabbit	13	Days	Oral		mg/kg bw/day	No effect				
24			Rat	16	Days	Oral		mg/kg bw/day	No effect				
15		Number of live births	Rat	24	Weeks	Oral	116.3	mg/kg bw/day	Decrease	For the F1 generation parents, there was a statistically significant decrease in the percentage of live pups born in the high dose only (94.0 mg/kg bw/day for males and 116.3 mg/kg bw/day for females).	Decreased the number of live pups born or number of male viable foetuses per litter in rats in ID:15 and ID:18.		
18			Rat	10	Days	Oral	75	mg/kg bw/day	Decrease	There was a statistically significant decrease in the number of male viable foetuses per litter from 75 mg/kg bw/day dose. In the high dose group there was a statistically significant reduction in the total number of viable foetuses per litter.			
24			Rat	16	Days	Oral		mg/kg bw/day	No effect				
15		Post implantation loss	Rat	24	Weeks	Oral	116.3	mg/kg bw/day	Increase	For the F1 generation parents, there was a statistically significant increase in percentage of post-implantation loss in the high dose only (94.0 mg/kg bw/day for males and 116.3 mg/kg bw/day for females).	Increased of post-implantation loss and pre-implantation loss in rats.		
17			Rat	16	Weeks	Oral	235.2	mg/kg bw/day	Increase	Statistically significantly increased post-implantation loss.			
18			Rat	10	Days	Oral	150	mg/kg bw/day	Increase	In the high dose group there was a statistically significant increase in the total number of resorptions/litter and an increase in the number of early resorptions/litter			
19			Rat	10	Days	Oral	120	mg/kg bw/day	Increase	In the high dose group there was a non-statistically significant but marked increase in the number of late resorptions and post-implantation loss.			

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17			Rat	16	Weeks	Oral	235.2	mg/kg bw/day	Increase	Statistically significantly increased pre-implantation loss.			
18		Presence of anomalies (external, visceral, skeletal	Rat	10	Days	Oral	25	mg/kg bw/day	Increase	Increased incidences of treatment related skeletal variations (mean percent of affected foetuses per litter and number of foetuses affected) as well as incidences of total variations (mean percent of affected foetuses per litter) were observed from 25 mg/kg bw/day dose level. The skeletal variations included partially ossified skull, partially ossified/unossified sternebra, partially ossified vertebra, wavy ribs, unossified hyoid and partially ossified pelvis and these variations indicated retarded/ skeletal development/delay ossification.	Effect on foetal development producing also malformations in rats ((two developmental toxicity studies) and rabbits (three out of four developmental toxicity studies)		
18			Rat	10	Days	Oral	150	mg/kg bw/day	Increase	The variations, i.e. partially ossified pelvis, partially ossified sternebra, unossified sternebra and wavy ribs, at the highest dose group (150 mg/kg bw/day) tested are considered to be treatment related and of toxicological relevance.			
18			Rat	10	Days	Oral	25	mg/kg bw/day	Increase	The percentage of affected foetuses per litter with any malformations (external visceral or skeletal) as well as the total number of foetuses affected was significantly increased from 25 mg/kg bw/day.			
18			Rat	10	Days	Oral	75	mg/kg bw/day	Increase	Increased incidences of malformations such as hemi vertebra were reported from 75 mg/kg bw/day and such malformations as exencephalic head and fused ribs at 150 mg/kg bw/day. These findings were above the range of historical control values declared, however, the relevance of the HCD was not evaluated by the RMS. Although incidences of these malformations observed were low, treatment-relation with respect to these findings cannot be excluded. Cleft sternebrae occurred at the upper two dose levels and the incidence was within the HCD. These effects were observed in the presence of maternal toxicity evident as statistically significant reduced maternal body weight, body weight gain and food consumption.			
19			Rat	10	Days	Oral	30	mg/kg bw/day	Increase	Concerning foetal skeletal alterations, incidences for minor anomalies (dumb-bell shaped thoracic vertebra 6/13) were shown to be statistically significantly increased and above the historical control data declared even at the lowest dose tested (30 mg/kg bw/day). These alterations are demonstrating an impact of the test material to the development of foetuses.			
19			Rat	10	Days	Oral	60	mg/kg bw/day	Increase	Several skeletal variations comprising incidences of delayed ossification (cervical vertebra: 7/7 and supraoccipital) and some related minor anomalies (hypoplasia of sternum: sternebra no. 1/2 and rudimentary 14th rib) were statistically significantly increased and were above the range			

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										of HCD from 60 mg/kg bw/day.			
19			Rat	10	Days	Oral		mg/kg bw/day	No effect				
20			Rabbit	13	Days	Oral		mg/kg bw/day	No effect				
21			Rabbit	13	Days	Oral	32	mg/kg bw/day	Increase	For the skeletal malformations, there were increases in the incidence of a vertebral and/or rib alterations, including hemivertebra, absent or fused vertebrae, misaligned vertebral centra/arches, fused/absent ribs, and various degrees of resulting scoliosis at the high dose of 32 mg/kg bw/day. Although these findings were not statistically significant, the percentage of foetuses with these malformations in the high dose group was above the HCD range submitted. However, the historical control data provided does not meet all requirements and is quite limited.			
21			Rabbit	13	Days	Oral	16	mg/kg bw/day	Increase	Although the finding "vertebral and other changes between upper cervical and mid-thoracic regions" was also not statistically significant, the percentage of foetuses affected in the mid and high dose groups was clearly above the HCD range declared based on results presented in the summary. These all skeletal alterations include malformations and other alterations that were classified as falling into a "borderline area" between malformation and anomaly allocating them into the category malformation. Diagnostic criteria for malformations and variations were not available in the study summary. The glossary of terminology under development by the International Federation of Teratology Societies was not considered by the Applicant. Since malformations and variations should be reported separately according to the Regulation (EU) No 283/2013, skeletal abnormality as "vertebral and other changes between upper cervical and mid-thoracic regions" is not considered as relevant.			
21			Rabbit	13	Days	Oral		mg/kg bw/day	No effect				
22			Rabbit	13	Days	Oral		mg/kg bw/day	Increase	Visceral malformation such as *hydrocephaly* was found in two foetuses of the highest dose group (32 mg/kg bw/day); the increased number of foetuses affected was without statistical significance but clearly above the range of historical control data. In addition, incidences of foetuses with cleft palates were found in the highest dose tested, the increased number of foetuses affected showed statistical significance and was above the range of historical control. These two visceral malformations (cleft palate and hydrocephaly) occurring in the highest dose			

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										group tested were found in two foetuses (i.e. each foetus with hydrocephaly and cleft palate) from dams that lost weight during the dosing period (i.e. 0.44 and 0.16 kg during 6-18 days) and showed anorexia (5 and 2 days during 12-19 days of gestation) indicating maternal toxicity. Hence, these visceral malformations (cleft palate and hydrocephaly) are considered to be of toxicological relevance and treatment related. Skeletal malformations like fused/asymmetric sternebra were shown in the two mid dose groups without statistical significance and dose relationship; furthermore, the increased incidences were within the range of historical control. Vertebra and/or rib alterations (malformed and absent vertebra, fused vertebra, hemivertebra, branched and fused ribs) were shown to be dose related increased; the increase of incidences for the two highest dose groups were of statistical significance but within the range of historical control.			
22			Rabbit	13	Days	Oral		mg/kg bw/day	No effect				
23			Rabbit	13	Days	Oral	25	mg/kg bw/day	Increase	Visceral variants such as slight renal pelvis dilation were statistically significantly increased for the high dose (25 mg/kg bw/day) foetuses showing a clear dose relationship. The incidence of dilation of heart ventricles was statistically significantly increased in the high dose animals and was above the historical control data. As dilation of heart ventricles must be classified as a structural change that could impair foetal survival, development or function, this alteration should be indicated as major visceral malformations rather than minor anomalies.			
23			Rabbit	13	Days	Oral	25	mg/kg bw/day	Increase	Regarding the skeletal alterations, the incidences of skeletal variants as incomplete/poor ossification of fore limb (middle phalange: 1/5) as well as skeletal minor anomalies as accessory floating rib no. 13 were also shown to be relevant at maternal toxic dose levels (25 mg/kg bw/day).			
18		Pup development	Rat	10	Days	Oral	150	mg/kg bw/day	Change	In the high dose group there was a statistically significant reduction by 16.7% in the mean foetal body weight	Reduced mean rat foetal body weight and increased number of pups found dead on Days 2-5/9-12, reduced lactation indexes and number of surviving pups/litter in rats		
19			Rat	10	Days	Oral	120	mg/kg bw/day	Change	In the high dose group there was statistically significant reduction by 11% in the mean male foetal body weight.			
24		Pup survival index	Rat	16	Days	Oral	100	mg/kg bw/day	Decrease	In pups of the highest dose (100 mg/kg bw/day) group, following treatment-related statistically significant changes were observed: statistically significantly increased number of pups (%) found dead or presumed cannibalized ((13.2% and 2.3% on Days 2-5/9-12, respectively), reduced lactation indexes (number of live pups			

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										on day 12 post-partum/number of live pups on day 5 post-partum; number of live pups on day 22 post-partum (weaning)/number of live pups in subsets 2 – 4 on day 12 post-partum), lower number of surviving pups/litter (from Day 5 post-partum to Day 22 post-partum) and live litter size (Days 12/18/22 post-partum).			
13	Target organ toxicity	Bone marrow histopathology	Mouse	18	Months	Oral	582	mg/kg bw/day	Change	Increased incidence of congestion in bone marrow of female mouse at 582 mg/kg bw/day .	Increased incidence of congestion in bone marrow in this Carcinogenicity study in female mouse at MTD	Sufficient evidence for blood (anaemia) and thymus toxicity in dogs following 90 days and 1 year exposure. Sufficient evidence for eyes toxicity in dogs following 1 year exposure. Sufficient evidence for systemic toxicity (clinical signs, decreased BW/BW gain and food consumption).	
2		Eyes histopathology	Mouse	28	Days	Oral	624.4	mg/kg bw/day	Change	An incidental condition of cataract in both the eyes was observed in one male. MTD dose	Adverse effects on eyes (cataract/lenticular degeneration/retinal atrophy) in dogs (90 days and 1 year studies) and rats (combined chronic toxicity/carcinogenicity study).		
8			Dog	52	Weeks	Oral	5.7	mg/kg bw/day	Change	Bilateral cataract (slight, grade 1) in one male. Since such finding is uncommon in young dogs (< 2 years), a relationship to treatment cannot be excluded. No information whether retina was included in histopathological examination of eyes.			
8			Dog	52	Weeks	Oral	3.1	mg/kg bw/day	Change	Unilateral cataract (slight, grade 1) in one female at 3.1 mg/kg bw /day. Since such finding is uncommon in young dogs (< 2 years), a relationship to treatment cannot be excluded. No information whether retina was included in histopathological examination of eyes.			
9			Dog	52	Weeks	Oral	5.6	mg/kg bw/day	Change	Lenticular degeneration of a slight degree was recorded in both eyes of one dog; since this finding may occur in untreated Beagle dogs at a very low incidence, a relationship to treatment cannot be excluded in this case.			
11			Rat	23	Months	Oral	30.3	mg/kg bw/day	Change	At study termination, the ocular examinations showed that retinal (photoreceptor cell) atrophy incidence was statistical significantly increased in both sexes at 700 and 2000 ppm (30.3 and 90.1 mg/kg bw/day for males): from 22% to 76.1/96.3% in males and from 60% to 90.4/98.2% in females, respectively. The incidence and severity of retinal atrophy in both males and females behaved in a dose response manner in 700 ppm and 2000 ppm rats. The atrophy occurred either bilaterally or unilaterally and ranged in severity from single discrete foci to complete loss of photoreceptor and outer nuclear layers. The 1-year interim sacrifice revealed statistically significant increases in the incidence of retinal atrophy in the 32.8 mg/kg bw/day males and 134 mg/kg bw/day males and females: 1/9, 1/10, 1/10, 4/10* and 9/10* in males groups and 3/10, 3/10, 5/10, 3/10 and 9/10* in female groups. This lesion was atypical in 1-year control rats and therefore, the RMS considers that a treatment related effect of cymoxanil to male retina at two high doses the			

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										first year of treatment (equal to 32.8 and 97.4 mg/kg bw/day, respectively) could not be excluded. 30.3 mg/kg bw/day is MTD for males and 126 mg/kg bw/day is MTD for females.			
12			Rat	24	Months	Oral		mg/kg bw/day	No effect				
14		Heart histopathology	Mouse	18	Months	Oral		mg/kg bw/day	Change	A statistically significant increase in the incidence of heart myocardial degeneration was seen in all treatment male groups found dead and sacrificed moribund (0/18 [0%], 5/22* [22.7%], 4/18* [22.2%], 8/12* [66.7%], 8/24* [33.3%]). This statistically significant increase was not dose related and it was not replicated in the males reaching terminal sacrifice. The total incidence of heart myocardial degeneration over the course of the study was also seen in all treatment male groups, however, values did not attain statistical significance. Based on the HCD provided, the incidences of heart myocardial degeneration in dead and moribund as well as terminally sacrificed male mice are not so rare case: six studies, overall 97 males - from 0% to 56% and overall 203 males - from 0% to 50%, respectively.	Increase in the incidence of heart myocardial degeneration in all treatment male groups found dead and sacrificed moribund in mouse carcinogenicity study		
1		Heart weight	Rat	28	Days	Oral	260.0	mg/kg bw/day	Increase	Relative weights of heart (15.9%) were higher; no histopathology was performed. Dose above MTD			
5		Kidney histopathology	Mouse	90	Days	Oral		mg/kg bw/day	No effect		Not consistent effect (decrease/increase) on kidney weight in short-term rat and mice studies were of limited toxicological relevance.		
1		Kidney weight	Rat	28	Days	Oral	143.5	mg/kg bw/day	Increase	Statistically significant increase in relative kidneys (11.8%, dose related); no histopathology was performed. MTD dose			
2			Mouse	28	Days	Oral	303.4	mg/kg bw/day	Decrease	Statistically significant increase in relative kidneys (11.8%, dose related); no histopathology was performed. MTD dose			
2			Mouse	28	Days	Oral	679.3	mg/kg bw/day	Decrease	The absolute weight of kidneys was statistical significantly lower (21.5%); however, the toxicological relevance of these changes were considered unclear, as the relative weight of kidney did not show statistically significant changes and no histopathology was performed in this study. MTD dose.			
4			Rat	90	Days	Oral	174.3	mg/kg bw/day	Increase	Statistically significant increase (7.8-17.3%) in relative kidney weight was observed at ≥ 85.1 mg/kg bw . Although this increase was dose related, relative kidney weight was higher than 15% of the control at 174.3 mg/kg bw only. No histopathological effects were seen in male kidney at high dose, except slight higher amount of hyaline casts (control 1/10, high dose 3/10).			
5			Mouse	90	Days	Oral		mg/kg bw/day	No effect				
5		Liver histopathology	Mouse	90	Days	Oral	256.6	mg/kg bw/day	Change	Vacuolar changes (minimal to mild/moderate) of liver cells have been observed in all treated	Histopathological changes in the liver of		

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										animals with highest incidences in the high dose groups. No statistical analysis has been performed with respect to histopathological changes. The aetiology of this change was uncertain. The macroscopic examination provided no information on damage to liver, there was no effect on liver enzymes in this study, there was the quite small size of the difference in numbers of affected animals compared to concurrent controls and vacuolar changes of liver cells were not reproducible in other toxicity studies on cymoxanil. Therefore, vacuolar changes of liver cells were disregarded in this study.	female rats and liver of mice ((Combined chronic toxicity/carcinogenicity studies) were accompanied by increased liver weight of female mice. Effects were observed below the MTD dose.		
5			Mouse	90	Days	Oral	302.5	mg/kg bw/day	Change	Vacuolar changes (minimal to mild/moderate) of liver cells have been observed in all treated animals with highest incidences in the high dose groups. No statistical analysis has been performed with respect to histopathological changes. The aetiology of this change was uncertain. The macroscopic examination provided no information on damage to liver, there was no effect on liver enzymes in this study, there was the quite small size of the difference in numbers of affected animals compared to concurrent controls and vacuolar changes of liver cells were not reproducible in other toxicity studies on cymoxanil. Therefore, vacuolar changes of liver cells were disregarded in this study.			
7			Dog	90	Days	Oral		mg/kg bw/day	No effect				
11			Rat	23	Months	Oral	38.4	mg/kg bw/day	Change	At study termination, substance related and adverse histopathological changes (inflammation and/or polyarteritis) were reported in the liver of female rats at 38.4 and 126 mg/kg bw/day. At 126 mg/kg bw/day the MTD was reached for females.			
12			Rat	24	Months	Oral		mg/kg bw/day	Change	The higher incidence of liver metastatic adenocarcinoma (MM) was observed in the high dose females of dead and moribund animals (D&M).It is noteworthy that these were not primary liver tumours in D&M females. In all these animals the uterine adenocarcinoma was metastatic to several organs including the liver. The primary liver tumour (hepatocellular carcinoma) was observed only in a single high dose terminally sacrificed female. For combined subgroup animals (i.e. animals found dead and moribund plus animals sacrificed at study termination), the following incidences of neoplasms were found to be increased with dose but revealed no statistically significance: liver adenocarcinoma (MM) in females. If a weight of			

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										the evidence approach is used, this very slight increase in female rats is not test substance related.			
13			Mouse	18	Months	Oral	42	mg/kg bw/day	Change	Statistically significant treatment-related histological findings in liver (centrilobular apoptotic hepatocytes, macrophages containing a pigment, granuloma, diffuse centrilobular hypertrophy) in males of three dose groups (42.0, 216 and 446 mg/kg bw/day) were not accompanied by liver weight changes. Effects were observed below the MTD dose.			
1		Liver weight	Rat	28	Days	Oral	143.5	mg/kg bw/day	Increase	Statistically significant increase in relative liver (12.7%); no histopathology was performed. MTD dose			
4			Rat	90	Days	Oral	174.3	mg/kg bw/day	Increase	A statistically significant increase (15.7%) in relative liver weight. No histopathological changes were seen at high dose. MTD dose			
5			Mouse	90	Days	Oral		mg/kg bw/day	No effect				
5			Mouse	90	Days	Oral	302.5	mg/kg bw/day	Increase	Statistically significant increase (11.2%) in relative liver weight			
7			Dog	90	Days	Oral	9.9	mg/kg bw/day	Increase	Relative liver weight was statistically significantly increased (>20%) at 9.9 mg/kg bw to 15.5 mg/kg bw doses with no histopathological correlation.			
13			Mouse	18	Months	Oral	298	mg/kg bw/day	Increase	Absolute and relative liver weight showed a statistically significant increase at the two high dose groups (10.3/18.1% and 13.0/26.8%, respectively) of females and this was accompanied by statistically significant treatment-related histological findings in liver			
11		Lymph nodes histopathology	Rat	23	Months	Oral	126	mg/kg bw/day	Change	Polyarteritis and cystic atrophy were observed in the mesenteric lymph node	Histopathological changes in the mesenteric lymph nodes (polyarteritis/haemorrhage) in female rats and male mice ((Combined chronic toxicity/carcinogenicity studies) at top dose.		
14			Mouse	18	Months	Oral	178.3	mg/kg bw/day	Change	A significant increase in the incidence of discolouration of the mesenteric lymph nodes in the highest dose males found dead and sacrificed moribund. This dicolouration of mesenteric lymph nodes was a red discolouration resulting from microscopically identified haemorrhage (3/18 [17%], 7/22 [32%], 1/18 [6%], 4/12 [33%], 11/24* [46%]). It should be noted that this gross finding as well as histopathological finding (haemorrhage) were not replicated in the animals reaching terminal sacrifice. The total incidence of haemorrhage in mesenteric lymph nodes over the course of the study was seen in all treatment male groups, however, values did not attain statistical significance. Based on the HCD provided, the incidence of haemorrhage in mesenteric lymph nodes in dead and moribund sacrificed male mice was below the value obtained in this study: six studies, from 0% to 40%, overall 97 males, overall 21 cases.			

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11		Lung histopathology	Rat	23	Months	Oral	90.1	mg/kg bw/day	Change	The incidence of the granulomatous inflammation and haemorage in the lung was elevated	Histopathological changes in the lung (inflammation/haemorage/bronchopneumonia) in Combined chronic toxicity/carcinogenicity rat studies		
11			Rat	23	Months	Oral	38.4	mg/kg bw/day	Change	In the lung, at the terminal sacrifice, the incidence of polyarteritis was statistical significantly increased at 38.4, 126 mg/kg bw/day. In addition, at 126 mg/kg bw/day females had significant increases in the incidence of histocytosis, alveolar inflammation, type II cell hyperplasia, alveolar wall squamous metaplasia and fibrosis/inflammation. Electron microscopy suggests test substance-induced phospholipidose. The incidence of the granulomatous inflammation was elevated in the females at 126 mg/kg bw/day. 126 mg/kg bw/day is MTD.			
12		Lung histopathology	Rat	24	Months	Oral	58.8	mg/kg bw/day	Change	Regarding histopathological examination of animals terminally sacrificed including animals found dead and sacrificed moribund, statistically significant increases in the following non-neoplastic incidences were seen: suppurative bronchopneumonia in lungs of the highest dose group			
12			Rat	24	Months	Oral	75.8	mg/kg bw/day	Change	Regarding histopathological examination of animals terminally sacrificed including animals found dead and sacrificed moribund, statistically significant increases in the following non-neoplastic incidences were seen: suppurative bronchopneumonia in lungs of the highest dose group			
11		Pancreas histopathology	Rat	23	Months	Oral	126	mg/kg bw/day	Change	Inflammation and/or polyarteritis were observed in the pancreas/stomach/small intestine/large intestine/urinary bladder was documented at 126 mg/kg bw/day. MTD dose.	Histopathological changes in the pancreas (Inflammation/polyarteritis/acinar cell necrosis) in female rats and mice in Combined chronic toxicity/carcinogenicity studies at or above MTD dose.		
13			Mouse	18	Months	Oral	582	mg/kg bw/day	Change	Based on pathological evaluation of 5 females which were sacrificed on or before test day 35, pancreatic acinar cell necrosis was considered an adverse effect at 582 mg/kg bw/day.			
11		Peripheral nerve histopathology	Rat	23	Months	Oral	38.4	mg/kg bw/day	Change	A statistically significant compound related increase in incidence of axon/myelin degeneration of the sciatic nerve occurred in females at 38.4 and 126 mg/kg bw/day , without any other signs of peripheral neuropathy.	Increase in incidence of axon/myelin degeneration of the sciatic nerve in female rats in combined chronic toxicity/carcinogenicity study below MTD.		
12		Small and large intestines histopathology	Rat	24	Months	Oral	23.5	mg/kg bw/day	Change	Regarding histopathological examination of animals terminally sacrificed including animals found dead and sacrificed moribund, statistically significant increases in the following non-	Histopathological non-neoplastic changes in the small and large intestines of rats and mice		

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										neoplastic incidences were seen: lymphoid hyperplasia in rectum of males of the mid and high dose group (23.5 and 58.8 mg/kg bw/day, respectively).	((Combined chronic toxicity/carcinogenicity studies).		
12			Rat	24	Months	Oral	75.8	mg/kg bw/day	Change	Regarding histopathological examination of animals terminally sacrificed including animals found dead and sacrificed moribund, statistically significant increases in the following non-neoplastic incidences were seen: lymphoid hyperplasia in colon in females of the highest dose group (75.8 mg/kg bw/day)			
13			Mouse	18	Months	Oral	216	mg/kg bw/day	Change	Increased incidence of cystic enteropathy in jejunum from 216 mg/kg bw/day			
13			Mouse	18	Months	Oral	58.1	mg/kg bw/day	Change	Increased incidence of cystic enteropathy in duodenum from 58.1 mg/kg bw/day. Increased incidence of cystic enteropathy in jejunum from 298 mg/kg bw/day.			
13		Spleen histopathology	Mouse	18	Months	Oral	582	mg/kg bw/day	Change	Increased incidence of diffuse atrophy in spleen of female mice above MTD.	Increased incidence of diffuse atrophy in spleen of female mice in carcinogenicity study above MTD.		
1		Spleen weight	Rat	28	Days	Oral	260.0	mg/kg bw/day	Increase	Relative weights of spleen (49.4%) were higher; no histopathology was performed. Dose above MTD			
13		Stomach histopathology	Mouse	18	Months	Oral	58.1	mg/kg bw/day	Change	Increased incidence of hyperplastic gastropathy in stomach of female mouse from 58.1 mg/kg bw/day.	Increased incidence of hyperplastic gastropathy in stomach of female mouse in carcinogenicity study below MTD.		
6		Thymus histopathology	Dog	92/93	Days	Oral		mg/kg bw/day	No effect	No changes, one animal from highest dose group was investigated only. Weight of thymus was not investigated.	Increase in thymus atrophy of male and female dogs in a 90 day study and in male dogs in a 1 year study.		
6			Dog	92/93	Days	Oral		mg/kg bw/day	No effect	No changes, one animal from highest dose group was investigated only. Weight of thymus was not investigated.			
7			Dog	90	Days	Oral	9.7	mg/kg bw/day	Change	A dose dependent increase in lymphoid atrophy of male thymus with increasing severity was reported from 9.7 mg/kg bw/day [2/4 (mild and moderate)] to 14.2 mg/kg bw/day [3/4 (2 moderate and 1 severe)]			
7			Dog	90	Days	Oral	9.9	mg/kg bw/day	Change	A dose dependent increase in lymphoid atrophy was reported from 9.9 mg/kg bw/day [2/4 (minimal and moderate)] to 15.5 mg/kg bw/day [4/4 (2 minimal and 2 moderate)].there were changes in other lymphoid tissues (e.g. of bene marrow and lymph nodes) of female at the highest dose (equal to 15.5 mg/kg bw/day), whereas these tissues were not examined at other doses. The following histopathological findings of lymphoid tissues were noted in one emaciated female: lymphoid atrophy in thymus (moderate), lymphoid atrophy in mesenteric lymph nodes (mild), atrophy in bone marrow (severe) and in sternum marrow (severe). No such histopathological findings of lymphoid tissues were noted in one emaciated male with lymphoid			

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
										atrophy in the thymus (severe) at high dose. There were not such histopathological findings of lymphoid tissues in both control groups.			
8			Dog	52	Weeks	Oral		mg/kg bw/day	No effect	No toxicologically significant changes.			
9			Dog	52	Weeks	Oral	1.3	mg/kg bw/day	Change	Microscopically, thymic lymphoid atrophy - involution was observed in three groups (1.3, 2.8, 5.6 mg/kg bw/day) of treated males but not in the control group (0/4; 2/4; 3/4 and 3/4, respectively). According to the grading system the severity of thymus atrophy from minimal to severe was reported: in Group 2 (2, 3), Group 3 (2, 2, 3) and Group 4 (1, 2, 4), where grade 1 - minimal, 2 - slight, 3 - moderate and 4 - severe. Furthermore, these microscopic observations correlated with thymus weights findings in male groups. Given that a dose-response relationship in absolute and relative thymus weights of males was observed, these thymus weights findings correlated with the microscopic observations (thymic lymphoid atrophy – involution) and the MTD have not been clear reached at the highest dose, these findings at least in males may indicate that a decrease of thymus weight in males of the high dose (5.6 mg/kg bw/day) group might be a substance-specific effect but not a result of generalised high-dose stress response.			
9			Dog	52	Weeks	Oral		mg/kg bw/day	No effect	Females were less affected than males with respect to thymus weight and thymus histopathology. Microscopically, thymic lymphoid atrophy - involution was observed in all groups of females including control (0, 0.8, 1.4, 2.9 mg/kg bw; 4/4; 2/4; 2/4; and 3/4, respectively) and these microscopic findings likely to be attributable to normal age-associated thymic involution. According to the grading system the severity of thymus atrophy from minimal to moderate was reported.			
13			Mouse	18	Months	Oral		mg/kg bw/day	Change	No toxicologically significant changes.			
13			Mouse	18	Months	Oral	582	mg/kg bw/day	Change	Increased incidence of thymus atrophy at 582 mg/kg bw/day .			
7		Thymus weight	Dog	90	Days	Oral	9.7	mg/kg bw/day	Decrease	Reduction in absolute (>52%) and relative (>30%) thymus weight at ≥ 9.7 mg/kg bw was non-statistically significant as well as not clear dose-dependent			
7			Dog	90	Days	Oral	5.2	mg/kg bw/day	Decrease	Clear dose-dependent and statistically significant reduction in relative thymus weight from 5.2 mg/kg bw dose (42%, 51.9% and 55.6%, respectively), whereas there was not significant loss of body weight gain as well as lymphoid atrophy in the thymus in females at 5.2 mg/kg bw dose. Dose-dependent and statistically significant reduction in absolute (>56%) and			

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
										relative (>50%) thymus weight at ≥ 9.9 mg/kg bw dose with histological evidence of lymphoid atrophy in the thymus was observed. Females affected were 2/4 (minimal and moderate) at 9.9 mg/kg bw/day, and 4/4 (2 minimal and 2 moderate) females at 14.2 mg/kg bw/day indicating a dose relationship.			
9			Dog	52	Weeks	Oral	5.6	mg/kg bw/day	Decrease	Statistically significant decrease of absolute thymus weight (52.7 %*). Dose-dependent decrease in mean absolute and relative thymus weights of all three male groups treated (equal to 1.3, 2.8 and 5.6 mg/kg bw) was observed (23.2-57.7% and 24.2-44.4%, respectively), however, the values did not attain statistical significance except the value mentioned above.			
9			Dog	52	Weeks	Oral		mg/kg bw/day	No effect				
1	Systemic toxicity	Body weight	Rat	28	Days	Oral	143.5	mg/kg bw/day	Decrease		Sufficient evidence for blood (anaemia) and thymus toxicity in dogs following 90 days and 1 year exposure. Sufficient evidence for eyes toxicity in dogs following 1 year exposure. Sufficient evidence for systemic toxicity (clinical signs, decreased BW/BW gain and food consumption).		
1			Rat	28	Days	Oral	287.8	mg/kg bw/day	Decrease				
2			Mouse	28	Days	Oral	303.4	mg/kg bw/day	Decrease				
2			Mouse	28	Days	Oral	679.3	mg/kg bw/day	Decrease				
3			Rat	90	Days	Oral	224	mg/kg bw/day	Decrease				
3			Rat	90	Days	Oral	333	mg/kg bw/day	Decrease				
4			Rat	90	Days	Oral	174.3	mg/kg bw/day	Decrease				
4			Rat	90	Days	Oral	187.7	mg/kg bw/day	Decrease				
5			Mouse	90	Days	Oral	256.6	mg/kg bw/day	Decrease				
5			Mouse	90	Days	Oral	302.5	mg/kg bw/day	Decrease				
6			Dog	92/93	Days	Oral	10.56	mg/kg bw/day	Decrease				
6			Dog	92/93	Days	Oral	5.27	mg/kg bw/day	Decrease				
7			Dog	90	Days	Oral	9.7	mg/kg bw/day	Decrease				
7			Dog	90	Days	Oral	9.9	mg/kg bw/day	Decrease				
8			Dog	52	Weeks	Oral		mg/kg bw/day	No effect				
8			Dog	52	Weeks	Oral		mg/kg bw/day	No effect				
9			Dog	52	Weeks	Oral	5.6	mg/kg bw/day	Decrease				
9			Dog	52	Weeks	Oral		mg/kg bw/day	No effect				

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
10			Rat	28	Days	Dermal		mg/kg bw/day	No effect				
11			Rat	23	Months	Oral	30.3	mg/kg bw/day	Decrease				
11			Rat	23	Months	Oral	126	mg/kg bw/day	Decrease				
12			Rat	24	Months	Oral	58.8	mg/kg bw/day	Decrease				
12			Rat	24	Months	Oral		mg/kg bw/day	No effect				
13			Mouse	18	Months	Oral	216	mg/kg bw/day	Decrease				
13			Mouse	18	Months	Oral	298	mg/kg bw/day	Decrease				
14			Mouse	18	Months	Oral	178.3	mg/kg bw/day	Change				
14			Mouse	18	Months	Oral	92.4	mg/kg bw/day	Change				
15			Rat	14-18	Weeks	Oral	94.0	mg/kg bw/day	Decrease				
15			Rat	24	Weeks	Oral	116.3	mg/kg bw/day	Decrease				
15			Rat	24	Weeks	Oral	31.6	mg/kg bw/day	Decrease				
16			Rat	16/32	Weeks	Oral	97.9	mg/kg bw/day	Decrease				
16			Rat	21	Weeks	Oral	103.0	mg/kg bw/day	Decrease				
17			Rat	16	Weeks	Oral	127.7	mg/kg bw/day	Decrease				
18			Rat	10	Days	Oral	25	mg/kg bw/day	Decrease				
19			Rat	10	Days	Oral	120	mg/kg bw/day	Decrease				
20			Rabbit	13	Days	Oral		mg/kg bw/day	No effect				
21			Rabbit	13	Days	Oral	32	mg/kg bw/day	Decrease				
22			Rabbit	13	Days	Oral		mg/kg bw/day	No effect				
23			Rabbit	13	Days	Oral	25	mg/kg bw/day	Decrease				
24			Rat	16	Days	Oral	50	mg/kg bw/day	Decrease				
24			Rat	16	Days	Oral		mg/kg bw/day	No effect				
4		Clinical chemistry and haematology	Rat	90	Days	Oral	85.1	mg/kg bw/day	Change		Adverse effect on haematology (reductions in haemoglobin) in dogs in the 90-days and 1-year studies (ID:6, ID:7 and ID:8).		
5			Mouse	90	Days	Oral	256.6	mg/kg bw/day	Change				
5			Mouse	90	Days	Oral	302.5	mg/kg bw/day	Change				

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
6			Dog	92/93	Days	Oral	5.13	mg/kg bw/day	Change				
6			Dog	92/93	Days	Oral	10.56	mg/kg bw/day	Change				
6			Dog	92/93	Days	Oral	10.51	mg/kg bw/day	Change				
6			Dog	92/93	Days	Oral	10.51	mg/kg bw/day	Change				
7			Dog	90	Days	Oral	14.2	mg/kg bw/day	Change				
7			Dog	90	Days	Oral	9.9	mg/kg bw/day	Change				
7			Dog	90	Days	Oral	5.2	mg/kg bw/day	Change				
8			Dog	52	Weeks	Oral	5.7	mg/kg bw/day	Change				
8			Dog	52	Weeks	Oral	5.7	mg/kg bw/day	Change				
8			Dog	52	Weeks	Oral		mg/kg bw/day	No effect				
9			Dog	52	Weeks	Oral		mg/kg bw/day	No effect				
9			Dog	52	Weeks	Oral	5.6	mg/kg bw/day	Change				
9			Dog	52	Weeks	Oral		mg/kg bw/day	No effect				
11			Rat	23	Months	Oral	90.1	mg/kg bw/day	Change				
11			Rat	23	Months	Oral		mg/kg bw/day	No effect				
11			Rat	23	Months	Oral		mg/kg bw/day	No effect				
12			Rat	24	Months	Oral		mg/kg bw/day	No effect				
12			Rat	24	Months	Oral		mg/kg bw/day	No effect				
13			Mouse	18	Months	Oral	216	mg/kg bw/day	Change				
13			Mouse	18	Months	Oral		mg/kg bw/day	No effect				
14			Mouse	18	Months	Oral		mg/kg bw/day	No effect				
14			Mouse	18	Months	Oral		mg/kg bw/day	No effect				
1		Clinical signs	Rat	28	Days	Oral	260.0	mg/kg bw/day	Change		Clinical signs in rats (weak, hyperreactivity, end of tail missing, sore and alopecia), mouse (weak, dull, pallor and stained fur), dogs (diarrhoea and weakness) and rabbits		
1			Rat	28	Days	Oral	415.9	mg/kg bw/day	Change				
2			Mouse	28	Days	Oral	624.4	mg/kg bw/day	Change				
6			Dog	92/93	Days	Oral	5.13	mg/kg bw/day	Induction				

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
6			Dog	92/93	Days	Oral	5.27	mg/kg bw/day	Induction		(anorexia/reduced faecal) were observed in short and long term studies.		
7			Dog	90	Days	Oral	9.7	mg/kg bw/day	Induction				
7			Dog	90	Days	Oral	9.9	mg/kg bw/day	Induction				
11			Rat	23	Months	Oral	30.3	mg/kg bw/day	Induction				
12			Rat	24	Months	Oral		mg/kg bw/day	Change				
13			Mouse	18	Months	Oral	446	mg/kg bw/day	Induction				
13			Mouse	18	Months	Oral	582	mg/kg bw/day	Induction				
14			Mouse	18	Months	Oral		mg/kg bw/day	No effect				
14			Mouse	18	Months	Oral		mg/kg bw/day	No effect				
15			Rat	14-18	Weeks	Oral		mg/kg bw/day	No effect				
15			Rat	24	Weeks	Oral	116.3	mg/kg bw/day	No effect				
16			Rat	16/32	Weeks	Oral	97.9	mg/kg bw/day	Induction				
16			Rat	21	Weeks	Oral	103.0	mg/kg bw/day	Induction				
16			Rat	21	Weeks	Oral	97.9	mg/kg bw/day	Induction				
18			Rat	10	Days	Oral	150	mg/kg bw/day	Induction				
21			Rabbit	13	Days	Oral	16	mg/kg bw/day	Induction				
22			Rabbit	13	Days	Oral		mg/kg bw/day	No effect				
24			Rat	16	Days	Oral	100	mg/kg bw/day	Induction				
1		Food consumption	Rat	28	Days	Oral	143.5	mg/kg bw/day	Decrease		Decreased food consumption was usually consistent with decreased body weight/ body weight gain in rat, mouse, dog and rabbit in 28-day, 90-day, chronic, multigenerational reproductive toxicity, prenatal developmental and developmental neurotoxicity studies.		
1			Rat	28	Days	Oral	287.8	mg/kg bw/day	Decrease				
2			Mouse	28	Days	Oral	303.4	mg/kg bw/day	Decrease				
2			Mouse	28	Days	Oral	679.3	mg/kg bw/day	Decrease				
3			Rat	90	Days	Oral	224	mg/kg bw/day	Decrease				
3			Rat	90	Days	Oral	333	mg/kg bw/day	Decrease				
4			Rat	90	Days	Oral	174.3	mg/kg bw/day	Decrease				
4			Rat	90	Days	Oral	187.7	mg/kg bw/day	Decrease				

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
5			Mouse	90	Days	Oral	256.6	mg/kg bw/day	Decrease				
5			Mouse	90	Days	Oral		mg/kg bw/day	No effect				
6			Dog	92/93	Days	Oral	10.56	mg/kg bw/day	Decrease				
6			Dog	92/93	Days	Oral	5.27	mg/kg bw/day	Decrease				
7			Dog	90	Days	Oral	9.7	mg/kg bw/day	Decrease				
7			Dog	90	Days	Oral	15.5	mg/kg bw/day	Decrease				
11			Rat	23	Months	Oral		mg/kg bw/day	No effect				
12			Rat	24	Months	Oral		mg/kg bw/day	Decrease				
13			Mouse	18	Months	Oral		mg/kg bw/day	No effect				
13			Mouse	18	Months	Oral		mg/kg bw/day	No effect				
15			Rat	14-18	Weeks	Oral	94.0	mg/kg bw/day	Decrease				
15			Rat	24	Weeks	Oral	116.3	mg/kg bw/day	Decrease				
16			Rat	16/32	Weeks	Oral	97.9	mg/kg bw/day	Decrease				
16			Rat	21	Weeks	Oral	103.0	mg/kg bw/day	Decrease				
17			Rat	16	Weeks	Oral	127.7	mg/kg bw/day	Decrease				
18			Rat	10	Days	Oral	25	mg/kg bw/day	Decrease				
19			Rat	10	Days	Oral	120	mg/kg bw/day	Decrease				
23			Rabbit	13	Days	Oral	15	mg/kg bw/day	Decrease				
24			Rat	16	Days	Oral	50	mg/kg bw/day	Decrease				
6		Mortality	Dog	92/93	Days	Oral	10.51	mg/kg bw/day	Increase		There were no dose related trends in mortality in chronic studies; no evidence of mortality in adults in other studies.		
11			Rat	23	Months	Oral	0	mg/kg bw/day	Increase				
11			Rat	23	Months	Oral	0	mg/kg bw/day	Increase				
12			Rat	24	Months	Oral	0	mg/kg bw/day	Increase				
12			Rat	24	Months	Oral	0	mg/kg bw/day	Increase				
13			Mouse	18	Months	Oral		mg/kg bw/day	Increase				
13			Mouse	18	Months	Oral	582	mg/kg bw/day	Increase				

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
14			Mouse	18	Months	Oral		mg/kg bw/day	Increase				
14			Mouse	18	Months	Oral		mg/kg bw/day	Increase				
15			Rat	14-18	Weeks	Oral		mg/kg bw/day	No effect				
15			Rat	24	Weeks	Oral	116.3	mg/kg bw/day	No effect				
15			Rat	24	Weeks	Oral	94.0	mg/kg bw/day	Increase				
16			Rat	16/32	Weeks	Oral		mg/kg bw/day	No effect				
16			Rat	21	Weeks	Oral		mg/kg bw/day	No effect				
16			Rat	21	Weeks	Oral	32.1	mg/kg bw/day	Increase				
17			Rat	16	Weeks	Oral	235.2	mg/kg bw/day	Increase				
18			Rat	10	Days	Oral		mg/kg bw/day	No effect				
19			Rat	10	Days	Oral		mg/kg bw/day	No effect				
21			Rabbit	13	Days	Oral		mg/kg bw/day	No effect				
22			Rabbit	13	Days	Oral		mg/kg bw/day	No effect				
23			Rabbit	13	Days	Oral		mg/kg bw/day	No effect				

2.2.2.1. Assessment of the integrated lines of evidence and weight of evidence for EAS-mediated adversity and endocrine activity

Table 5: WoE for EAS-mediated adversity

- There were numerous effects of cymoxanil on **testis and epididymis** in rats, mice and dogs. Testis and epididymis are the EAS-mediated organs with the most reported effects (considering histopathology and weight). There were effects consistently observed in these organs in the available mammalian studies (see Table 2-1), but there is an uncertainty regarding the human health relevance of these effects. These effects in testes included elongate spermatid degeneration, atrophy of the seminiferous tubules, atrophy of testes, aspermatogenesis and multinucleate spermatids which was secondary to the elongate spermatid abnormality; while the effects in epididymis included seminiferous cell debris, multinucleated spermatids, sperm granuloma, atrophy, hypospermia, oligospermia and aspermia. The most relevant studies for effects of cymoxanil on testis were rat (90 days dietary study, **ID:3**) and dog (1 year dog study **D:9**) studies, while for effects of cymoxanil on epididymis was mouse study (carcinogenicity mouse study, **ID:13**).
- **Rat**
  - In 90 days dietary study **(ID:3)**, there were dose-related increases in mean relative testis weights at 47.6, 102, 224 mg/kg bw/day (9.9%, 17.7%* and 15.6%*, respectively); however, the increase at low-intermediate dose was non-statistically significant. These changes in testis weight were correlated with histopathological findings: increased bilateral elongate spermatid degeneration occurred at ≥ 47.6 mg/kg bw/day, the incidence and severity increasing with dosage. Increased elongate spermatid degeneration was observed in three animals of the 47.6 mg/kg bw/day dose group, five of the 102 mg/kg bw/day dose group and seven animals of the 224 mg/kg bw/day dose group: the increased incidence showed a clear dose-relationship and was statistically significant at the highest dose level. Furthermore, one male rat each from the 102 mg/kg bw/day and 224 mg/kg bw/day dose group had multinucleated spermatids: despite of no statistical significance the finding supports a compound related effect to male reproductive organ. The following histopathological changes have been observed with respect to epididymis: cell debris (1 animal and 6 animals of the two highest dose groups, respectively), bilateral hypospermia (4 animals of the highest dose group) and multinucleated spermatids (one animal each of the two highest dose groups tested); statistical significance was shown for cell debris and hypospermia of the highest dose group (224 mg/kg bw/day). No weight of epididymis was assessed in the study. At 224 mg/kg bw/day the MTD was reached for males.
  - In 90 days dietary study **(ID:4)**, neither weight of epididymis/prostate/seminal vesicles with coagulating glands was recorded nor histopathological examination of these organs/tissues was performed. Neither changes in weight of testes nor macroscopic/histopathological damage/changes in testes were observed in this study at the highest dose tested (174.3 mg/kg bw/day). However, in additional recovery group (173.5 mg/kg bw/day) small sized (-1.5 cm) and flabby bilateral testes were observed in one male (ten animals were investigated) during gross necropsy and later histopathological examination showed atrophy of seminiferous tubules (severe and diffuse)/calcification (mild and multifocal) in this male testes (testes tissues of other males were not examined). At the 174.3 mg/kg bw/day dose the MTD was reached for males.
  - In a 2 years dietary rat study **(ID:11)**, a statistically significant increase (30.7%*) of relative testes weight in the high dose group (90.1 mg/kg bw/day) was reported; however, weight of epididymis was not investigated. Histological findings with respect to testes (statistically significant elongate spermatid degeneration) were observed from 30.3 mg/kg bw/day (7/63, 5/62, 4/62, 17/56* and 29/62* at 0, 1.98, 4.08, 30.3 and 90.1 mg/kg bw/day) at termination (all animals excluding interim sacrifice). Although this lesion could be found in control animals, the incidence and severity increased with increasing dietary concentration of the test compound, e.g. grades of lesions were 14 minimal, 3 mild at 30.3 mg/kg bw/day and 20 minimal, 4 mild and 5 moderate at 90.1 mg/kg bw/day. In addition to the elongate spermatid lesion there was a statistically significant increase in multinucleate spermatids in the 90.1 mg/kg bw/day male testes

which was secondary to the elongate spermatid abnormality. Furthermore, these histopathological findings in testes were evident also in animals at the one year interim sacrifice: statistically significant increase of elongate spermatid degeneration at 32.8 mg/kg bw/day and 97.4 mg/kg bw/day [1/9, 0/10, 0/10, 4/10* and 5/10*] as well as multinucleated spermatids in testes in one male at 97.4 mg/kg bw/day. Additionally, at the one year interim sacrifice, multinucleated spermatids in epididymis had a statistically significantly increased occurrence in males at 97.4 mg/kg bw/day (0/9, 0/10, 0/10, 0/10 and 3/10* at 0, 2.25, 4.58, 32.8, 97.4 mg/kg bw/day, respectively); however, at study termination increased bilateral oligospermia in epididymis at high dose (10/63, 8/62, 11/62, 8/56 and 23/62 at 0, 1.98, 4.08, 30.3, 90.1 mg/kg bw/day, respectively) did not attain statistical significance. Therefore, these results confirmed the similar microscopic findings in testes in a previous 90-day rat study (ID:3). At 30.3 mg/kg bw/day (32.8 mg/kg bw/day for interim sacrifice) the MTD was reached for males.

- In a 2 years dietary study **(ID:12)**, histopathological examination of testes after 24 months with cymoxanil (animals terminally sacrificed including animals found dead and sacrificed moribund) revealed statistically significantly increased incidence of mild-to-moderate seminiferous tubule atrophy in the testis at 58.8 mg/kg bw/day. It was seen in both testes (bilaterally) in 3 of the 12 cases at 1200 ppm. It should be noted that, however, histopathological examinations wasn't performed from all animals in all dose groups. There was a clear increased combined incidence of epididymal oligospermia with aspermia (a low sperm count and the complete lack of semen) at the highest dose level (58.8 mg/kg bw/day); however, no statistical analysis has been performed. In all cases aspermia was exceptionally identified in terminal sacrificed males: this finding was not identified in animals found dead and sacrificed moribund. Due to methodological limitation (no histopathological examination was performed from all animals in all dose groups) the NOAEL cannot be set properly in this assay with respect to findings on male reproductive organs. At 58.8 mg/kg bw/day the MTD was reached for males.

- **Mouse**
  - In the 90 days dietary study **(ID:5)**, neither weight of epididymis/prostate/seminal vesicles with coagulating glands was recorded nor histopathological examination of these organs/tissues was performed. At 256.6 mg/kg bw/day the MTD seems to be reached for males. Neither changes in weight of testes nor macroscopic/histopathological damage/changes in testes were observed in this study at the highest dose tested (256.6 mg/kg bw/day). However, in additional recovery group (equal to 266.7 mg/kg bw/day) histopathological examination showed decreased spermatogenesis in testes in one male (ten animals were investigated).
  - In the 18 months study **(ID:13)**, absolute testes weight in highest dose (446 mg/kg bw/day) group was statistically significant reduced (19.8%) and macroscopic examination showed increased incidences of small and "soft" testes at this dose. Histopathological examination of testes revealed statistical significantly increased incidence of tubular atrophy of testes at the top dose (446 mg/kg bw/day). Furthermore, from 42.0 mg/kg bw/day tubular dilatation, aggregate lymphoid and sperm cysts/cystic dilation of epididymis were statistically significantly increased in a dose-dependent manner. From 216 mg/kg bw/day statistically significantly increased unilateral and bilateral oligospermia and sperm granuloma in epididymis were observed. All these changes are considered test substance related and adverse. At 216 mg/kg bw/day the MTD was reached for males.

- **Dog**
  - A statistically significant decrease of relative (24.1%) and absolute (48.4%) epididymis weight of animals in the high dose (10.56 mg/kg bw/day) group were noted in the 90 days study **(ID:6)**, however, no histopathological findings were reported in all groups tested. Non-statistically significant decrease of relative (9.8%) and absolute (35.7%) testes weight of animals in the high dose group was also observed and at the macroscopic examination "small testes" of one high dose male was reported. Histopathology showed aspermatogenesis in the testes of 2 animals of the high dose group. For Male 1 moderate (grade 3) aspermatogenesis bilateral, no evidence of development beyond primary spermatocyte were reported; for Male 2 mild (grade 2)

aspermatogenesis, minimal spermatid formation were reported (at the scheduled necropsy "small testes" were described too). Findings reported in male reproductive organs at 10.56 mg/kg bw/day were observed in the presence of general toxicity (the MTD).

- A statistically significant dose-dependent decrease of absolute testes weight in the mid (9.7 mg/kg bw/day) and high (14.2 mg/kg bw/day) dose group (30.3% and 38.1%, respectively) was reported in the 90 days study **(ID:7)**. No histopathological changes were observed in these groups. Histopathological examination revealed some changes in prostate: prostate hypoplasia (0/4, 1/4, 1/4 and 2/4) was reported in the 0, 4.9, 9.7 and 14.2 mg/kg bw/day dose groups, respectively. No histopathological findings were reported in epididymis in the control and the highest dose groups tested. At 9.7 mg/kg bw/day the MTD might have been reached for males.
- Considering the top dose selection, the amount of compound administered in the 1 year study **(ID:8)** was not high enough to elicit evidence of toxicity with respect to findings on male reproductive organs weights. A non-statistically significant and non-dose-dependent decrease of absolute and relative testes weight in the high dose (5.7 mg/kg bw/day) group (10.1% and 19.2%, respectively) was reported; however, no histopathological changes testes were observed in this group. Histopathological examination revealed prostate benign adenoma (0/4, 0/4, 0/4 and 1/4) and mild periarteritis in epididymis (0/4, 0/4, 0/4 and 1/4) in this group. Effects on testes and epididymis found in a 90 days study on dogs at 10.56 mg/kg bw/day (ID:6) could not be confirmed in this 1 year dog study due to low amount administered: the highest dose administered was much lower than the "effect dose" in the 90 days study (10.56 mg/kg bw/day). The MTD has not been reached up to 5.7 mg/kg bw/day (highest dose tested) for males.
- In the second 1 year dog study **(ID:9)**, the amount of compound administered was not high enough to elicit clear evidence of toxicity. The MTD have not been clear reached for males at the highest dose. From week 5 until study termination, body weight of males at highest dose (5.6 mg/kg bw/day) was approximately less 10-14% than the control; however, these values did not attain statistical significance. No test article related statistically significant effects on testes/epididymis weights (relative and absolute) were apparent at any concentration. With respect to pathological findings on male reproductive organs, macroscopic examination exhibited a reduced size of testis and/or flaccid testis in one the same animal of the high dose group as well as reduced size of epididymis in the same male and thickened epididymis in another animal in the high dose group. Regarding histopathological changes, there was an apparent trend in the incidence of atrophy of testes, with two (out of four) and three males (out of four) in 2.8 and 5.6 mg/kg bw/day dose groups, respectively, showing from minimal to severe grade in the absence of such findings in control animals. The histological findings in the epididymis were bilateral seminiferous cell debris (minimal) and unilateral atrophy (moderate) with aspermia of one animal of the high dose group each. Based on some scientific data (*Michael J. Goedken et al., 2008*) atrophy/hypoplasia of seminiferous tubules is decreasing with age to 14%-17% in dogs twelve to thirty-six months old and dogs less than eight months of age have lower testicular weights, incomplete filling of epididymal tails with sperm and abnormal epididymal content. Furthermore, atrophy/ hypoplasia was found in 7.4% of dogs and findings in epididymis consisted mainly of reduced spermatids (5.6%), based on the RMS selected 54 dogs over one year of age from background control data provided by the Applicant. It means that changes in testes/epididymis of this study are outside the range of background pathology in Beagle dogs of this age. Based on the study results and some scientific data, the RMS considers that a treatment related effect of cymoxanil to testes at two high doses could not be excluded as well as effect to epididymis at the highest dose. The NOAEL was proposed at 1.3 mg/kg bw/day based on histological findings in the testes (minimal/slight bilateral atrophy) at 2.8 mg/kg bw/day with an apparent trend in the incidence and severity.

- In two studies only, effects on **histopathology of testis** were observed in the absent of general systemic toxicity (below the MTD), i.e. in rat 90 days rat study (ID:3) and in 1 year dog study (ID:9).
- Effects on **epididymis histopathology** in rats were observed at MTD (90 day ID:3, 1 year interim sacrifice ID:11 and combined chronic toxicity/carcinogenicity ID:12 studies). Effects on epididymis

histopathology in dogs were observed most likely below the MTD (1 year ID:9 study), however, some uncertainty remains. Effects on epididymis histopathology in mice were observed below the MTD (carcinogenicity ID:13 study), however, concomitant effects on histopathological findings in liver (apoptosis, pigment, granuloma, diffuse centrilobular hypertrophy) were observed at the same level.

- Not consistent effect (decrease/increase) on **relative/absolute testis weight** was identified: absolute (and in some cases relative) testis weight decreased in rat, dog and mouse at or above the MTD (ID:6, ID:7, ID:8, ID:13 and ID:16), while relative testis weight increased below or at the MTD in rat only (ID:1, ID:3, ID:11 and ID:16). Overall, there is limited evidence of testis weight in rat, mouse and dog.
- Not consistent effect (decrease/increase) on **epididymis relative weight** in rat and dog was observed in two short-term rat studies (ID:1 and ID:6).

Table 5-1: Summary table of histopathologic changes in the testes and epididymides

Study/Species Dose levels	Observed effects, concomitant effects (in males) NOAEL/LOAEL (for sexual function and fertility, parents)
ID:3 90 days rat oral 0, 100, 750, 1500, 3000 ppm equal to 0, 6.54, 47.6, 102, 224 mg/kg bw /day	NOAEL = 6.54 (♂) LOAEL = 47.6 (♂) based on histopathological findings in **testes** (bilateral elongate spermatid degeneration) and ↑ relative testes weight (10%) **MTD at 224 mg/kg bw /day** **Epididymis:** cell debris and hypospermia at 224 mg/kg bw /day
ID:4 90 days rat oral 0, 500, 1000, 2000 ppm equal to 0, 42.6, 85.1, 174.3 mg/kg bw /day *Additional recovery subgroups* (control and high dose), 28 days: equal to 0, 173.5 mg/kg bw /day	NOAEL = 85.1 (♂) LOAEL = 174.3 (♂) based on ↓ body weight (11.3%), ↓ body weight gain (14.6%), ↓ food consumption (15.6%), ↑ creatinine (34%), ↑ total bilirubin (85.8%), ↑ relative kidney weight (17.3%), ↑ relative liver weight (15.7%) **MTD at 174.3 mg/kg bw /day** Recovery: **Testes:** atrophy of seminiferous tubules (severe and diffuse)/calcification (mild and multifocal) in one male from 10 at 173.5 mg/kg bw/ day (testes tissues of other 9 males were not examined) **Epididymis:** no histopathological examination
ID:5 90 days mice oral 0, 150, 450, 1350 ppm equal to 0, 28.7, 84.4, 256.6 mg/kg bw / day *Additional recovery subgroups* (control and high dose), 28 days: equal to 0, 266.7 mg/kg bw	NOAEL = 84.4 (♂) LOAEL = 256.6 (♂) based on ↓ body weight gain (21.4%), ↑ total bilirubin (114.8%) **MTD at 256.6 mg/kg bw /day** Recovery: **Testes:** decreased spermatogenesis in one male from ten at 266.7 mg/kg bw/ day **Epididymis:** no histopathological examination
ID: 6 90 days dog oral 0, 100, 200, 250/500 ppm equal to 0, 3.13, 5.13, 10.56 mg/kg bw /day	NOAEL = 3.13 (♂) LOAEL = 5.13 (♂) based on clinical signs (↓ defecation), alterations of haematological parameters [↓ RBC (15.9%**), ↓ Hb (16.7%**), ↓ Ht (14.9%)] **MTD at 10.56 mg/kg bw /day** **Testes:** "small testes" of one male and aspermatogenesis in the testes (2 animals) at 10.56 mg/kg bw/ day **Epididymis:** decrease of relative (24.1%) and absolute (48.4%) weight at

	10.56 mg/kg bw/ day, however, no histopathological findings were reported in all groups tested.
ID:7 90 days dog oral 0, 200, 400, 800 ppm equal to 0, 4.9, 9.7 and 14.2 mg/kg bw /day	NOAEL = 4.9 (♂) LOAEL = 9.7 (♂) based on clinical signs ('weakness'), loss body weight gain, ↓ food consumption (g/animal/day) during 5 weeks (23.2 – 46.7%*); ↓ absolute (>55%) and relative (>45%) thymus weight; histological alterations in thymus (lymphoid atrophy) with increasing severity **MTD might have been reached at 9.7 mg/kg bw/day** **Testes:** 30.3% - 38.1% decrease of absolute testes weight from 9.7 mg/kg bw/day; however, no histopathological changes were observed in these groups. **Epididymis:** No histopathological findings were reported in the control and the highest dose groups tested **Prostate:** prostate hypoplasia (1/4, 1/4 and 2/4) was reported at 4.9 mg/kg bw/ day, 9.7 mg/kg bw /day and 14.2 mg/kg bw /day, respectively
ID:8 1 year dog oral 0, 50, 100, 200 ppm equal to 0, 1.8, 3.0, 5.7 mg/kg bw /day	NOAEL = 3.0 (♂) LOAEL = 5.7 (♂) based on alterations of haematological parameters [↑MCV (4.2%**) at termination, ↓ RBC (18.3%*/10.2%*) at week 12/25, ↓ Hb (11.0%*/18.8%**/10.8%*) at week 2/12/25]; alterations of clinical chemistry [↓potassium (13.7%**); bilateral cataract **MTD has not been reached up to 5.7 mg/kg bw/day** **Testes:** non-statistically significant and non-dose-dependent decrease of absolute and relative testes weight at 5.7 mg/kg bw/day; no histopathological changes in testes **Epididymis:** mild periarteritis (1 animal) at 5.7 mg/kg bw/day **Prostate:** benign adenoma (1 animal) at 5.7 mg/kg bw/day *Not high enough dose levels were selected*
ID:9 1 year dog oral 0, 50, 100, 200 ppm equal to 0, 1.3, 2.8, 5.6 mg/kg bw /day	NOAEL = 1.3 (♂) LOAEL = 2.8 (♂) based on histological changes in **testes** (minimal/slight bilateral atrophy) with an apparent trend in the incidence and severity **MTD has not been clear reached at 5.6 mg/kg bw /day** **Epididymis:** bilateral seminiferous cell debris (minimal) and unilateral atrophy (moderate) with aspermia at 5.6 mg/kg bw/day *Not high enough dose levels were selected* *Histological examination of epididymis was not specified*
ID:11 Combined chronic toxicity /carcinogenicity rat study 0, 50, 100, 700, 2000 ppm equal to 0, 1.98, 4.08, 30.3, 90.1 mg/kg bw/day	Long-term NOAEL = 4.08 (♂) Long-term LOAEL= 30.3 (♂), based on clinical findings (↑hyperreactivity), ↓ body weight (15.3%), ↓ body weight gain (21.8%), histopathological findings (elongate spermatid degeneration, retinal atrophy) **MTD at 30.3 mg/kg bw/day** **Testes:** elongate spermatid degeneration in testes at 30.3 mg/kg bw/day; increase in multinucleate spermatids at 90.1 mg/kg bw/day **Epididymis:** non-statistically significant increase in bilateral oligospermia at 90.1 mg/kg bw/day
ID:11 Interim sacrifice (12 months, 357 days):	**MTD at 32.8 mg/kg bw/day**

10/sex/dose 0, 50, 100, 700, 2000 ppm equal to 0, 2.25, 4.58, 32.8, 97.4 mg/kg bw/day	**Testes:** elongate spermatid degeneration at 32.8 mg/kg bw/day **Epididymis:** increase in multinucleate spermatids at 97.4 mg/kg bw/day
ID:12 Combined chronic toxicity /carcinogenicity rat study 0, 100, 500, 1200 ppm equal to 0, 4.7, 23.5, 58.8 mg/kg bw/day Interim sacrifice (12 months) 0, 1200 ppm equal to 0, 67.6 mg/kg bw/day	Long-term NOAEL = 4.7 (♂) Long-term LOAEL= 23.5 (♂), based on histopathological findings (lymphoid hyperplasia in rectum)* * - *Due to deviations of the study the NOAEL (♂) cannot be set properly with respect to findings on male reproductive organs.* **MTD at 58.8 mg/kg bw/day** **Testes:** seminiferous tubule atrophy at 58.8 mg/kg bw/day **Epididymis:** increased combined incidence of epididymal oligospermia with aspermia at top dose (control 3/50 and 11/50 at 58.8 mg/kg bw/day); however, no statistical analysis has been performed
ID:13 Carcinogenicity mouse study 0, 30, 300, 1500, 3000 ppm equal to 0, 4.19, 42.0, 216, 446 mg/kg bw/day	Long-term NOAEL = 4.19 (♂) Long-term LOAEL= 42.0 (♂), based on histopathological findings in liver (apoptosis, pigment, granuloma, diffuse centrilobular hypertrophy) and epididymis (tubular dilatation, aggregate lymphoid and sperm cysts) **MTD at 216 mg/kg bw/day** **Testes:** tubular atrophy at 446 mg/kg bw/day **Epididymis:** tubular dilatation, aggregate lymphoid and sperm cysts at 42.0 mg/kg bw/day; oligospermia and sperm granuloma in epididymis from 216 mg/kg bw/day

- No clear toxicologically significant changes were reported in the repeated exposure studies in the other EAS mediated target organs, i.e. in weights and histopathology of the uterus, ovaries, prostate, seminal vesicles (+ coagulating glands):
  - Reduced absolute weight of ovaries and limited histopathologic changes in the **ovaries** (ovaries follicular cysts) were observed in the mice below the MTD in repeated dose 28-day and carcinogenicity studies (ID:2 and ID:14), respectively.
  - Limited evidence of absolute and relative weight of **uterus** was observed in dog in 90-day study (ID:7).
  - Limited evidence of **prostate** histopathology (prostate hypoplasia) in the dogs at the MTD in the repeated dose 90-day study (ID:7) was observed. In addition, in 1-year dog study (ID:8) histopathological examination revealed prostate benign adenoma (0/4, 0/4, 0/4 and 1/4) at the 5.7 mg/kg bw/day dose only (below the MTD).
  - There were no changes in **seminal vesicles** histopathology.
- In addition, in the rat developmental neurotoxicity study (ID:24) **sexual maturation** (e.g. age at vaginal opening and preputial separation) was explored (a sensitive indicator of androgenic/antiandrogenic effects), but no changes were seen. Doses there were up to 100 mg/kg bw/day, which encompassed the effect level of 48 mg/kg bw/d seen in rats (ID:3).
- In the two 2-generation reproductive studies (ID:15 and ID:16), while specific sexual maturation endpoints were not examined, there was no evidence of androgenic/antiandrogenic changes such as effects on genital development of offspring. No such evidence was seen also in the prenatal developmental toxicity studies in rats and rabbits (ID:18-ID:23).

- Additionally, minor effects of cymoxanil on **fertility parameters** were reported in the F0 and F1 generation parents.
    - In one out of the 2-generation studies **(ID:15)** minor effects on fertility parameters were reported in the F1 generation parents, there were a statistically significant decrease in mean litter size, reduction in the percentage of live pups born together with a reduced mean number of corpora lutea and reduced mean number of implantations in the high dose only (116.3 mg/kg bw/day for females). It should be noted that at the high dose female parents of the F1 slight maternal effects were observed: reduced initial body weight that persisted about 10% for the remainder of the study; reduced body weight gain by 20% during gestation and reduced food consumption by 8-26% during all phases.
    - Based on the results obtained in the second 2-generation study **(ID:16)**, the reproductive parameters investigated did not indicate a possible reproductive influence caused by the test substance up to 97.9 mg/kg bw/day.
    - In the one generation study **(ID:17)** minor effects on fertility parameters were reported: the statistically significantly reduced the female fertility index (%), reduced numbers of corpora lutea, increased pre-implantation, reduced mean number of implantations as well as reduced mean litter size at 240.8 mg/kg bw/day. The effects reported can be related to maternal toxicity. The body weight gain of females was statistically significantly reduced by 12% and 43% (respectively) during gestation (0 – 20 days) at the mid (136.1 mg/kg bw/day) and high doses levels ; food consumption was statistically significantly reduced (>10%) during all gestation and all lactation period from the mid dose. Food consumption was statistically significantly reduced (12.9 – 28.4%) at top dose in parental females throughout all premating period. Furthermore, at necropsy 5 parent males in the 226.2 mg/kg bw/day group had bilateral small and flaccid testes, rendering them infertile. However, microscopic examination of testes was not performed.
- It should be noted that a harmonised classification and labelling for cymoxanil for fertility as Repr.2, H361f is based on the adverse effects on testes and epididymis in the repeated dose toxicity studies, especially in rats.

- Additionally, the target of orally administered cymoxanil was **thymus**. A dose-dependent increase in male and female thymus atrophy from 9.7 mg/kg bw/day was reported in a 90 day study in dogs (ID:7). Male thymus atrophy was repeated at 5.6 mg/kg bw/day in a 1 year dog study (ID:9). Some findings indicate that changes in thymus might be a substance-specific effect but not a result of generalised high-dose stress response.
- Additional target of orally administered cymoxanil was **eyes**. Effects on eyes were observed in two 1 year dog studies (ID:8 and ID:9): slight bilateral cataract in one male from five at 5.7 mg/kg bw/day dose and bilateral lenticular degeneration of a slight degree was recorded in a single male from four at 5.6 mg/kg bw/day dose, respectively. In addition, slight unilateral cataract in one female (from five) was recorded at 3.1 mg/kg bw/day dose level in one 1 year dog study (ID:8). Given small amount of dogs, young age of animals and similar dose level in two studies, it is considered that three incidents of eye damage were not incidental and might represent an effect of substance treatment. Additionally, at from 30.3 mg/kg bw/day and above, both males and females showed statistically significant retinal atrophy in a two years study in rats (ID:11). It is noteworthy that the 1-year interim sacrifice revealed statistically significant increases in the incidence of retinal atrophy in males from the 32.8 mg/kg bw/day.
- Additional target of orally administered cymoxanil was **blood** (anaemia). In the first 90 days study in dogs (ID:6) statistically significant reductions in haemoglobin in males (24.4%) at 10.56 mg/kg bw/day and females (22.2%) at 10.51 mg/kg bw/day were reported. A similar but weaker effect was repeated in a second 90 days study in dogs (ID:7): a dose-related and statistically significant reduction (13.9%) in haemoglobin in female was reported at 15.5 mg/kg bw/day. In addition, at highest dose (5.7 mg/kg bw/day) a statistically significant reduction in haemoglobin in male was reported at week 2, 12 and 25

(11.0%, 18.8% and 10.8%, respectively) in 1 year dog dietary study (ID:8).

- It should be noted that the effects on haematology and the thymus atrophy reported are in accordance with a harmonised classification of cymoxanil in **STOT-RE 2 H373 (blood, thymus)**. It is considered by the RMS that effects reported on blood parameters, the thymus and eyes in dogs following 90 days and 1 year exposure to cymoxanil are relevant for a classification for STOT RE 2 H373 (blood, thymus, eyes).

- Effect on foetal development producing also **malformations** (endpoint potentially sensitive to, but not diagnostic of, EATS modalities) in rats (hemi vertebra, fused ribs and exencephalic head in two developmental toxicity studies, ID:18 and ID:19) and rabbits (hydrocephaly and cleft palates, dilation of heart ventricles, vertebra and/or rib alterations linked with scoliosis in three out of four developmental toxicity studies, ID:21, ID:22 and ID:23) was observed. It should be noted that a harmonised classification and labelling for cymoxanil for adverse effects on development is Repr. 2, H361d, based on marked effects reported in five developmental toxicity studies in rats and rabbits as well as an increased percentage of post-implantation loss in rats in a 2-generation (ID:15) and two developmental toxicity studies (ID:18 and ID:19).

Table 6: WoE for EAS-mediated endocrine activity

- There are no data on hormone measurements (e.g. oestradiol, testosterone, luteinising hormone (LH) and follicle stimulating hormone (FSH)) available for EAS-mediated modalities.

- **E-modality:**
- Cymoxanil was tested in 17 out of 18 relevant Estrogen related assays (as is indicated in the guidance) in the ToxCast database. Except for a single ER assay (ATG_ERE_CIS_up, ID:41), cymoxanil was inactive in all. Inspection of the activity curves for the positive assay revealed that only a single point at the highest concentration was above the cut-off value for a positive result. All other values were negative. This implied that the AC_{50} value (concentration at which 50% of the activity occurred) derived by this assay (46.7) was artefactual and the correct interpretation was a negative result overall.
- In accordance with the testing strategy to evaluate the endocrine activity in the guidance, it can be concluded that E-mediated activity is sufficiently investigated with the predictions of the ToxCast ER Bioactivity Model.

- **A-modality:**
- Cymoxanil was tested in 9 out of 11 relevant Androgen related assays (as is indicated in the guidance) in the ToxCast database. Cymoxanil was inactive in all. Two Androgen related assays (NVS_NR_cAR and NVS_NR_rAR) were not available.
- It should be noted that in line with the Guidance the ToxCast Androgen related assays do not replace the AR STTA assay (OECD TG 458) for the A-modality.

- **S-modality:**
- Cymoxanil was tested in 13 out of 14 relevant Steroidogenesis related assays (as is indicated in the guidance) in the ToxCast database. The effects of cymoxanil on steroidogenesis (activation or induction) in all assays were negative including is the result of the aromatase inhibition assay. One Steroidogenesis related assay (NVS_ADME_hCYP19A1) was not available.
- It is noteworthy that in line with the Guidance the ToxCast Steroidogenesis related assays do not replace the H295R Steroidogenesis Assay (OECD TG 456) and OPPTS 890.1200 (Aromatase assay) for the S modality.

Several changes that can be potentially considered as a result of an endocrine mode of action were observed in the rat 90 day study and 1 year dog study, involving the testis as a target organ. Indeed, when considering these findings in a whole, a relationship to an AS mediated mode of action cannot be excluded. However, when adding to the weight of evidence the information a convincing pattern of adversity, indicative of endocrine disruption, cannot be drawn.

- All the available *in vitro* studies were negative for the EAS mediated endocrine activity. However, the overall dataset for evaluating A- and/or S-mediated adversity currently has data gaps and uncertainties. Required male mammary gland histopathology, weight examination and the optional evaluation of circulating levels of EAS-relevant hormones are missing in the available mammalian studies. Furthermore, effects on sexual development and maturation were not completely investigated in the available 2-generation rat reproduction studies.
- In repeated dose toxicity studies in rats, mice and dogs, studies were also reported that induced minor or no effects on male reproductive organs. However, it is noteworthy that in some studies neither weight of epididymis/prostate/seminal vesicles with coagulating glands was recorded nor histopathological examination of these organs/tissues was performed (e.g. ID:4;ID:5); whereas in one study histological examination of epididymis was not specified (ID:9). It is noteworthy that some studies had several methodological limitations: despite the fact that male reproductive organs (testes and epididymis) were target tissues of cymoxanil and these tissues showed treatment-related changes in the high dose group, no histopathological examination was performed from all animals in all dose groups and no statistical analysis has been performed (e.g. ID:12). Additionally, regarding the dose level spacing selected, in some studies wide intervals were used, instead of recommended shorter intervals for setting the descending dose levels (e.g. ID:11*;* ID:12; ID:13). Furthermore, considering the top dose selection, not high enough dose levels were selected in some studies (e.g. ID:8; ID:9; ID:14). For example, effects on testes/epididymis found in studies on dogs (at 10.56 mg/kg bw/ day, ID:6) could not be confirmed in other dog study due to low amount of substance administered (highest dose applied was 5.7 mg/kg bw/ day, ID:8). The difference in the results in the rat and mouse studies could also have been due to difference in the rat (Crl:CDBR and HsdCpb:WU) or mouse (Crl:CD-1®BR and HsdOla:MF 1) strains used in the various studies.
- The changes in the testes and epididymides were not accompanied by toxicologically significant effects on other androgen- and steroidogenesis-related organs such as the seminal vesicles, prostate, uterus and ovaries. The effects in the testes and epididymides appear to be specific, indicating that they likely are not a result of disturbance of the androgen hormonal system.
- In addition, in the rat developmental neurotoxicity study (ID:24) sexual maturation was explored (a sensitive indicator of androgenic/antiandrogenic effects), but no changes were seen. Doses there were up to 100 mg/kg bw/day, which encompassed the effect level of 48 mg/kg bw/d seen in rats (ID:3).
- In the two 2-generation reproductive studies (ID:15 and ID:16), while specific sexual maturation endpoints were not examined, there was no evidence of androgenic/antiandrogenic changes such as effects on genital development of offspring. No such evidence was seen also in the prenatal developmental toxicity studies in rats and rabbits (ID:18-ID:23).
- It is noteworthy that according to some scientific data (*O'Donnell, Mechanisms of spermiogenesis and spermiation and how they are disturbed, 2015; Spermatogenesis. 2015 Jan 26;4(2):e979623.)* degeneration of spermatid elongation is generally seen as a result of direct toxicological action rather than of endocrine activity.
- Testicular toxicity is of interest for human health risk assessment especially in terms of reproductive and developmental toxicity, however, the testicular toxicity has not fully elucidated. The AOP 212 [Histone deacetylase inhibition (HDI) leading to testicular atrophy] could be mentioned as non endocrine activity case. The HDIs inhibit deacetylation of the histone, leading to the increase in histone acetylation. The apoptosis induced by disrupted cell cycle leads to spermatocyte depletion and testis atrophy.
- There is the uncertainty regarding the human health relevance of these effects on male reproductive organs. With respect to human relevance, it is considered that the mammalian toxicology data available are insufficient to address the potential for the findings on testes/epididymis being mediated by endocrine activity of cymoxanil.

2.2.3. Initial analysis of the evidence and identification of relevant scenario for the ED assessment of EAS-modalities

Table 7: Selection of relevant scenario

Adversity based on EAS-mediated parameters	**Positive mechanistic OECD CF level 2/3 Test**	**Scenario**	**Next step of the assessment**	**Scenario selected** *(indicate with an "x" the scenario selected based on the assessed lines of evidence)*
No (sufficiently investigated)	Yes/No	1a	Conclude: ED criteria not met because there is not "**EAS-mediated**" adversity	
Yes (sufficiently investigated)	Yes/No	1b	Perform MoA analysis	
No (not sufficiently investigated)	Yes	2a (i)	Perform MoA analysis (additional information may be needed for the analysis)	
No (not sufficiently investigated)	No (sufficiently investigated)	2a (ii)	Conclude: ED criteria not met because no **EAS-mediated endocrine activity** observed	
No (not sufficiently investigated)	No (not sufficiently investigated)	2a (iii)	Generate missing level 2 and 3 information. Alternatively, generate missing "EATS-mediated" parameters. Depending on the outcome move to corresponding scenario	X
Yes (not sufficiently investigated)	Yes/No	2b	Perform MoA analysis	

2.2.4. Conclusion of the assessment of EAS-modalities

The E-modality is considered to be sufficiently investigated. No evidence of oestrogen-mediated activity was reported based on the negative ToxCast ER bioactivity Model. Therefore, in accordance with the ECHA/EFSA Guidance on identifying ED, cymoxanil does not meet the ED criteria for the E-modality.

Based on scenario 2a (iii), the endocrine activity was not sufficiently investigated for the A- and S-modalities:

- There is no AR STTA assay (OECD TG 458) for the A-modality
- There is no H295R Steroidogenesis Assay (OECD TG 456) for the S modality

In addition to these missing assays, there were no data on circulating levels of EAS-relevant hormone measurements, i.e. testosterone, oestradiol, luteinising hormone (LH) and follicle stimulating hormone (FSH), available for the evaluation.

For assessment of A-mediated activity, only data from ToxCast was available, and even though the data seem to indicate no androgenic activity, this alone is not sufficient to conclude on the androgenic activity. Similarly, only data from ToxCast was available for the assessment of S-mediated activity, which is not sufficient for the investigation.

The overall dataset for evaluating A- and/or S-mediated adversity currently has data gaps and uncertainties. Required male mammary gland histopathology, weight examination and the optional evaluation of circulating levels of EAS-relevant hormones are missing in the available mammalian studies. Furthermore, effects on sexual development and maturation were not completely investigated in the available 2-generation rat reproduction studies. There were numerous testicular effects observed in the rat, mouse and dog in 90 day, 1 year, 2-year chronic toxicity and carcinogenicity studies, however, only in two studies effects on histopathology of testis were observed in the absent of general systemic toxicity (below the MTD), i.e. in rat 90 days rat study and in 1 year dog study. In addition, it cannot be concluded whether the observed effects on epididymis are attributable to

general systemic toxicity: only in one study, effects on histopathology of epididymis were observed in the absent of general systemic toxicity (below the MTD), i.e. in carcinogenicity mouse study; however, concomitant effects on histopathological findings in liver (apoptosis etc) were observed at the same level. Therefore, a convincing pattern of adversity, indicative of endocrine disruption, cannot be drawn.

Therefore, according to the guidance, additional information should be generated (Scenario 2a(iii)). Level 2 studies are required for A- and S-modality:

- OECD TG 458 (AR STTA assays),
- OECD TG 456 (H295R Steroidogenesis Assay)
- OPPTS 890.1200 (Aromatase assay)
- A study in line with OECD TG 441 (Hershberger Assay) in case OECD TG 456 and OECD TG 458 and OPPTS 890.1200 are negative.
- If the above studies are negative, the scenario 2a(ii) applies and ED criteria are not met.
- If endocrine activity is observed, the scenario 2a(i) applies and further data will be needed to support the MoA analysis i.e. extended one-generation study with inclusion of the cohort 1a and 1b including the mating of cohort 1b to produce the F2 generation (OECD TG 443, Level 5) or an OECD TG 416.

2.3. Overall conclusion on the ED assessment for humans

In conclusion, based on the available evidence, the T-modality is considered sufficiently investigated and a pattern of adversity was not observed. Therefore, the substance does not meet the ED criteria for the T modality.

Based on the available data, the E-modality is considered sufficiently investigated and no adversity has been observed. Therefore, the substance does not meet the ED criteria for the E modality.

For the A- and S-modalities, endocrine activity has not been sufficiently investigated, there are uncertainties regarding effects on testes/epididymis and the overall dataset for evaluating A- and/or S-mediated adversity currently has data gaps and uncertainties. A convincing pattern of adversity, indicative of endocrine disruption, cannot be drawn. Therefore, further data should be generated before a conclusion on whether the ED criteria are met for the A- and/or S-modalities can be drawn.

3. ED assessment for non-target organisms

According to the ECHA/EFSA guidance document (2018), since cymoxanil does not meet the criteria for mammals for the T-modality (see section 2.1.4), further consideration on the potential ED properties of cymoxanil on non-target organisms other than mammals is required for this modality.

In the case of the E modality, based on the available data, the E modality was considered sufficiently investigated and no adversity has been observed. Therefore, cymoxanil does not meet the ED criteria for mammals for the E modality. In case of the A and S modalities, the dataset for mammals was considered not sufficient to address the adversity and endocrine activity of cymoxanil. Therefore, for E, A and S modalities, further consideration on the potential ED properties on non-target organisms other than mammals is required.

3.1. ED assessment for T-modality

For assessing the ED properties through the T-modality for non-target organisms other than mammals, only in vitro data retrieved from ToxCast were available. For mammals as non-target organisms, see section 2.1.

3.1.1. **Lines of evidence for adverse effects and endocrine activity related to T-modality**

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
57	In vitro mechanistic	Thyroid receptor	Human	24	Hours		1000	µM	No effect	ToxCast assay: No TR-mediated activity	No TR mediated activity (in vitro)	Supportive evidence, No TR mediated activity (in vitro)	T
58		Thyroid receptor	rat	28			83,8	µM					
59		Thyroid receptor	rat	28			1000	µM					

3.1.1.1. Assessment of the integrated lines of evidence and weight

Four studies OECD TG 210 ELS study on Rainbow trout, Sheepshead minnow and *Brachydanio rerio* are available.

According to OECD 150, ELS study could be considered in the assessment for T-modality when parameters like swim- bladder inflation and metamorphosis are investigated. However, no T-modality relevant parameters like swim-bladder inflation and metamorphosis were measured and reported.

According to EFSA/ECHA ED guidance, T modality is not investigated for NTO other than mammals, only in vitro assays (TOX CAST) are available, which is considered as supportive only. No conclusion can be drawn for T modality.

3.1.2. Initial analysis of the evidence and identification of the relevant scenario

Table 8: Selection of relevant scenario

Adversity based on T-mediated parameters	**Positive mechanistic OECD CF level 2/3 Test**	**Scenario**	**Next step of the assessment**	**Scenario selected** *(indicate with an "x" the scenario selected based on the assessed lines of evidence)*
No (sufficiently investigated)	Yes/No	1a	Conclude: ED criteria not met because there is not "**T-mediated**" adversity	
Yes (sufficiently investigated)	Yes/No	1b	Perform MoA analysis	
No (not sufficiently investigated)	Yes	2a (i)	Perform MoA analysis (additional information may be needed for the analysis)	
No (not sufficiently investigated)	No (sufficiently investigated)	2a (ii)	Conclude: ED criteria not met because no **T-mediated endocrine activity** observed	
No (not sufficiently investigated)	No (not sufficiently investigated)	2a (iii)	Generate missing level 2 and 3 information. Alternatively, generate missing "EATS-mediated" parameters. Depending on the outcome move to corresponding scenario	**X**
Yes (not sufficiently investigated)	Yes/No	2b	Perform MoA analysis	

3.1.3. Conclusion on the ED assessment for T-modality

The conclusion drawn in section 2.1.4 that, for the T-modality, cymoxanil does not meet the ED criteria for T-modality for humans also applies to wild mammals as non-target organisms.

Regarding non-target organisms other than mammals, the available evidence is not sufficient to conclude either on T-mediated endocrine activity or on the T-mediated adversity.

Based on scenario 2a (iii), the endocrine activity/endocrine adversity was not sufficiently investigated for the T-modality.

Therefore, according to the guidance, additional information should be generated (Scenario 2a(iii)). A level 3 study according to OECD TG 231 is required. Two cases are possible:

1. If the above study is negative, the scenario 1a applies and ED criteria are not met.
2. If positive, the scenario 2a(i) applies and further data will be needed to support the MoA analysis, i.e. OECD TG 241 (Level 4).

3.2. ED assessment for EAS-modality

For assessing the ED properties through the EAS-modalities, in this case, a level 4 four studies according to OECD TG 210 (ELS) on fish were available. In addition, two reproductive toxicity studies on birds (OECD TG 206) were available. However, only “sensitive to, but not diagnostic of EATS” parameters were investigated in the provided studies. Therefore no conclusion can be drawn on EAS-modalities.

3.2.1. **Lines of evidence for adverse effects and endocrine activity related to EAS-modalities**

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
31	In vitro mechanistic	Androgen receptor	Human	24	Hours		1000	µM	No effect	No AR-mediated activity.	No AR-mediated activity.	Overall, negative evidence for endocrine	EAS
32			Human	20			1000	µM	No effect				
33			Chinese Hamster	24			1000	µM	No effect				
34			Human	8			1000	µM	No effect				
35			Human	16			1000	µM	No effect				
36			Human	24			1000	µM	No effect				
37			Human	24			1000	µM	No effect				
38			Human	24			1000	µM	No effect				
39			Human	24			1000	µM	No effect				
74							0		No effect				
40			Human	80			1000	µM	No effect	.			
41			Human	24			46,7	µM	Change				
42			Human	24			1000	µM	No effect				
43			bovine	18			1000	µM	No effect				
44			Human	18			1000	µM	No effect				

45		Estrogen receptor	Human	8	Hours		1000	µM	No effect	No ER mediated activity	No ER mediated activity	activity.	
46			Human	24			1000	µM	No effect				
47			Human	8			1000	µM	No effect				
48			Human	24			1000	µM	No effect				
49			Human	8			1000	µM	No effect				
50			Human	24			1000	µM	No effect				
51			Human	2			1000	µM	No effect				
52			Human	8			1000	µM	No effect				
53			Human	24			1000	µM	No effect				
54			Human	24			1000	µM	No effect				
55			Human	48			1000	µM	No effect				
56			Human	48			1000	µM	No effect				
73							0		No effect				
60		Steroidogenesis (genes/enzyme changes) (in	Human	48			1000	µM	No effect	No SR mediated activity	No SR mediated activity		
61			Human	48			1000	µM	No effect				
62			Human	48			1000	µM	No effect				
63			Human	48			1000	µM	No effect				
64			Human	48			1000	µM	No effect				
65			Human	48			1000	µM	No effect				

66		vitro)	Human	48			1000	µM	No effect				
67			Human	48			1000	µM	No effect				
68			Human	48			1000	µM	No effect				
69			Human	48			1000	µM	No effect				
70			Human	48			1000	µM	No effect				
71			Human	48	Hours		1000	µM	No effect				
72			Human	24			1000	µM	No effect				
25			Bobwhite quail	21	week	Oral	1200	ppm	Decrease	decrease only observed at the highest tested concentrations	decrease only observed at the highest tested concentrations		
25		Body weight (bird)	Bobwhite quail	21	week	Oral	> 1200	ppm	No effect	no effects observed up to the highest tested concentrations	no effects observed up to the highest tested concentrations		
26			Mallard duck	21	week	Oral	600/450	ppm	Decrease	decrease only observed at the highest tested concentrations	decrease only observed at the highest tested concentrations		
25		Cracked eggs	Bobwhite quail	21	week	Oral	> 1200	ppm	No effect	no effects observed up to the highest tested concentrations	no effects observed up to the highest tested concentrations	Overall, the available evidence from the reproductive studies with birds and	
26			Mallard duck	21	week	Oral	>600/450	ppm	No effect	no effects observed up to the highest tested	no effects observed up to the highest tested		

										concentrations	concentrations	from the studies with early life stages of fish are only considered supportive for the lack of ED related adversity since those studies provide little information concerning potential ED-related effects.	
25	Sensitive to, but not diagnostic of, EATS	Egg production	Bobwhite quail	21	week	Oral	> 1200	ppm	No effect	no effects observed up to the highest tested concentrations	no effects observed up to the highest tested concentrations		
26			Mallard duck	21	week	Oral	300	ppm	Decrease	Decrease observed in a dose response manner	Decrease observed in a dose response manner		
25		Egg viability (% viable embryo of egg set)	Bobwhite quail	21	week	Oral	1200	ppm	Decrease	decrease only observed at the highest tested concentrations	decrease only observed at the highest tested concentrations		
26			Mallard duck	21	week	Oral	600/450	ppm	Decrease	decrease only observed at the highest tested concentrations	decrease only observed at the highest tested concentrations		
25		Eggshell thickness	Bobwhite quail	21	week	Oral	> 1200	ppm	No effect	no effects observed up to the highest tested concentrations	no effects observed up to the highest tested concentrations		
26			Mallard duck	21	week	Oral	>600/450	ppm	No effect	no effects observed up to the highest tested concentrations	no effects observed up to the highest tested concentrations		
25		Hatchability	Bobwhite quail	21	week	Oral	1200	ppm	Decrease	decrease only observed at the highest tested concentrations	decrease only observed at the highest tested concentrations		
26			Mallard duck	21	week	Oral	300	ppm	Decrease	Decrease observed in a dose response manner	Decrease observed in a dose response manner		
25		No of 14 day-	Bobwhite	21	week	Oral	1200	ppm	Decrease	decrease only observed at the	decrease only observed at the		

		old survivors	quail							highest tested concentrations	highest tested concentrations		
26			Mallard duck	21	week	Oral	300	ppm	Decrease	Decrease observed in a dose response manner	Decrease observed in a dose response manner		
25		Gross pathology (bird)	Bobwhite quail	21	week	Oral	> 1200	ppm	No effect	no effects observed up to the highest tested concentrations	no effects observed up to the highest tested concentrations		
26			Mallard duck	21	week	Oral	300	ppm	Increase	Slight increase of regressed ovaries in a dose response manner	Slight increase of regressed ovaries in a dose response manner		
26			Mallard duck	21	week	Oral	600/450	ppm	Increase	Increase of smaller testes at the highest tested concentrations	Increase of smaller testes at the highest tested concentrations		
27		Body weight (fish)	Rainbow trout	90	day	Uptake from water	0,031	mg/L water	Decrease	Decrease observed in a dose response manner	Decrease observed in a dose response manner		
28			Sheepshead minnow	36	day	Uptake from water	> 0,767	mg/L water	No effect	no effects observed up to the highest tested concentrations	no effects observed up to the highest tested concentrations		
29			Rainbow trout	97	day	Uptake from water	>0,12	mg/L water	No effect	no effects observed up to the highest tested concentrations	no effects observed up to the highest tested concentrations		
30			Zebra fish	34	day	Uptake from water	10	mg/L water	Increase	Increase only observed at the highest tested concentrations	Increase only observed at the highest tested concentrations		

27		Hatching success	Rainbow trout	90	day	Uptake from water	0,59	mg/L water	Decrease	Decrease observed in a dose response manner	Decrease observed in a dose response manner		
28			Sheepshead minnow	36	day	Uptake from water	> 0,767	mg/L water	No effect	no effects observed up to the highest tested concentrations	no effects observed up to the highest tested concentrations		
29			Rainbow trout	97	day	Uptake from water	>0,12	mg/L water	No effect	no effects observed up to the highest tested concentrations	no effects observed up to the highest tested concentrations		
30			Zebra fish	34	day	Uptake from water	10	mg/L water	Decrease	no effects observed up to the highest tested concentrations	no effects observed up to the highest tested concentrations		
27		Larval survival and length	Rainbow trout	90	day	Uptake from water	0,25	mg/L water	Decrease	Decrease observed in a dose response manner	Decrease observed in a dose response manner		
27			Rainbow trout	90	day	Uptake from water	0,031	mg/L water	Decrease	Decrease observed in a dose response manner	Decrease observed in a dose response manner		
28			Sheepshead minnow	36	day	Uptake from water	0,178	mg/L water	Decrease	Decrease observed in a dose response manner	Decrease observed in a dose response manner		
28			Sheepshead minnow	36	day	Uptake from water	> 0,767	mg/L water	No effect	no effects observed up to the highest tested concentrations	no effects observed up to the highest tested concentrations		
29			Rainbow trout	97	day	Uptake from water	>0,12	mg/L water	No effect	no effects observed up to the highest tested concentrations	no effects observed up to the highest tested concentrations		

30			Zebra fish	34	day	Uptake from water	3,2	mg/L water	Decrease	Decrease observed in a dose response manner	Decrease observed in a dose response manner		
29		Length (fish)	Rainbow trout	97	day	Uptake from water	>0,12	mg/L water	No effect	no effects observed up to the highest tested concentrations	no effects observed up to the highest tested concentrations		
30			Zebra fish	34	day	Uptake from water	>10	mg/L water	No effect	no effects observed up to the highest tested concentrations	no effects observed up to the highest tested concentrations		
25	Systemic toxicity	Mortality	Bobwhite quail	21	week	Oral	> 1200	ppm	No effect	no effects observed up to the highest tested concentrations	no effects observed up to the highest tested concentrations		
26			Mallard duck	21	week	Oral	600/450	ppm	Increase	increase only observed at the highest tested concentrations	increase only observed at the highest tested concentration		
29		Survival (fish)	Rainbow trout	97	day	Uptake from water	>0,12	mg/L water	No effect	no effects observed up to the highest tested concentrations	no effects observed up to the highest tested concentrations		

3.2.1.1. Assessment of the integrated lines of evidence and weight of evidence

The available evidence from the reproductive studies with birds and from the studies with early life stages of fish are only considered supportive for the lack of ED related adversity since those studies provide little information concerning potential ED-related effects.

The studies from ToxCast were available. However, in line with the ECHA/EFSA (2018) the dataset is considered insufficient for the assessment of the E, A and S modalities regarding endocrine activity for non-target organisms. The lines of evidence and their evaluation as reported in section 2.2.2 are also relevant for mammals as non-target organisms.

3.2.2. Initial analysis of the evidence and identification of the relevant scenario

Table 9: Selection of relevant scenario

Adversity based on T-mediated parameters	**Positive mechanistic OECD CF level 2/3 Test**	**Scenario**	**Next step of the assessment**	**Scenario selected** *(indicate with an "x" the scenario selected based on the assessed lines of evidence)*
No (sufficiently investigated)	Yes/No	1a	Conclude: ED criteria not met because there is not "**T-mediated**" adversity	
Yes (sufficiently investigated)	Yes/No	1b	Perform MoA analysis	
No (not sufficiently investigated)	Yes	2a (i)	Perform MoA analysis (additional information may be needed for the analysis)	
No (not sufficiently investigated)	No (sufficiently investigated)	2a (ii)	Conclude: ED criteria not met because no **T-mediated endocrine activity** observed	
No (not sufficiently investigated)	No (not sufficiently investigated)	2a (iii)	Generate missing level 2 and 3 information. Alternatively, generate missing "EATS-mediated" parameters. Depending on the outcome move to corresponding scenario	X
Yes (not sufficiently investigated)	Yes/No	2b	Perform MoA analysis	

3.2.3. Conclusion on the ED assessment for EAS-modality

The available mammalian dataset for the ED assessment for E modality is sufficiently investigated and no E-mediated activity of cymoxanil on humans was observed. However, AS-modalities did not allow to conclude on the ED properties of cymoxanil on humans and further data were requested (see section 2.2.3). This conclusion also applies to wild mammals.

The available dataset for non-target organisms other than mammals for cymoxanil was not complete since EAS-mediated parameters were not sufficiently investigated; only four studies according to OECD TG 210 (ELS) and two reproductive toxicity studies on birds (OECD TG 206) were available.

Based on scenario 2a (iii), the endocrine activity/endocrine adversity was not sufficiently investigated for the EAS-modalities.

Therefore, according to the guidance, additional information should be generated (Scenario 2a(iii)). A level 3 study according to OECD TG 229 is required. Two cases are possible:

1. If the above study is negative, the scenario 2a(ii) applies and ED criteria are not met.
2. If positive, the scenario 2a(i) applies and further data will be needed to support the MoA analysis, i.e. OECD TG 240 (Level 5).

4. Overall conclusion on the ED assessment

Based on the available evidence, the T-modality is considered sufficiently investigated and a pattern of adversity was not observed. Therefore, the substance does not meet the ED criteria for the T modality.

Based on the available data, the E-modality is considered sufficiently investigated and no adversity has been observed. Therefore, the substance does not meet the ED criteria for the E modality.

For the A- and S-modalities, endocrine activity has not been sufficiently investigated, there are uncertainties regarding effects on testes/epididymis and the overall dataset for evaluating A- and/or S-mediated adversity currently has data gaps and uncertainties. A convincing pattern of adversity, indicative of endocrine disruption, cannot be drawn. Therefore, further data should be generated before a conclusion on whether the ED criteria are met for the A- and/or S-modalities can be drawn.

For non-target organisms, the dataset was not sufficient to assess the ED properties of cymoxanil through the E, A, S and T- modalities.

EFSA notes that the 'Guidance for the identification of endocrine disruptors in the context of Regulations (EU) No 528/2012 and (EC) No 1107/2009' recommends a tiered assessment strategy. Therefore, according to the guidance, additional information should be generated. Level 2 and level 3 studies would be required:

- OECD TG 458 (AR STTA assays),
- OECD TG 456 (H295R Steroidogenesis Assay)
- OPPTS 890.1200 (Aromatase assay)
- A study in line with OECD TG 441 (Hershberger Assay) in case OECD TG 456 and OECD TG 458 and OPPTS 890.1200 are negative.
- A study in line with the OECD TG 231 (AMA) (see section 3.1.3);
- A study in line with the OECD TG 229 (FSTRA) (see section 3.2.3).

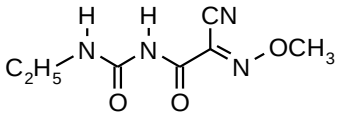
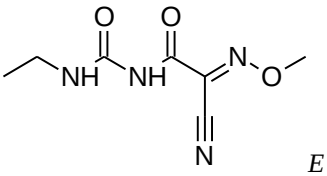
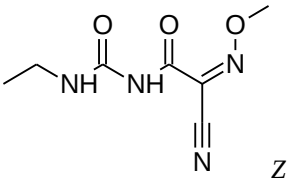
If endocrine activity is observed, further data will be needed to support the MoA analysis i.e. extended one-generation study with inclusion of the cohort 1a and 1b including the mating of cohort 1b to produce the F2 generation (OECD TG 443, Level 5) or an OECD TG 416. In this case for non-target organisms OECD TG 241 (Level 4) and OECD TG 240 (Level 5) might be needed to further investigate the adversity.

2.11 PROPOSED HARMONISED CLASSIFICATION AND LABELLING ACCORDING TO THE CLP CRITERIA *[SECTIONS 1-6 OF THE CLH REPORT]*

2.11.1 Identity of the substance [section 1 of the CLH report]

2.11.1.1 Name and other identifiers of the substance

Table 166: Substance identity and information related to molecular and structural formula of the substance

Name(s) in the IUPAC nomenclature or other international chemical name(s)	1-[*(E/Z)*-2-cyano-2-methoxyiminoacetyl]-3-ethylurea
Other names (usual name, trade name, abbreviation)	2-cyano-N-[(ethylamino)carbonyl]-2-(methoxyimino)acetamide (CA name)
ISO common name (if available and appropriate)	Cymoxanil
EC number (if available and appropriate)	261-043-0
EC name (if available and appropriate)	2-cyano-N-[(ethylamino)carbonyl]-2-(methoxyimino)acetamide (CA name)
CAS number (if available)	57966-95-7 (*E* isomer) 93195-85-8 (*Z*-isomer)
Other identity code (if available)	CIPAC no. 419
Molecular formula	$C_7H_{10}N_4O_3$
Structural formula	 Cymoxnil isomers (*E/Z*), *E/Z* ratio min.99:1  *E* isomer  *Z* isomer
SMILES notation (if available)	
Molecular weight or molecular weight range	198.2 g/mol
Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	Cymoxnil isomers (*E/Z*), *E/Z* ratio min.99:1
Description of the manufacturing process and identity of the source (for UVCB substances only)	Confidential information – data provided in Volume 4.
Degree of purity (%) (if relevant for the entry in Annex VI)	min.purity 970 g/kg

2.11.1.2 Composition of the substance

Table 167: Constituents (non-confidential information)

Constituent (Name and numerical identifier)	Concentration range (% w/w minimum and maximum in multi-constituent substances)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)
Cymoxanil		*Commission Regulation (EU) No 605/2014 (6th adaptation to technical and scientific progress of Regulation (EC) No 1272/2008):* Acute Tox., 4 H302; Skin Sens. 1, H317; STOT RE 2, H373 (blood, thymus); Repr. 2, H361fd Aquatic Acute 1, H400 Aquatic Chronic 1, H410	-

Table 168: Impurities (non-confidential information) if relevant for the classification of the substance

Impurity (Name and numerical identifier)	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)	The impurity contributes to the classification and labelling

Table 169: Additives (non-confidential information) if relevant for the classification of the substance

Additive (Name and numerical identifier)	Function	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)	The additive contributes to the classification and labelling

Table 170: Test substances (non-confidential information)

Identification of test substance	Purity	Impurities and additives (identity, %, classification if available)	Other information	The study(ies) in which the test substance is used

2.11.2 Proposed harmonized classification and labelling

2.11.2.1 Proposed harmonised classification and labelling according to the CLP criteria

Table 171: Proposed harmonised classification and labelling according to the CLP criteria

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATE	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	616-035-00-5	cymoxanil (ISO); 2-cyano-N-[(ethylamino)carbonyl]-2-(methoxyimino)acetamide	261-043-0	57966-95-7	Repr. 2 Acute Tox. 4 STOT RE 2 Skin Sens. 1 Aquatic Acute 1 Aquatic Chronic 1	H361fd H302 H373 (blood, thymus) H317 H400 H410	GHS08 GHS07 GHS09 Wng	H361fd H302 H373 (blood, thymus) H317 H410		M=1 M=1	
Dossier submitters proposal	616-035-00-5	cymoxanil (ISO); 2-cyano-N-[(ethylamino)carbonyl]-2-(methoxyimino)acetamide	261-043-0	57966-95-7	Modify: STOT RE 2 Skin Sens. 1A Aquatic Acute 1 Aquatic Chronic 1	Modify: H373 (blood, thymus, eye) H317 H400 H410		Modify: H373 (blood, thymus, eye)		M=1 M=1 Add: oral; ATE = 356 mg/kg bw	
Resulting Annex VI entry if agreed by RAC and COM	616-035-00-5	cymoxanil (ISO); 2-cyano-N-[(ethylamino)carbonyl]-2-(methoxyimino)acetamide	261-043-0	57966-95-7	Repr. 2 Acute Tox. 4 STOT RE 2 Skin Sens. 1A Aquatic Acute 1 Aquatic Chronic 1	H361fd H302 H373 (blood, thymus, eye) H317 H400 H410	GHS08 GHS07 GHS09 Wng	H361fd H302 H373 (blood, thymus, eye) H317 H410		M=1 M=1	

2.11.2.2 Additional hazard statements / labelling

Table 172: Reason for not proposing harmonised classification and status under CLH public consultation

Hazard class	Reason for no classification	Within the scope of CLH consultation
Explosives	Conclusive but not sufficient for classification	YES
Flammable gases (including chemically unstable gases)	Hazard class not applicable	YES
Oxidising gases	Hazard class not applicable	YES
Gases under pressure	Hazard class not applicable	YES
Flammable liquids	Hazard class not applicable	YES
Flammable solids	Conclusive but not sufficient for classification	YES
Self-reactive substances	Conclusive but not sufficient for classification	YES
Pyrophoric liquids	Hazard class not applicable	YES
Pyrophoric solids	Conclusive but not sufficient for classification	YES
Self-heating substances	Conclusive but not sufficient for classification	YES
Substances which in contact with water emit flammable gases	Conclusive but not sufficient for classification	YES
Oxidising liquids	Hazard class not applicable	YES
Oxidising solids	Conclusive but not sufficient for classification	YES
Organic peroxides	Hazard class not applicable	YES
Corrosive to metals	Conclusive but not sufficient for classification	YES
Acute toxicity via oral route	Acute Tox. 4 H302	YES
Acute toxicity via dermal route	Conclusive but not sufficient for classification	YES
Acute toxicity via inhalation route	Conclusive but not sufficient for classification	YES
Skin corrosion/irritation	Conclusive but not sufficient for classification	YES
Serious eye damage/eye irritation	Conclusive but not sufficient for classification	YES
Respiratory sensitisation	Conclusive but not sufficient for classification	YES
Skin sensitisation	Skin Sens. 1A	YES
Germ cell mutagenicity	Conclusive but not sufficient for classification	YES
Carcinogenicity	Conclusive but not sufficient for classification	YES
Reproductive toxicity	Repr. 2 H361fd	YES
Specific target organ toxicity-single exposure	Conclusive but not sufficient for classification	YES
Specific target organ toxicity-repeated exposure	STOT RE 2 H373 (blood, thymus, eye)	YES
Aspiration hazard	Not applicable	YES
Hazardous to the aquatic environment	Aquatic acute 1 Aquatic chronic 1	YES
Hazardous to the ozone layer	Not applicable	YES

2.11.3 History of the previous classification and labelling

A harmonised classification and labelling for cymoxanil has been adopted by the ECHA Committee for Risk Assessment (RAC) in 14 September 2012 (ECHA/RAC/CLH-O-0000002970-73-01/F). All the human health hazard classes (except respiratory sensitisation, aspiration hazard and adverse effects on or via lactation as well as endocrine disruption properties) were reviewed by the ECHA RAC. The resulting classification is available in Commission Regulation (EU) No 605/2014 (6th adaptation to technical and scientific progress of Regulation (EC) No 1272/2008) and the classification for human health is the following: Acute Tox., 4 H302; Skin Sens. 1, H317; STOT RE 2, H373 (blood, thymus); Repr. 2, H361fd; Aquatic Acute 1, H400, M=1; Aquatic Chronic 1, H410, M=1.

Cymoxanil was first approved for use as an agricultural fungicide under Council Directive 91/414/EEC in 2008, with Austria as Rapporteur Member State. It is approved for use under Regulation (EC) 1107/2009 and is being reviewed for the renewal of its approval under the AIR(IV) renewal programme with Lithuania as the RMS.

Based on re-evaluation of all old and new studies, some changes of the harmonised classification of cymoxanil is proposed by the RMS: Acute Tox. 4, H302; Skin Sens. 1A, H317; STOT RE 2, H373 (blood, thymus, eye); Repr. 2, H361fd. Aquatic Acute 1, H400, M=1; Aquatic Chronic 1, H410, M=1.

RAC general comment

Cymoxanil is an agricultural, vinicultural and horticultural fungicide used in plant protection products for the control of late blight (on potatoes and tomatoes) and mildew on grapes.

It was first approved in 2008 and is now reviewed for the renewal of its approval under the AIR(IV) renewal programme.

Following a RAC opinion on Cymoxanil in 2012, it received a harmonised classification as Acute Tox. 4, H302; Skin Sens. 1, H317; STOT RE 2, H373 (blood, thymus); Repr. 2, H361fd; Aquatic Acute 1, H400, M=1; Aquatic Chronic 1, H410, M=1. In this process, all human health hazard classes except respiratory sensitisation and aspiration hazard were reviewed.

Since then, new studies have been submitted by the applicants in the renewal process, relevant for the CLH dossier: three *in vitro* comparative metabolism studies, three acute (oral, dermal and eye irritation) toxicity studies, two phototoxicity assays, eight *in vitro* and *in vivo* genotoxicity studies, one reduced one generation study, and two QSAR studies. The Dossier Submitter nevertheless chose to re-evaluate all hazard classes .

Toxicokinetic data showed a rapid absorption after oral administration and extensive metabolism that didn't qualitatively differ between species. Most of the applied oral doses were excreted via urine (up to 80%) and faeces (up to 30%) within the first 48 hours after administration. No potential for bioaccumulation was shown.

2.11.4 Identified uses

Cymoxanil is used as an agricultural, vinicultural and horticultural fungicide.

The product "Rival Duo" is used for late blight control on potatoes. The plant protection product is intended for outdoor use.

The product "Dauphin 45/FDJ03" is used as a fungicide in potatoes, tomatoes and grapes against a variety of fungal pests. The plant protection product is intended for outdoor use.

The product "Cymoxanil 45 WG" is used for the control of late blight (*Phytophthora infestans*) on potatoes and tomatoes and mildew on vines. All of these crops are grown in field and the product is intended for outdoor use. Also, the product is used for the control of late blight on tomatoes grown in greenhouses.

Please refer to 1.5 for the full details on identified uses.

2.11.5 Data sources

All data were submitted for the renewal of approval of cymoxanil under Regulation 1107 (EC) 2009. Five applicants – Belchim, Du Pont, Indofil, Oxon, UPL submitted their joint Task Force dossier of cymoxanil. Two applicants - Agria and SFP - submitted their separate dossiers for the EU renewal of cymoxanil approval. Lithuania as RMS made assessment and prepared the RAR, Finland acting as Co-RMS, agreed to review the RAR before the submission to EFSA.

2.12 RELEVANCE OF METABOLITES IN GROUNDWATER

According to the "Guidance document on the Assessment of the relevance of Metabolites in Groundwater of Substances regulated under Council Directive" (SANCO/221/2000 – rev.10 – final, 25 February 2003), all major degradation products of cymoxanil in soil, plus metabolites found lysimeter leachate at > 0.1 µg/L are considered for their relevance for groundwater, following a sequential assessment which is presented in this document.

Table 2.12-1 Key substance specific input parameters of cymoxanil and its metabolites for calculating PECgw

2.12.1 STEP 1: Exclusion of degradation products of no concern

According to the Guidance Document on the Assessment of the Relevance of Metabolites in Groundwater of Substances Regulated under Council Directive 91/414/EEC (SANCO/221/2000 - rev. 10, final) organic compounds of aliphatic structure with a chain length of 4 or less, which consist only of C, H, N, O atoms and which has no "alerting structures" such as epoxide, nitrosamine, nitrile or other functional groups of known toxicological concern, are considered substances of no concern. Two soil degradation productsof cymoxanil, IN-18474 (oxamic acid) and ethyl urea meet these citeria and can therefore be excluded from risk assessment for potential groundwater contamination as products aof no concern.

2.12.2 STEP 2: Quantification of potential groundwater contamination

Based on laboratory studies, the remaining major degradation products of cymoxanil in soil are IN-U3204, IN-W3595, IN-JX915, IN-T4226, IN-Q960. Following further kinetic assessment none of these metabolites were found exceed the 0.1 µg/Ltrigger value and therefore warrant further assessment or have the potential to reach groundwater. Full details are provided in document CP8 Section 8.3.

In all cases, PEC_{GW} for cymoxanil and metabolites IN-3204, IN-W3595, IN-JX915 were <0.1 µg/L. For metabolite IN-KQ960 the majority of the modelled scenarios indicated it to be below <0.1 µg/L. Only in the Jokionen scenario for bothearly and late application patterns PECgw values for IN-KQ960 were above 0.1 µg/L (0.159 µg/L in PEARL and 0.167 µg/L in PELMO for early usage applications and 0.198 µg/L in PEARL and 0.210 µg/L in PELMO for late usage applications). Similarly, in the late application scenario only PECgw values for IN-KQ960 were marginally above 0.1 µg/L in Hamburg (0.199 µg/L and 0.188 µg/L in PEAL and PELMO respectively).

Indicative data in Hamburg and Jokionen scenarios only, no use in potatoes and grapes in Northern zone intended.

None of cymoxanil metabolites found in soil are predicted to exceed the 0.1 µg/L trigger value and therefore no need for futher assessment.

2.12.3 STEP 3: Hazard assessment – identification of relevant metabolites

Not required

2.12.3.1 STEP 3, Stage 1: screening for biological activity

Not required

2.12.3.2 STEP 3, Stage 2: screening for genotoxicity

Not required

2.12.3.3 STEP 3, Stage 3: screening for toxicity

Not required

2.12.4 STEP 4: Exposure assessment – threshold of concern approach

Not required

2.12.5 STEP 5: Refined risk assessment

Not required

2.12.6 Overall conclusion

2.13 CONSIDERATION OF ISOMERIC COMPOSITION IN THE RISK ASSESSMENT

It is stated by the Applicant (CTF) that: *"Cymoxanil is manufactured as the (E) isomer and the reference technical specification is for the cymoxanil content to comprise at least a 99:1 ratio of E isomer to Z isomer. The (eco)toxicological endpoints are derived from studies using this technical material. A number of the studies evaluating the metabolism of cymoxanil in plants or the fate and behaviour of cymoxanil in the environment included the Z-isomer (IN-Q8761) as an analytical target and this was never detected in those studies. Additionally, the parent stereochemistry is maintained in all of the identified metabolites. Therefore, there is no indication of the formation of (Z) isomer or other such stereoselective metabolism. In any case, cymoxanil is very labile in the environment and in plants and livestock such that it is rapidly degraded to glycine and other natural products. Therefore, structural fragments containing the intact imino functionality are very short lived and are of no relevance for the human and environment exposure assessment."*

Z-isomer was not detected or only very tentatively identified in metabolism in plants studies (studies CA 6.2.1/04 (CTF), CA 6.2.1/06 (CTF), CA 6.2.1/08 (CTF), CA 6.2.1/05 (CTF)). Based on the outcome of environmental fate study of Cooper (2017) there is no need to include the Z-isomer in the risk assessment.

2.13.1 Identity and physical chemical properties

Cymoxanil isomers (*E/Z*) considered, *E/Z* ratio min.99:1

2.13.2 Methods of analysis

Cymoxanil isomers (*E/Z*) considered.

2.13.3 Mammalian toxicity

2.13.4 Operator, Worker, Bystander and Resident exposure

2.13.5 Residues and Consumer risk assessment

Not relevant for risk assessment. Z-isomer was not detected (in studies CA 6.2.1/04 (CTF), CA 6.2.1/06 (CTF), CA 6.2.1/08 (CTF)) or only very tentatively identified (in study CA 6.2.1/05 (CTF)) in metabolism in plants studies.

2.13.6 Environmental fate

The study (Cooper, 2017) was specifically designed to look at IN-Q8761 (the Z-isomer of cymoxanil) that was reported at a maximum of 6.9 % AR with no significant difference between the irradiated and dark control samples during the Annex I inclusion. As IN-Q8761 was reported at >5 % at two or more consecutive time points, data requirements under EC/1107/2009 would now require that it be assessed in the environmental fate risk assessment for annex renewal.

It can be concluded that in study of Copper (2017) IN-Q8761 was not generated during the course of the dry soil photolysis investigation, thus supported the suggesting that its reported presence in a previous study was an effect of the chromatographic methodology. Consequently IN-Q8761 would not need to be included in the environmental risk assessment. The present study was therefore designed to investigate wheater IN-Q8761 is caused by photolytic conditions.

2.13.7 Ecotoxicology

2.14 RESIDUE DEFINITIONS

2.14.1 Definition of residues for exposure/risk assessment

Food of plant origin: cymoxanil

Food of animal origin: not proposed, not necessary

Soil: Cymoxanil, N-U3204, IN-W3595, IN-JX915, IN-T4226, IN-KQ960

Groundwater: Cymoxanil, IN-KQ960, IN-U3204, IN-W3595, IN-JX915

Surface water: Cymoxanil, IN-W3595, IN-KQ960, IN-U3204, IN-R3274, IN-KP533, IN-JX915, IN-T4226, IN-R3273, ASS999

Sediment: Cymoxanil.

Air: Cymoxanil

2.14.2 Definition of residues for monitoring

Food of plant origin: cymoxanil

Food of animal origin: not proposed, not necessary

Soil: Cymoxanil

Groundwater: Cymoxanil

Surface water: Cymoxanil

Sediment: Cymoxanil

Air: Cymoxanil

Level 3

CYMOXANIL

3 PROPOSED DECISION WITH RESPECT TO THE APPLICATION

3.1 BACKGROUND TO THE PROPOSED DECISION

3.1.1 Proposal on acceptability against the decision making criteria – Article 4 and annex II of regulation (EC) No 1107/2009

3.1.1.1 Article 4				
		Yes	No	
i)	It is considered that Article 4 of Regulation (EC) No 1107/2009 is complied with. Specifically the RMS considers that authorisation in at least one Member State is expected to be possible for at least one plant protection product containing the active substance for at least one of the representative uses.	X		CTF product/uses: A safe use has not be identified as there are unacceptable/unresolved risks to herbivorous mammals for tomatoes use and to honeybees larvae for all uses for weeds scenario. Agria product/uses: A safe use has not be identified as there are unacceptable/unresolved risks to herbivorous mammals, non-target arthropods for potatoes use. SFP product/uses: A safe use has not be identified as there are unacceptable/unresolved risks to residents for all uses and operator spraying tomatoes by manual application. A safe use has not be identified as there are unacceptable/unresolved risks to mammals for grapes, tomatoes and potatoes uses and to honeybees for all uses for weeds scenario. Risk to mammals and honeybees could be mitigated or refined further with additional data at Member State level.
3.1.1.2 Submission of further information				
		Yes	No	
i)	It is considered that a complete dossier has been submitted		X	Insufficient information has been available to support the proposed reference specification
ii)	It is considered that in the absence of a full dossier the active substance may be approved even though certain information is still to be submitted because: (a) the data requirements have been amended or refined after the	X		Therer are data gaps identified (see Level 3.1.4)

	submission of the dossier; or (b) the information is considered to be confirmatory in nature, as required to increase confidence in the decision.			
3.1.1.3 Restrictions on approval				
		Yes	No	
	It is considered that in line with Article 6 of Regulation (EC) No 1107/2009 approval should be subject to conditions and restrictions.		X	
3.1.1.4 Criteria for the approval of an active substance				
Dossier				
		Yes	No	
	It is considered the dossier contains the information needed to establish, where relevant, Acceptable Daily Intake (ADI), Acceptable Operator Exposure Level (AOEL) and Acute Reference Dose (ARfD).	X		The data submitted are sufficient to establish an Acceptable Daily Intake (ADI), Acceptable Operator Exposure Level (AOEL), an Acute Reference Dose (ARfD) and acute acceptable operator exposure level (AAOEL). Please refer to Level 2.6.10.
	It is considered that the dossier contains the information necessary to carry out a risk assessment and for enforcement purposes (relevant for substances for which one or more representative uses includes use on feed or food crops or leads indirectly to residues in food or feed). In particular it is considered that the dossier: (a) permits any residue of concern to be defined; (b) reliably predicts the residues in food and feed, including succeeding crops (c) reliably predicts, where relevant, the corresponding residue level reflecting the effects of processing and/or mixing; (d) permits a maximum residue level to be defined and to be determined by appropriate methods in general use for the commodity and, where appropriate, for products of animal origin where the commodity or parts of it is fed to animals; (e) permits, where relevant, concentration or dilution factors due to processing and/or mixing to be defined.	X		The data submitted are sufficient to carry out a risk assessment and for enforcement purposes. No risk to consumer safety identified. Please refer to level 2.7.
	It is considered that the dossier submitted is sufficient to permit, where relevant, an estimate of the fate and distribution of the active substance in the environment, and its impact on non-target species.	X		It is considered that the dossier contains the sufficient information
Efficacy				
		Yes	No	

	It is considered that it has been established for one or more representative uses that the plant protection product, consequent on application consistent with good plant protection practice and having regard to realistic conditions of use is sufficiently effective.	X		Representative uses are already authorized on national level and have been evaluated according to uniform principles. Based upon the efficacy summary provided now, sufficient efficacy (min. 60.1%, max. 90.7 %) of Propamocarb hydrochloride 400 g/L + Cymoxanil 50 g/L SC against late blight (*Phytophthora infestans*) on potatoes was demonstrated.
Relevance of metabolites				
		Yes	No	
	It is considered that the documentation submitted is sufficient to permit the establishment of the toxicological, ecotoxicological or environmental relevance of metabolites.		X	There are no metabolites formed in amount triggering a groundwater risk assessment.
Composition				
		Yes	No	
	It is considered that the specification defines the minimum degree of purity, the identity and maximum content of impurities and, where relevant, of isomers/diastereo-isomers and additives, and the content of impurities of toxicological, ecotoxicological or environmental concern within acceptable limits.		X	Insufficient information has been available to support the proposed reference specification at renewal of cymoxanil with respect to the identity and content of impurities in the specification.
	It is considered that the specification is in compliance with the relevant Food and Agriculture Organisation specification, where such specification exists.		X	FAO specification of cymoxanil (2006) produced by the Oxon Italia and ny DuPont under the new procedure is confidential and therefore not available for the assessment.
	It is considered for reasons of protection of human or animal health or the environment, stricter specifications than that provided for by the FAO specification should be adopted	X		Explain as necessary
Methods of analysis				
		Yes	No	
	It is considered that the methods of analysis of the active substance, safener or synergist as manufactured and of determination of impurities of toxicological, ecotoxicological or environmental concern or which are present in quantities greater than 1 g/kg in the active substance, safener or synergist as manufactured, have been validated and shown to be sufficiently specific, correctly calibrated, accurate and precise.	X		*Please refer to level 2.5.1*
	It is considered that the methods of residue analysis for the active substance and relevant metabolites in plant, animal and environmental matrices and drinking water, as appropriate, shall have been validated and shown to be sufficiently sensitive with respect to the levels of concern.	X		*Please refer to level 2.5.2*
	It is confirmed that the evaluation has been carried out in accordance with the uniform principles for evaluation and authorisation of plant protection products referred to in Article 29(6) of Regulation	X		

	1107/2009.			
Impact on human health				
Impact on human health - ADI, AOEL, ARfD				
		Yes	No	
	It is confirmed that (where relevant) an ADI, AOEL and ARfD can be established with an appropriate safety margin of at least 100 taking into account the type and severity of effects and the vulnerability of specific groups of the population.	X		The ADI is set at 0.013 mg/kg bw/day, based on the 1-year stud in dog and by using a safety factor of 100 (see Level 2.6.10.1). The AOEL is set at 0.01 mg/kg bw/ day, based on the 1-year study in dog, by using a safety factor of 100 and corrected by the limited oral absorption of 76% (see Level 2.6.10.3). The ARfD is set at 0.08 mg/kg bw/day, based on the rabbit developmental toxicity study and by using a safety factor of 100 (see Level 2.6.10.2). The AAOEL is set at 0.061 mg/kg bw/ day, based on the rabbit developmental toxicity study, by using a safety factor of 100 and corrected by the limited oral absorption of 76% (see Level 2.6.10.4).
Impact on human health – proposed genotoxicity classification				
		Yes	No	
	It is considered that, on the basis of assessment of higher tier genotoxicity testing carried out in accordance with the data requirements and other available data and information, including a review of the scientific literature, reviewed by the Authority, **the substance SHOULD BE classified or proposed for classification**, in accordance with the provisions of Regulation (EC) No 1272/2008, **as mutagen category 1A or 1B.**		X	Based on the results of *in vitro* and *in vivo* genotoxicity studies, cymoxanil unlikely to be genotoxic *in vivo* (see Level 2.6.4).
Impact on human health – proposed carcinogenicity classification				
		Yes	No	
i)	It is considered that, on the basis of assessment of the carcinogenicity testing carried out in accordance with the data requirements for the active substances, safener or synergist and other available data and information, including a review of the scientific literature, reviewed by the Authority, **the substance SHOULD BE classified or proposed for classification**, in accordance with the provisions of Regulation (EC) No 1272/2008, **as carcinogen category 1A or 1B.**		X	Cymoxanil is not considered to be carcinogen as no oncogenic effects were observed in studies conducted with cymoxanil, either in rat or in mouse carcinogenicity studies (see Level 2.6.5). This conclusion is in line with RAC Opinion 2012 and Commission Regulation (EU) No 605/2014 (6th adaptation to technical and scientific progress of Regulation (EC) No 1272/2008). No change of classification is considered necessary (see Level 2.6.5).
ii)	Linked to above classification proposal. It is considered that exposure of humans to the active substance,			Not applicable

	safener or synergist in a plant protection product, under realistic proposed conditions of use, is negligible, that is, the product is used in closed systems or in other conditions excluding contact with humans and where residues of the active substance, safener or synergist concerned on food and feed do not exceed the default value set in accordance with Article 18(1)(b) of Regulation (EC) No 396/2005.			
Impact on human health – proposed reproductive toxicity classification				
		Yes	No	
i)	It is considered that, on the basis of assessment of the reproductive toxicity testing carried out in accordance with the data requirements for the active substances, safeners or synergists and other available data and information, including a review of the scientific literature, reviewed by the Authority, **the substance SHOULD BE classified or proposed for classification**, in accordance with the provisions of Regulation (EC) No 1272/2008, **as toxic for reproduction category 1A or 1B.**		X	The harmonised classification of cymoxanil is Repr. 2, H361fd based on the adverse effects on male reproductive organs in repeated dose toxicity studies in rats, mice and dogs as well as on malformations demonstrated in the developmental toxicity studies in rats and in rabbits. This conclusion is in line with RAC Opinion 2012 and Commission Regulation (EU) No 605/2014 (6th adaptation to technical and scientific progress of Regulation (EC) No 1272/2008). No change of classification is considered necessary (see Level 2.6.5).
ii)	Linked to above classification proposal. It is considered that exposure of humans to the active substance, safener or synergist in a plant protection product, under realistic proposed conditions of use, is negligible, that is, the product is used in closed systems or in other conditions excluding contact with humans and where residues of the active substance, safener or synergist concerned on food and feed do not exceed the default value set in accordance with Article 18(1)(b) of Regulation (EC) No 396/2005.			Not applicable
Impact on human health – proposed endocrine disrupting properties classification				
		Yes	No	
i)	It is considered that **the substance SHOULD BE identified as having endocrine disrupting properties** in accordance with the provisions of point 3.6.5 in Annex II of Regulation (EC) No 1107/2009		X	According to the EFSA/ECHA guidance and based on the information currently available, it cannot be concluded if cymoxanil meets the ED criteria with respect to humans for A- and S- modalities. Therefore, further data should be generated before a conclusion could be drawn.
ii)	Linked to above identification proposal. It is considered that exposure of humans to the active substance, safener or synergist in a plant protection product, under realistic proposed conditions of use, is negligible, that is, the product is used in closed systems or in other conditions excluding contact with humans and where residues of the active substance, safener or synergist concerned on food and feed do not exceed the default value set in			Not applicable

	accordance with Article 18(1)(b) of Regulation (EC) No 396/2005.			
Fate and behaviour in the environment				
Persistent organic pollutant (POP)				
		Yes	No	
	It is considered that the active substance **FULFILS** the criteria of a persistent organic pollutant (POP) as laid out in Regulation 1107/2009 Annex II Section 3.7.1.		X	According to the Commission Implementing Regulation (EU) 2015/408 of 11 March 2015 on implementing Article 80(7) of Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market and establishing a list of candidates for substitution Cymoxanil meet the criteria to be considered a toxic substance. The criteria for persistence and bioaccumulation were not fulfilled.
Persistent, bioaccumulative and toxic substance (PBT)				
		Yes	No	
	It is considered that the active substance **FULFILS** the criteria of a persistent, bioaccumulative and toxic (PBT) substance as laid out in Regulation 1107/2009 Annex II Section 3.7.2.		X	According to the Commission Implementing Regulation (EU) 2015/408 of 11 March 2015 on implementing Article 80(7) of Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market and establishing a list of candidates for substitution Cymoxanil meet the criteria to be considered a toxic substance. The criteria for persistence and bioaccumulation were not fulfilled.
Very persistent and very bioaccumulative substance (vPvB).				
		Yes	No	
	It is considered that the active substance **FULFILS** the criteria of a a very persistent and very bioaccumulative substance (vPvB) as laid out in Regulation 1107/2009 Annex II Section 3.7.3.		X	According to the Commission Implementing Regulation (EU) 2015/408 of 11 March 2015 on implementing Article 80(7) of Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market and establishing a list of candidates for substitution Cymoxanil meet the criteria to be considered a toxic substance. The criteria for persistence and bioaccumulation were not fulfilled.
Ecotoxicology				
		Yes	No	
i	It is considered that the risk assessment demonstrates risks to be acceptable in accordance with the criteria laid down in the uniform principles for evaluation and authorisation of plant protection products referred to in Article 29(6) under realistic proposed conditions of use of a plant protection product containing the active substance, safener or synergist. The RMS is content that the assessment takes into account		X	CTF A low risk was demonstrated to birds, aquatic organisms other than algae and aquatic plants, non-target arthropods, soil micro, meso and macrofauna and sewage treatment organisms, without the requirements for risk mitigation measures. The risk to algae and aquatic plants could not be resolved due to the lack of valid data to meet the data requirements.

	the severity of effects, the uncertainty of the data, and the number of organism groups which the active substance, safener or synergist is expected to affect adversely by the intended use.			The long-term risk to herbivorous mammals from cymoxanil could not be resolved for the representative use in tomato. Agria A low risk was demonstrated to birds, aquatic organisms other than algae and aquatic plants, soil and meso and macrofauna and sewage treatment organisms, without the requirements for risk mitigation measures. The long-term risk to herbivorous mammals from cymoxanil and the acute risk to herbivorous mammals for representative formulation could not be resolved for the representative use in potato. The in-field risk to non-target terrestrial arthropods for the intended uses of representative formulation in potatoes could not be resolved. The risk to algae and aquatic plants could not be resolved due to the lack of valid data to meet the data requirements. SFP A low risk was demonstrated toaquatic organisms other than algae and aquatic plants, non-target arthropods, soil and meso and macrofauna and sewage treatment organisms, without the requirements for risk mitigation measures. A low acute risk to birds and mammals was demonstrated for the representative uses. A low long term risk for birds was demonstrated for the representative uses in grapes and tomato The long-term risk to birds from cymoxanil could not be resolved for the representative use in tomatoes. The long-term risk to herbivorous and frugivorous mammals from cymoxanil could not be resolved for the representative uses in grapes, potato and tomato. The risk to algae and aquatic plants could not be resolved due to the lack of valid data to meet the data requirements.
ii	It is considered that the substance **SHOULD BE identified as having**		X	According to the EFSA/ECHA guidance and based on the information

	endocrine disrupting properties that may cause adverse effects on non-target organisms in accordance with the provisions of point 3.8.2 in Annex II of Regulation (EC) No 1107/2009.			currently available, it cannot be concluded if cymoxanil meets the ED criteria with respect to non-target organisms for T-, A- and S- modalities. Therefore, further data should be generated before a conclusion could be drawn.
iii	Linked to the consideration of the endocrine properties immediately above. It is considered that the exposure of non-target organisms to the active substance in a plant protection product under realistic proposed conditions of use is negligible.			Not applicable
iv	It is considered that it is established following an appropriate risk assessment on the basis of Community or internationally agreed test guidelines, that the use under the proposed conditions of use of plant protection products containing this active substance, safener or synergist: — will result in a negligible exposure of honeybees, or — has no unacceptable acute or chronic effects on colony survival and development, taking into account effects on honeybee larvae and honeybee behaviour.		X	CTF The evaluation of the risk for bees was performed in accordance with the recommendations of the "EFSA Guidance Document on the risk of plant protection products on bees (*Apis mellifera*, *Bombus* spp. And solitary bees)", as provided by EFSA (EFSA Journal 2013; 11(7): 3295; updated version published on 4 July 2014). The acute and chronic risk to adult honeybees and ther acute risk to bumblebees from the active substance cymoxanil is considered acceptable for the proposed uses in grapevines, potato and tomato. However, the risk to honeybee larvae is unacceptable for the proposed use of Cymoxanil in grapevines, potatoes and tomatoes when bees are foraging on the weeds in the field. The risk from consumption of contaminated water is acceptable. SFP The evaluation of the risk for bees was performed in accordance with the recommendations of the "EFSA Guidance Document on the risk of plant protection products on bees (*Apis mellifera*, *Bombus* spp. And solitary bees)", as provided by EFSA (EFSA Journal 2013; 11(7): 3295; updated version published on 4 July 2014). The acute risk from the active substance cymoxanil is considered acceptable for the proposed uses in potato, grapevines and tomato, however the chronic risk to adult honeybees and the risk to honeybee larvae are not acceptable in the treated crop in grapevines and in potato and tomato when bees are foraging on weeds in the field. A higher tier assessment could not be performed as no suitable data were available. The risk from consumption of contaminated water was acceptable for acute risk, however not acceptable

				for chronic risk. Agria The evaluation of the risk for bees was performed in accordance with the recommendations of the "Guidance Document on Terrestrial Ecotoxicology", as provided by the Commission Services (SANCO/10329/2002 rev.2 (final), October 17, 2002) and EPPO 2010. No risk assessment to bees was provided according to the EFSA Bee Guidance Document (EFSA Journal 2013; 11(7): 3295; updated version published on 4 July 2014).
Residue definition				
		Yes	No	
	It is considered that, where relevant, a residue definition can be established for the purposes of risk assessment and for enforcement purposes.	X		The proposed residue definitions for plant commodities are: - Enforcement: cymoxanil; - Risk assessment: cymoxanil. Based on available livestock metabolism studies and as livestock exposure was below the trigger value, no proposal has been done for livestock residue definitions (both enforcement and risk assessment). Please refer to section 2.7.3 in level 2.
Fate and behaviour concerning groundwater				
		Yes	No	
	It is considered that it has been established for one or more representative uses, that consequently after application of the plant protection product consistent with realistic conditions on use, the predicted concentration of the active substance or of metabolites, degradation or reaction products in groundwater complies with the respective criteria of the uniform principles for evaluation and authorisation of plant protection products referred to in Article 29(6) of Regulation 1107/2009.	X		The 80th percentile of the mean annual leachate concentration at 1 m soildepth (PECgw) for cymoxanil were below 0.1 µg/L for all intended uses with both PELMO and PEARL models in all scenarios with application pattern every year.

3.1.2 Proposal – Candidate for substitution

Candidate for substitution			
	Yes	No	

	It is considered that the active substance shall be approved as a candidate for substitution		X	Cymoxanil does not meet any of the candidate for substitution criteria

3.1.3 Proposal – Low risk active substance

Low-risk active substances				
		Yes	No	
	It is considered that the active substance **shall be considered of low risk.** If the active substance is not a micro-organism, in particular it is considered that: (a) the substance **should NOT be classified or proposed for classification** in accordance to Regulation (EC) No 1272/2008 as any of the following: — carcinogenic category 1A, 1B or 2, — mutagenic category 1A, 1B or 2, — toxic to reproduction category 1A, 1B or 2, — skin sensitiser category 1, — serious damage to eye category 1, — respiratory sensitiser category 1, — acute toxicity category 1, 2 or 3, — specific Target Organ Toxicant, category 1 or 2, — toxic to aquatic life of acute and chronic category 1 on the basis of appropriate standard tests, — explosive, — skin corrosive, category 1A, 1B or 1C; (b) it has **not been identified as priority substance under Directive 2000/60/EC**; (c) it is **not deemed to be an endocrine disruptor** in accordance to Annex II of Regulation (EC) No 1107/2009; (d) it **has no neurotoxic or immunotoxic effects**; (e) it **is not persistent** (half-life in soil is more than 60 days) or its **bio-concentration factor is lower than 100**. (f) it is a **semiochemical** and verifies points (a) to (d).		X	May not be regarded as low risk active substance because the harmonised classification of cymoxanil for human health is: Skin Sens. 1, H317; STOT RE 2, H373 (blood, thymus) and Repr. 2, H361fd. Toxic to aquatic life –Aquatic acute category 1and aquatic chronic category 1.

	Paragraph (e) doesn't apply to naturally occurring active substances. If the active substance is a micro-organism, in particular it is considered that at strain level the micro-organism has not demonstrated multiple resistance to anti-microbials used in human or veterinary medicine. If the active substance is a baculovirus, in particular it has not demonstrated adverse effects on non-target insects.			

3.1.4 List of studies to be generated, still ongoing or available but not peer reviewed

Data gap	Relevance in relation to representative use(s)	Study status		
		No confirmation that study available or on-going.	Study on-going and anticipated date of completion	Study available but not peer-reviewed
3.1.4.1 Identity of the active substance or formulation				
None				
3.1.4.2 Physical and chemical properties of the active substance and physical, chemical and technical properties of the formulation				
None				
3.1.4.3 Data on uses and efficacy				
None				
3.1.4.4 Data on handling, storage, transport, packaging and labelling				
None				
3.1.4.5 Methods of analysis				
3.1.4.6 Toxicology and metabolism				
In accordance with the ECHA/EFSA Guidance on identifying ED Level 2 study for A- modality:	Relevant for all representative uses.			

OECD TG 458 (AR STTA assays)				
In accordance with the ECHA/EFSA Guidance on identifying ED Level 2 study for S-modality: OECD TG 456 (H295R Steroidogenesis Assay)	Relevant for all representative uses.			
In accordance with the ECHA/EFSA Guidance on identifying ED Level 2 study for S-modality: OPPTS 890.1200 (Aromatase assay)	Relevant for all representative uses.			
In accordance with the ECHA/EFSA Guidance on identifying ED a study in line with OECD TG 441 (Hershberger Assay) in case OECD TG 456 and OECD TG 458 and OPPTS 890.1200 are negative. If endocrine activity is observed, further data to support the MoA analysis i.e. extended one-generation study with inclusion of the cohort 1a and 1b including the mating of cohort 1b to produce the F2 generation (OECD TG 443, Level 5) or an OECD TG 416 in accordance with the ECHA/EFSA Guidance on identifying ED	Relevant for all representative uses.			
A literature search and QSAR analysis on some metabolites/impurities	Relevant for all representative uses.			
Further data to exclude the relevance of some impurities or to support their maximum content in the proposed reference specification	Relevant for all representative uses.			
Equivalence between technical materials once a proposed reference specification is supported by toxicity studies	Relevant for all representative uses.			
Measurement of resident exposure	Relevant for all representative uses.			

3.1.4.7 Residue data				
3.1.4.8 Environmental fate and behaviour				
3.1.4.9 Ecotoxicology				
In accordance with the ECHA/EFSA Guidance on identifying ED level 3 study according to OECD TG 231 is required	Relevant for all representative uses.			
In accordance with the ECHA/EFSA Guidance on identifying ED level 3 study according to OECD TG 229 is required	Relevant for all representative uses.			
If endocrine activity is observed, studies OECD TG 241 (Level 4) and OECD TG 240 (Level 5) might be needed to further investigate the ED adversity for non-targetr organisms	Relevant for all representative uses.			
CTF, Agria and SFP do not have valid algae ecotox studies for cymoxanil	Relevant for all representative uses.		March 2020	
CTF, Agria and SFP do not have valid aquatic plants ecotox studies for cymoxanil	Relevant for all representative uses.	X		
SFP does not have soil-macro-organisms (*Folsomia candida and Hypoaspis aculeifer*) ecotox studies for cymoxanil	Relevant for all representative uses.	X		

3.1.5 Issues that could not be finalised

An issue is listed as an issue that could not be finalised where there is not enough information available to perform an assessment, even at the lowest tier level, for the representative uses in line with the Uniform Principles, as laid out in Commission Regulation (EU) No 546/2011, and where the issue is of such importance that it could, when finalised, become a concern (which would also be listed as a critical area of concern if it is of relevance to all representative uses).

Area of the risk assessment that could not be finalised on the basis of the available data	**Relevance in relation to representative use(s)**
According to the EFSA/ECHA guidance and based on the information currently available, it cannot be concluded if cymoxanil meets the ED criteria with respect to humans for A- and S- modalities	All uses
According to the EFSA/ECHA guidance and based on the information currently available, it cannot be concluded if cymoxanil meets the ED criteria with respect to non-target organisms other than mammals for T-, E-, A- and S-modalities	All uses

3.1.6 Critical areas of concern

An issue is listed as a critical area of concern:
(a) where the substance does not satisfy the criteria set out in points 3.6.3, 3.6.4, 3.6.5 or 3.8.2 of Annex II of Regulation (EC) No 1107/2009 and the applicant has not provided detailed evidence that the active substance is necessary to control a serious danger to plant health which cannot be contained by other available means including non-chemical methods, taking into account risk mitigation measures to ensure that exposure of humans and the environment is minimised, or
(b) where there is enough information available to perform an assessment for the representative uses in line with the Uniform Principles, as laid out in Commission Regulation (EU) 546/2011, and where this assessment does not permit to conclude that for at least one of the representative uses it may be expected that a plant protection product containing the active substance will not have any harmful effect on human or animal health or on groundwater or any unacceptable influence on the environment.

An issue is also listed as a critical area of concern where the assessment at a higher tier level could not be finalised due to a lack of information, and where the assessment performed at the lower tier level does not permit to conclude that for at least one of the representative uses it may be expected that a plant protection product containing the active substance will not have any harmful effect on human or animal health or on groundwater or any unacceptable influence on the environment.

Critical area of concern identified	**Relevance in relation to representative use(s)**
The proposed reference specification at renewal is not covered by the toxicological assessment	Relevant for all representative uses
Residents	An acceptable risk has not been demonstrated for all uses (SFP)
Mammals	An acceptable risk has not been demonstrated for

	tomatoes (CTF), for all uses (SFP) and for potatoes (Agria).
Birds	An acceptable risk has not been demonstrated for tomatoes (SFP).
The risk to birds and mammals from the plant metabolite IN-U3204	Relevant for all representative uses
Bees	An acceptable risk has not been demonstrated for crop and weeds scenario (SFP).
Honeybees larvae	An acceptable risk has not been demonstrated for weeds scenario (CTF) and for crop and weeds scenario (SFP).

3.1.7 Overview table of the concerns identified for each representative use considered

(If a particular condition proposed to be taken into account to manage an identified risk, as listed in 3.3.1, has been evaluated as being effective, then 'risk identified' is not indicated in this table.)
All columns are grey as the material tested in the toxicological studies has not been demonstrated to be representative of the technical specification.

Representative use		Grapes (CTF) (X^1)	Tomatoes (CTF) (X^1)	Potatoes (CTF) (X1)	Grapes (SFP) (X^1)	Tomatoes (SFP) (X^1)	Potatoes (SFP) (X^1)	Potatoes (Agria) (X^1)
Operator risk	Risk identified					X^{1a}		
	Assessment not finalised							
Worker risk	Risk identified							
	Assessment not finalised							
Bystander risk	Risk identified							
	Assessment not finalised							
Resident risk	Risk identified				X^1	X^1	X^1	
	Assessment not finalised							
Consumer risk	Risk identified							

	Assessment not finalised							
Risk to wild non target terrestrial vertebrates	Risk identified		X- A low long-term risk to small herbivorous mammals from cymoxanil could not be concluded.		X- A low long-term risk to small herbivorous mammals from cymoxanil could not be concluded.	X- A low long-term risk to frugivorous birds and mammals from cymoxanil could not be concluded.	X- A low long-term risk to small and large herbivorous mammals from cymoxanil could not be concluded.	X- A low acute and long-term risk to small and large herbivorous mammals from cymoxanil could not be concluded.
	Assessment not finalised							
Risk to wild non target terrestrial organisms other than vertebrates	Risk identified	X- A low chronic risk to honeybees larvae could not be confirmed for weeds scenario	X- A low chronic risk to honey bee larvae could not be confirmed for weeds scenario	X- A low chronic risk to honeybee larvae could not be confirmed for weeds scenario	X- A low chronic risk to adult honeybees and honey bee larvae could not be confirmed for crop and weeds scenario	X- A low chronic risk to adult honeybees and honey bee larvae could not be confirmed for weeds scenario	X- A low chronic risk to adult honeybees and honey bee larvae could not be confirmed for weeds scenario	X-A low in-field risk to non-target arthropods could not be confirmed for representative formulation.
	Assessment not finalised							X-No risk assessment to bees was performed according to EFSA bee guidance document

Risk to aquatic organisms	Risk identified							
	Assessment not finalised	X -No valid study and hence no risk assessment to address the risk from active substance to algae and aquatic plants	X -No valid study and hence no risk assessment to address the risk from active substance to algae and aquatic plants	X -No valid study and hence no risk assessment to address the risk from active substance to algae and aquatic plants	X- No valid study and hence no risk assessment to address the risk from active substance to algae and aquatic plants	X- No valid study and hence no risk assessment to address the risk from active substance to algae and aquatic plants	X -No valid study and hence no risk assessment to address the risk from active substance to algae and aquatic plants	X -No valid study and hence no risk assessment to address the risk from active substance to algae and aquatic plants
Groundwater exposure active substance	Legal parametric value breached							
	Assessment not finalised							
Groundwater exposure metabolites	Legal parametric value breached							
	Parametric value of 10µg/L[(a)] breached							
	Assessment not finalised							
Comments/Remarks								

The superscript numbers in this table relate to the numbered points indicated within chapter 3.1.5 and 3.1.6. Where there is no superscript number, see level 2 for more explanation.
(a): Value for non relevant metabolites prescribed in SANCO/221/2000-rev 10-final, European Commission, 2003
X[1a]: unacceptable risks to the operator are only to spraying tomatoes indoors.

3.1.8 Area(s) where expert consultation is considered necessary

It is recommended to organise a consultation of experts on the following parts of the assessment report:

Area(s) where expert consultation is considered necessary	**Justification**

3.1.9 Critical issues on which the Co RMS did not agree with the assessment by the RMS

Points on which the co-rapporteur Member State did not agree with the assessment by the rapporteur member state. Only the points relevant for the decision making process should be listed.

Issue on which Co-RMS disagrees with RMS	**Opinion of Co-RMS**	**Opinion of RMS**
The endpoints for *Folsomia candida*	Co-RMS FI is of the opinion that EC_{10} value is preferred over a NOEC value if EC_{10} value is a valid value. Therefore, FI suggest using EC_{10} - 72.3 mg a.s./kg dws in the risk assessment.	RMS is of the opinion that the lowest one should be used in the risk assessment. Therefore, as EC_{10}> NOEC then NOECrep = 52.9 mg/kg dws is the relevant endpoint to use in the risk assessment.
Studies on arthropods (Malecki, S., 2018a; Cockroft, R., 2014) Rival Duo (Agria)	Co-RMS FI is of the opinion as the results showed no clear dose response they question the overall validity of the studies**.**	RMS thinks as all provided studies with representative formulation do not show high toxicity to tested species and the results are comparable and validity criteria are met, these studies can be treat as valid and can be used in the risk assessment.

3.2 PROPOSED DECISION

<confidential>

3.3 RATIONAL FOR THE CONDITIONS AND RESTRICTIONS TO BE ASSOCIATED WITH THE

APPROVAL OR AUTHORISATION(S), AS APPROPRIATE

3.3.1 Particular conditions proposed to be taken into account to manage the risks identified

Proposed condition/risk mitigation measure	Relevance in relation to representative use(s)
<confidential>	<confidential>
<confidential>	<confidential>
<confidential>	<confidential>

3.4 APPENDICES

GUIDANCE DOCUMENTS USED IN THIS ASSESSEMENT

General

Section identity, physical chemical and analytical methods

Section identity and physico chemical properties

European Commission, 2012. Guidance document on the assessment of the equivalence of technical materials of substances regulated under Regulation (EC) No 1107/2009. SANCO/10597/2003-rev. 10.1, 13 July 2012.

Manual on development and use of FAO and WHO specifications for pesticides. First edition - third revision, Rome, March 2016.

United nations recommendations on the transport of dangerous goods (UN RTDG) manual of tests and criteria

Section analytical methods

European Commission, 2000a. Residues: guidance for generating and reporting methods of analysis in support of pre-registration data requirements for Annex II (Part A, Section 4) and Annex III (Part A, Section 5) of Directive 91/414. SANCO/3029/99-rev. 4, 11 July 2000

European Commission, 2000b. Technical material and preparations: guidance for generating and reporting methods of analysis in support of pre- and post-registration data requirements for Annex II (Part A, Section 4) and Annex III (Part A, Section 5) of Directive 91/414. SANCO/3030/99-rev. 4, 11 July 2000

European Commission, 2010. Guidance Document on residue analytical methods. SANCO/825/00-rev. 8.1, 16 November 2010

Technical guideline on the evaluation of extraction efficiency of residue analytical methods, SANTE 2017/10632, rev.3, 22 November 2017

Section Data on application and efficacy

SANCO/2012/11251 – rev. 5 (22 March 2019): Guidance document on the renewal of approval of active substances to be assessed in compliance with Regulation (EU) No 844/2012 (the Renewal Regulation)

Section Toxicology

EFSA, 2014. Guidance on the assessment of exposure of operators, workers, residents and bystanders in risk assessment for plant protection products. EFSA Journal 2014; 12(10):3874, 55 pp., doi: 10.2903/j.efsa.2014.3874

EFSA (European Food Safety Authority), Buist H, Craig P, Dewhurst I, Hougaard Bennekou S, Kneuer C, Machera K, Pieper C, Court Marques D, Guillot G, Ruffo F and Chiusolo A, 2017. Guidance on dermal absorption. EFSA Journal 2017;15(6):4873, 60 pp.

ECHA/EFSA Guidance for the identification of endocrine disruptors in the context of Regulations (EU) No 528/2012 and (EC) No 1107/2009

Guidance document on standardised test guidelines for evaluating chemicals for endocrine disruption, OECD No. 150, 2012

European Food Safety Authority; Submission of scientific peer-reviewed open literature for the approval of pesticide active substances under Regulation (EC) No 1107/2009 (OJ L 309, 24.11.2009, p. 1-50). EFSA Journal 2011;9(2):2092. [49 pp.]. doi:10.2903/j.efsa.2011.2092

SANCO/221/2000 – rev.10- final (2003): Guidance Document on the Assessment of the Relevance of Metabolites in Groundwater of Substances Regulated under Council Directive 91/414/EEC

SANCO/10597/2003 – rev. 10.1 (13 July 2012): Guidance Document on the Assessment of the Equivalence of Technical Materials of Substances Regulated under Regulation (EC) No 1107/2009

Guidance on the Application of the CLP Criteria, ECHA, Version 5 (July 2017)

OECD (2010): Guidance for the Derivation of an Acute Reference Dose. Series on Testing and Assessment No. 124; 8 June 2010

[European Commission: Draft Guidance for the setting of an acute reference dose (ARfD); 7199/VI/99 rev. 5 05/07/2001]

EFSA (European Food Safety Authority), 2016. Technical Report on the outcome of the pesticides peer review meeting on general recurring issues in mammalian toxicology. EFSA Supporting publication 2016:EN-1074. 24 pp.

European Food Safety Authority, 2019. Administrative guidance on submission of dossiers and assessment reports for the peer-review of pesticide active substances, EFSA supporting publication 2019:EN-1612. 49 pp. doi:10.2903/sp.efsa.2019.EN-1612

EFSA Scientific Committee, Hardy A, Benford D, Halldorsson T, Jeger M, Knutsen HK, More S, Naegeli H, Noteborn H, Ockleford C, Ricci A, Rychen G, Silano V, Solecki R, Turck D, Younes M, Aquilina G, Crebelli R, G€urtler R, Hirsch-Ernst KI, Mosesso P, Nielsen E, van Benthem J, Carfi M, Georgiadis N, Maurici D, Parra Morte J and Schlatter J, 2017. Scientific Opinion on the clarification of some aspects related to genotoxicity assessment. EFSA Journal 2017;15(12):5113, 25 pp.

JMPR Guidance Document for WHO monographers and reviewers, 2015; 106 pp.

European Food Safety Authority, 2019. Administrative guidance on submission of dossiers and assessment reports for the peer-review of pesticide active substances, EFSA supporting publication 2019:EN-1612. 49 pp. doi:10.2903/sp.efsa.2019.EN-1612

Section Residue and consumer risk assessment

European Commission, 2013. Working document on the nature of pesticide residues in fish. SANCO/11187/2013. 35 pp.

OECD, 2007. Guideline for the testing of chemicals. 501 Metabolism in crops. 20 pp.

OECD, 2007. Guideline for the testing of chemicals. 502 Metabolism in rotational crops. 18 pp.

OECD, 2007. Guideline for the testing of chemicals. 503 Metabolism in livestock. 21 pp.

OECD, 2007. Guideline for the testing of chemicals. 504 Residues in rotational crops (Limited field studies). 9 pp.

OECD, 2007. Guideline for the testing of chemicals. 505 Residues in livestock. 21 pp.

OECD, 2007. Guideline for the testing of chemicals. 506 Stability of pesticide residues in stored commodities. 12 pp.

OECD, 2007. Guideline for the testing of chemicals. 507 Nature of the pesticide residues in processed commodities – high temperature hydrolysis. 15 pp.

OECD, 2008. Guideline for the testing of chemicals. 508 Magnitude of the pesticide residues in processed commodities. 15 pp.

OECD, 2008. Guidance document on magnitude of pesticide residues in processed commodities, Series on Testing and Assessment No. 96. ENV/JM/MONO(2008)23, 44 pp.

OECD, 2009. Guidance document on the definition of residue, Series on Pesticides No. 31, Series on Testing and Assessment No. 63. ENV/JM/MONO(2009)30, 38 pp.

OECD, 2009. Guideline for the testing of chemicals. 509 Crop field trial. 44 pp.

OECD, 2013. Guidance document on residues in livestock, Series on Pesticides No.73. ENV/JM/MONO(2013)8, 77pp.

OECD, 2016. Guidance document on crop field trials, Series on Pesticides No. 66. Series on Testing and Assessment No. 164. ENV/JM/MONO(2011)50/REV1, 43 pp.

Section fate and behavior in environment

OECD, 1981. Guideline for the testing of chemicals. 101 UV-VIS Absorption spectra (Spetrophotometric method), 6 pp.

OECD, 2000. Guideline for the testing of chemicals. 106 Adsorption- desorption using a batch equilibrium method, 45 pp.

OECD, 2006. Guideline for the testing of chemicals. 111 Partition coefficient (1-Octanol/Water): slow-stirring method, 15 pp.

OECD, 1992. Guideline for the testing of the chemicals. 301 Ready biodegradability, 62 pp.

OECD, 1982. Guideline for the testing of the chemicals. 302A Inherent biodegradability: modified SCAS test, 7 pp.

OECD, 2002. Guideline for the testing of the chemicals. 307 Aerobic and anaerobic transformation in soil, 17 pp.

OECD, 2002. Guideline for the testing of the chemicals. 308 Aerobic and Anaerobic transformation in aquatic sediment systems, 19 pp.

OECD, 2004. Guideline for the testing of the chemicals. 309 Aerobic mineralisation in surface water – simulation biodegradation test, 21 pp.

OECD, 2014. Guideline for the testing of the chemicals. 310 Ready biodegradability – CO_2 in vessels (Headspace test), 18 pp.

OECD, 2008. Guideline for the testing of the chemicals. 316 Phototransformation of chemicals in water – direct photolysis, 53 pp.

EFSA (2017). Technical report on the outcome of the pesticides peer review meeting on the OECD 106 evaluators checklist. EFSA Journal 2017; 18:1326.

SANCO/221/2000 –rev.10- final (2003): Guidance Document on the Assessment of the Relevance of Metabolites in Groundwater of Substances Regulated under Council Directive 91/414/EEC.

EFSA (2014) Guidance Document for evaluating laboratory and field dissipation studies to obtain DegT50 values of active substances of plant protection products and transformation products of these active substances in soil. EFSA Journal 2014; 12(5):3662.

SANCO/10058/2005 version 2.0 (final) (2014): Generic Guidance for Estimating Persistence and Degradation Kinetics from Environmental Fate Studies in Pesticides in EU Registration: Based on the official guidance document of FOCUS Degradation Kinetics in the context of 91/414/EEC and Regulation (EC) No 1107/2009.

SANCO/4802/2001 rev.2 (final) (2015): Generic Guidance for Surface Water Scenarios (version 1.4, May 2015): Based on official guidance document of FOCUS Surface Water Scenarios in the context of 91/414/EEC and Regulation (EC) No 1107/2009.

SANCO/321/2000 rev.2 (2000). FOCUS groundwater scenarios in the EU review of active substances.

Section ecotoxicology

EFSA (2009). Guidance Document on Risk Assessment for Birds and Mammals. EFSA Journal 2009; 7(12):1438

EFSA (2013). Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters. EFSA Journal 2013;11(7):3290

EFSA draft (2013). Guidance Document on the risk assessment of plant protection products on bees (Apis mellifera, Bombus spp. and solitary bees). EFSA Journal 2013;11(7):3295

EU (2002). Guidance Document on Terrestrial Ecotoxicology Under Council Directive 91/414/EEC. SANCO/10329/2002 rev 2 final.

3.5 REFERENCE LIST

Section identity, physical chemical and analytical methods

Section data on application and efficacy

None

Section toxicology

Section residue and consumer risk assessment

Austria 2013 - Draft Evaluation Report Prepared under Article 12 of Regulation (EC) No 396/2005 - Review of the existing MRLs for Cymoxanil;

EFSA 2008 - Conclusion regarding the peer review of the pesticide risk assessment of the active substance cymoxanil Issued on 17 September 2008 (EFSA Scientific Report (2008) 167, 1-116);

EFSA 2015 - Review of the existing maximum residue levels for cymoxanil according to Article 12 of Regulation (EC) No 396/2005 (EFSA Journal 2015;13(12):4355);

EFSA 2019 - Evaluation of confirmatory data following the Article 12 MRL review for cymoxanil (EFSA Journal 2019;17(10):5823); proposed MRLs not yet implemented.(RMS LT does not agree with the proposed lowered MRL of 0.05 mg/kg for grapes).

Section fate and behavior in environment

Section ecotoxicology