Committee for Risk Assessment

RAC

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

benalaxyl (ISO); methyl *N*-(2,6-dimethylphenyl)-*N*-(phenylacetyl)-*DL*-alaninate

EC Number: 275-728-7
CAS Number: 71626-11-4

CLH-O-0000007053-82-01/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

26 November 2021

CLH report

Proposal for Harmonised Classification and Labelling

Based on Regulation (EC) No 1272/2008 (CLP Regulation),
Annex VI, Part 2

International Chemical Identification:

Benalaxyl (ISO);

methyl N-(2,6-dimethylphenyl)-N-(phenylacetyl)-DL-alaninate

EC Number: 275-728-7

CAS Number: 71626-11-4

Index Number: 616-104-00-X

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ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON BENALAXYL (ISO); METHYL *N*-(2,6-DIMETHYLPHENYL)-*N*-(PHENYLACETYL)-*DL*-ALANINATE

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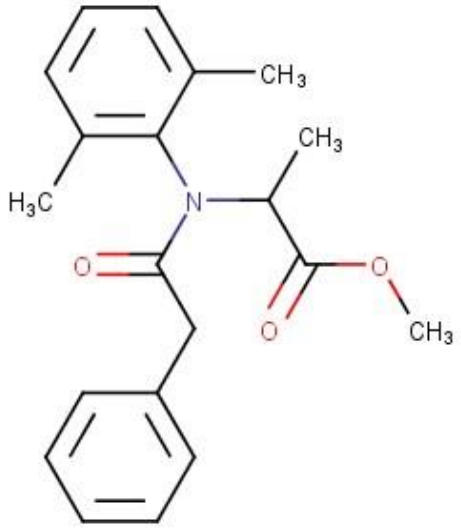
ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON BENALAXYL (ISO); METHYL *N*-(2,6-DIMETHYLPHENYL)-*N*-(PHENYLACETYL)-*DL*-ALANINATE

1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 1: Substance identity and information related to molecular and structural formula of the substance

Name in the C&L Inventory **Name in the IUPAC nomenclature**	benalaxyl (ISO); methyl N-phenylacetyl-N-2,6-xylyl-DL-alaninate methyl N-(phenylacetyl)-N-(2,6-xylyl)-DL-alaninate
Other names	benalaxyl
ISO common name	benalaxyl
EC number	275-728-7
EC name	methyl N-(2,6-dimethylphenyl)-N-(phenylacetyl)-DL-alaninate
CAS number	71626-11-4
CAS name	methyl N-(2,6-dimethylphenyl)-N-(phenylacetyl)-DL-alaninate
CIPAC number	416
Index number	616-104-00-X
Molecular formula	C20H23NO3
Structural formula	
SMILES notation (if available)	
Molecular weight or molecular weight range	325.40
Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	Racemic mixture of “D” - and “ L” I - isomers. “D” - isomer is the most biologically active.
Description of the manufacturing process and identity of the source (for UVCB substances only)	Not relevant; benalaxyl is not a UVCB substance
Minimum purity of active substance (%)	96.0 (Benalaxyl)

1.2 Composition of the substance

Table 2: 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)**
benalaxyl	Min 960g/kg	Aquatic Acute 1, H400 Aquatic Chronic 1, H410	

2 PROPOSED HARMONISED CLASSIFICATION AND LABELLING

2.1 Proposed harmonised classification and labelling according to the CLP criteria

Table 3:

	Index No	Chemical name	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATEs	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	616-104-00-X	benalaxyl (ISO); methyl N-phenylacetyl-N-2,6-xylyl-DL-alaninate	275-728-7	71626-11-4	Aquatic Acute 1 Aquatic Chronic 1	H400 H410	GHS09 Wng	H410			
Dossier submitters proposal	616-104-00-X	benalaxyl (ISO); methyl N-(2,6-dimethylphenyl)-N-(phenylacetyl)-DL-alaninate	275-728-7	71626-11-4	**Retain** Aquatic Acute 1 Aquatic Chronic 1 **Add** Carc. 2 Acute Tox. 4 STOT SE 2	**Retain** H400 H410 **Add** H351 H302 H371 (nervous system)	**Retain** GHS09 Wng **Add** GHS07 GHS08	**Retain** H410 **Add** H351 H302		**Add** oral; ATE=2000 mg/kg bw M=1 M=1	
Resulting entry in Annex VI if adopted by RAC and agreed by Commission	616-104-00-X	benalaxyl (ISO); methyl N-(2,6-dimethylphenyl)-N-(phenylacetyl)-DL-alaninate	275-728-7	71626-11-4	Acute Tox. 4 Carc. 2 STOT SE 2 Aquatic Acute 1 Aquatic Chronic 1	H302 H351 H371 (nervous system) H400 H410	GHS07 GHS08 GHS09 Wng	H302 H351 H410		oral; ATE=2000 mg/kg bw M=1 M=1	

Table 4: Reason for not proposing harmonised classification and status under public consultation

Hazard class	Reason for no classification	Within the scope of public consultation
Explosives	Data conclusive but not sufficient for classification	yes
Flammable gases (including chemically unstable gases)	Hazard class not applicable	No
Oxidising gases	Hazard class not applicable	No
Gases under pressure	Hazard class not applicable	No
Flammable liquids	Hazard class not applicable	No
Flammable solids	Data conclusive but not sufficient for classification	yes
Self-reactive substances	Hazard class not applicable	No
Pyrophoric liquids	Hazard class not applicable	No
Pyrophoric solids	Data conclusive but not sufficient for classification	yes
Self-heating substances	Data conclusive but not sufficient for classification	yes
Substances which in contact with water emit flammable gases	Data lacking	No
Oxidising liquids	Hazard class not applicable	No
Oxidising solids	Data conclusive but not sufficient for classification	yes
Organic peroxides	Data lacking	No
Corrosive to metals	Data lacking	No
Acute toxicity via oral route	Harmonised classification proposed	Yes
Acute toxicity via dermal route	Hazard class not addressed in this proposal	No
Acute toxicity via inhalation route	Hazard class not addressed in this proposal	No
Skin corrosion/irritation	Hazard class not addressed in this proposal	No
Serious eye damage/eye irritation	Hazard class not addressed in this proposal	No
Respiratory sensitisation	Hazard class not addressed in this proposal	No
Skin sensitisation	Hazard class not addressed in this proposal	No
Germ cell mutagenicity	Hazard class not addressed in this proposal	no
Carcinogenicity	Harmonised classification proposed	Yes
Reproductive toxicity	Hazard class not addressed in this proposal	No
Specific target organ toxicity-single exposure	Harmonised classification proposed	yes
Specific target organ toxicity-repeated exposure	Hazard class not addressed in this proposal	No
Aspiration hazard	Data lacking	No
Hazardous to the aquatic environment	Harmonised classification proposed	Yes
Hazardous to the ozone layer	Hazard class not addressed in this proposal	No

3 HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING

Benalaxyl is a fungicide used as an active substance in plant protection products (PPP). Benalaxyl was included in Annex I to Directive 91/414/EEC by Commission Directive 2008/58/EC and has been deemed to be approved under Commission Implementing Regulation (EU) No 540/2011 in accordance with Regulation (EC) No 1107/2009, which was amended in accordance with the Commission Implementing Regulation 1100/2011.

Commission Implementing Regulation (EU) 2020/869 (7) extended the approval period of benalaxyl to 31 July 2021 in order to allow the renewal process to be completed before the expiry of the approval period of that substance.

On 19 December 2019, the EFSA communicated to the Commission its conclusion on whether benalaxyl can be expected to meet the approval criteria provided for in Article 4 of Regulation (EC) No 1107/2009.

In its conclusion, the EFSA identified a number of concerns. In particular, the potential groundwater contamination by relevant metabolites could not be excluded. Furthermore, a long-term risk to to birds and earthworm-eating birds from secondary poisoning from benalaxyl was identified as a critical area of concern. Concerns were also identified in relation to the long-term risk to non-target arthropods for all representative uses. Finally, following the request by the Authority for additional information to assess the endocrine disrupting potential of benalaxyl, the applicant confirmed that no additional studies would be performed or submitted. Consequently, it cannot be concluded that the substance has no endocrine disrupting properties.

Following the EFSA request for additional information the applicant confirmed that no additional studies will be performed.
However, given that a decision on the non-renewal of the approval is being taken ahead of the expiry of that extended approval period, a new COMMISSION IMPLEMENTING REGULATION (EU) 2020/1280 of 14 September 2020 concerning the non-renewal of the approval of the active substance benalaxyl, in accordance with Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market, and amending the Annex to Commission Implementing Regulation (EU) No 540/2011, has been promoted.

Benalaxyl is currently listed in Annex VI of Regulation (EC) 1272/2008. The current harmonized classification (2005) is Aquatic Acute 1, H400 and Aquatic Chronic 1, H410.

RAC general comment
Benalaxyl is a fungicide which is currently classified as Aquatic Acute 1, H400 and Aquatic Chronic 1, H410 in Annex VI of the CLP Regulation. No M-factors have been set previously.

4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

Specific justification that action is needed at EU level is required, since benalaxyl, an active substance for plant protection products under Regulation (EC) 1107/2009 has been followed the procedure of the renewal of the approval, under Article 14 of Regulation (EC) No 1107/2009. Therefore, new values of endpoints have been identified for parent, benalaxyl and its metabolites

EFSA had concluded that exists dose-related evidence on carcinogenicity and neurotoxicity of benalaxyl is involved in classification of benalaxyl and Carc. Cat.2; H351 based on observed astrocytomas may be

warranted according to CLP Regulation criteria.This proposal for classification was reported in the RAR and in the EFSA conclusion.

The Acute toxicity Cat.4; H302 based on the oral acute studies (Anonymous. (2013a; Anonymous (2014a,b,c)) identified, too and reported in the RAR and in the EFSA conclusion (EFSA Journal 2020;18(1):5985).

Given the current classification of benalaxyl and the outcomes of the European renewal peer review of this active substance, a proposal for a classification as Carc.2:H351, STOT SE 2; H371 and Acute tox.4; H302; ATE = 2000 mg/kg bw has been done.

5 IDENTIFIED USES

Benalaxyl is an active substance for pesticide belonging to the phenylamide group name and acylalanine chemical group of systemic fungicide with apoplastic translocation which inhibits mycelial growth of fungi and germination of zoospores (fungistatic action). The mode of action of Benalaxyl is described by nucleic acid synthesis on RNA polymerase. Benalaxyl has specific activity against Peronosporales (Oomycetes) both on; (a) species with saprophytic phase such as Pythium spp. and; (b) on obligate parasitic plant pathogens such as the downy mildews and late blight (Bremia, Peronospora, Phytophthora, Plasmopara, Pseudoperonospora). The mode of action of benalaxyl is twofold:

- Interference with rRNA synthesis by affecting the endogenous RNA polymerase activity;

- Interference with membrane function by inducing the leakage of amino acids from mycelium.

6 DATA SOURCES

Benalaxyl was evaluated for renewal of approval as a pesticide active substance according to Commission Regulation (EU) No 844/2012 under the 3rd active ingredient renewal process, AIR 3.

The primary sources of data are the following:

Dossier from Applicant (FMC,USA)

The Renewal Assessment Report (RAR) plus associated documentation published by EFSA and available at https://efsa.onlinelibrary.wiley.com

EFSA RAR Volumes 1-4 (2018)

EFSA LoEP (2018)

EFSA ED Assessment and Conclusion (2019)

EFSA Conclusion (2020) at https://www.efsa.europa.eu/en/efsajournal/pub/5985

7 PHYSICOCHEMICAL PROPERTIES

Table 5: Summary of physicochemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
Physical state at 20°C and 101,3 kPa	Purified active substance: White, microcrystalline solid. Technical active substance: Whitish, microcrystalline solid.	Anonymous (1995), N. 94/1087.B CA B 2.3	Visual assessment
Melting/freezing point	Melting point: 76.8°C.	Anonymous (1995), N. 94/1087.B	Method EEC A.1

Property	Value	Reference	Comment (e.g. measured or estimated)
		CA B.2.1	
Boiling point	The test substance starts boiling at 302-307 ºC, showing changes from 250 ºC upwards	Anonymous (1995) N. 030/95 CA B.2.1	Method EEC A.2
Relative density	Data not available		
Vapour pressure	5.72×10^{-4} Pa at 20°C 6.64×10^{-4} Pa at 25°C Henry's law constant (calculated) = 6.50×10^{-3} $Pa.m^3.mol^{-1}$ at 20°C	Anonymous (1995), N. 94/1087.B CA B.2.2	Method EEC A.4
Surface tension	The surface tension of benalaxyl, at 22°C, was determined to be 47.0 mN/m.	Anonymous (1995), N. 94/1087.C CA B.2.12	Method EEC A.5
Water solubility	0.0286 g/l at 20°C	Anonymous (1995), N. 94/1087.B CA B.2.5	Method EEC A6
Partition coefficient n-octanol/water	Log P_{ow} for Benalaxyl: 3.54 at 20°C and at pH=6.1	Anonymous (1995), N. 94/1087.B CA B.2.7	Method EEC A8
Flash point	Not applicable (melting point > 40°C).	-	-
Flammability	Not flammable	Anonymous (1993), 102 CA B.2.9	Method EEC A10
Explosive properties	Not explosive	Anonymous (1995), N. 94/1087.C CA B.2.11	Method EEC A14
Self-ignition temperature	Data not available	-	-
Oxidising properties	Maximum combustion velocity of benalaxyl (0.78 mm/s) is slightly higher then the maximum one shown by the reference mixture (0.60 mm/s).	Anonymous (1995), N. 94/1087.C CA B.2.13	Method EEC A17
Granulometry	Data not available	-	-
Solubility in organic solvents	Solubility in the following organic solvents at 22 °C: n-heptane 19.4 g/kg Xylene > 250 g/kg Methanol > 250 g/kg Acetone > 250 g/kg 1,2-dichloroethane > 250 g/kg ethyl acetate > 250 g/kg	Anonymous AnonymusAnonymusAnonymus(1995), N. 94/1087.C CA B.2.6	In house method
Dissociation constant	Data not available	-	-
Viscosity	Data not available	-	-

8 EVALUATION OF PHYSICAL HAZARDS

8.1 Explosives

Table 6: Summary table of studies on explosive properties

Method	Results	Reference
EEC A.14	Not explosive	Anonymous (1995), N. 94/1087.C

8.1.1 Short summary and overall relevance of the information provided on exploziv properties

Benalaxyl was tested for explosive properties using the EC Method A.14 and was found not to be explosive.

8.1.2 Comparison with the CLP criteria

Benalaxyl does not carry functional groups listed in tables A6.1 in Annex 6 (Screening Procedures) of the Manual of Tests and Critereia associated with explosive properties. CLP criteria are not met.

8.1.3 Conclusion on classification and labelling for explosive properties

Not classified.

8.6. Flammable solids

Table 7: Summary table of studies on flammable solids

Method	Results	Reference
EEC A.10	Not flammable	Anonymous s(1993) 102

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

Benalaxyl was tested for flammable properties using the EC Method A.10. The method used for classification purposes according to CLP criteria is the UN Test N.1 described in the UN RTDG, Manual of Tests and Criteria (7th revision). However, as reflected in the CLP Guidance and ECHA Guidance on Information Requirements and Chemical Safety Asssessment (R.7.1.10.3), if the result of an A.10 method indicates that a classification as a flammable solid does not apply (result: not flammable), no more testing is necessary.

8.6.2 Comparison with the CLP criteria

Benalaxyl is not met the flammable solids CLP criteria.

8.6.3 Conclusion on classification and labelling for flammable solids

Not classified.

8.7 Self-reactive substances

-

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

-

8.7.2 Comparison with the CLP criteria

Benalaxyl does not carry chemical groups listed in tables A6.1 in Annex 6 (Screening Procedures) of the Manual of Tests and Criteria associated with explosive or self-reactive properties. CLP criteria are not met.

8.7.3 Conclusion on classification and labelling for self-reactive substances

Not classified.

8. 8. Pyrophoric liquids-

Not applicable

8.9 Pyrophoric solids

Benalaxyl does not ignite spontaneously in contact with air based on experience of handling and use.

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

According to Section 2.10.4.1 of Annex 1 of CLP, the classification procedure for pyrophoric solids need not be applied when experience in manufacture and handling shows that the substance does not spontaneously ignite upon coming into contact with air at normal temperatures. There are no reports in the available studies of benalaxyl spontaneously igniting when in contact with air.

8.9.2 Comparison with the CLP criteria

Benalaxyl does not meet the criteria for classification as a pyrophoric solid.

8.9.3 Conclusion on classification and labelling for pyrophoric solids

Not classified.

8. 10 Self-heating substances

Table 8: Summary table of studies on self-heating substances

Method	Results	Remarks	Reference
EEC A16	No ignition or significant difference between sample and oven temperatures were observed by heating until melting occurs.	Not	Anonymous (1995), N. 94/1087.C

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

Benalaxyl is not an auto-inflammable substance when tested for auto-flammability using the method EC A.16

8.10.2 Comparison with the CLP criteria

According to the ECHA Guidance on the Application of the CLP Criteria (version 5.0 July 2017), the test method A.16 is not deemed appropriate to evaluate the self-heating property of solids towards a CLP

classification. However, substances with a low melting point (< 160ºC) should not be considered for classification in this hazard class. Benalaxyl has a measured melting point of 76.8ºC.

8.10.3 Conclusion on classification and labelling for self-heating substances

Not classified.

8.11 Substances which in contact with water emit flammable gases

The chemical structure of the substance does not contain metals or metalloids.

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

8.11.2 Comparison with the CLP criteria

According to the CLP Criteria laid down in Annex I, 2.12.1 is not deemed appropriate to evaluate the substances which in contact with water emit flammable gases towards a CLP classification. However, Benalaxyl is substance with a chemical structure without metals or metalloids.

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

Not classified.

8.13. Oxidising solids

Table 9: Summary table of studies on oxidising solids

Method	Results	Reference
EEC A.17	Maximum combustion velocity of benalaxyl (0.78 mm/s) is slightly higher then the maximum one shown by the reference mixture (0.60 mm/s).	Anonymous (1995), N. 94/1087.C

8.13.1 Short summary and overall relevance of the provided information onoxidising solids

Benalaxyl was tested for its oxidizing properties according to the method EEC A.17 and the result shows to be a weak oxidizing substance.

8.13.2 Comparison with the CLP criteria

According to Section 2.14.4.1 point b) of Annex I of CLP, for organic substances the classification procedure for this hazard class shall not apply if the substance or mixture contains oxygen, fluorine or chlorine and these elements are chemically bonded only to carbon or hydrogen. Benalaxyl does not contain such chemical groups and it was not an oxidizing substance according to test method EC A.17. Therefore, classification for this class is not applicable to benalaxyl

8.13.3 Conclusion on classification and labelling for oxidising solids

Not classified.

RAC evaluation of physical hazards

Summary of the Dossier Submitter's proposal

Explosives

A negative A.14 test (Constantini, 1995) is available for benalaxyl. The Dossier Submitter (DS) proposed no classification based on absence of chemical groups associated with explosive properties.

Flammable solids

The DS proposed no classification based on a negative A.10 test (Jonas, 1993). A negative result in an A.10 test can be used for classification under CLP (see Guidance on information requirements and chemical safety assessment, R.7.1.10.3).

Pyrophoric solids

The DS proposed no classification based on experience in handling and use.

Self-heating substances

An A.16 test (Constantini, 1995) showed no significant difference between sample and oven temperatures until the melting point (76.8°C). The DS proposed no classification based on a melting point below 160°C.

Oxidising solids

An A.17 test (Constantini, 1995) was positive. The maximum combustion velocity of benalaxyl was 0.78 mm/s, while the maximum combustion velocity of the reference mixture was 0.60 mm/s. The DS proposed no classification based on chemical structure (the substance contains oxygen, but the oxygen is chemically bonded only to carbon).

Comments received during consultation

A manufacturer supported no classification for physical hazards.

Assessment and comparison with the classification criteria

Explosives, Flammable solids, Pyrophoric solids, Self-heating substances

RAC agrees with the DS's assessment that **no classification** is warranted for the above hazard classes.

Oxidising solids

The substance contains oxygen atoms but these are only bonded to carbon. Thus, the

substance meets the waiving criteria of the CLP based on structure (CLP, Annex I, 2.14.4.1)

In the A.17 test benalaxyl showed a combustion velocity comparable to that of the reference substance. The interpretation of the result according to the Risk Assessment Report (RAR) is that 'the test sample proved to be a weak oxidising substance'. However, the structure does not indicate oxidising properties and a follow-up test to exclude a false positive (with a non-combustible substance in place of cellulose, or in an inert atmosphere) is not available. Therefore, the result is considered equivocal rather than positive. Further, a result of an A.17 test cannot be directly compared with the CLP criteria.

As the only test available is an equivocal A.17 test and the CLP waiving criteria have been met, RAC agrees with the DS's proposal that **no classification** is warranted**.**

8.14 Organic peroxides

Benalaxyl is not an organic peroxide. It does not contain the bivalent O-O-structure and it is not thermally unstable.

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

-

8.14.2 Comparison with the CLP criteria

-

8.14.3 Conclusion on classification and labelling for organic peroxides

Not classified.

8.15 Corrosive to metals

No data derived in accordance with the recommended test method in CLP (test in Part III, sub-section 37.4 of the UNRTDG Manual of Tests and Criteria) have been provided. According to the ECHA Guidance on the Application of the CLP Criteria (version 5.0 July 2017), the UN Test C.1 excludes solids while it considers 'solids that may become liquid upon transportation'. Benalaxyl is supplied as a dry solid and its measured melting point is > 55ºC, which is the test temperature required in the UN Test C.1 test.

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

-

8.15.2 Comparison with the CLP criteria

According to the ECHA Guidance on the Application of the CLP Criteria (version 5.0 July 2017), the UN Test C.1 excludes solids while it considers 'solids that may become liquid upon transportation'. Benalaxyl is supplied as a dry solid and its measured melting point is > 55ºC, which is the test temperature required in the UN Test C.1 test.

8.15.3 Conclusion on classification and labelling for corrosive to metals

Not classified.

9 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

A summary of the toxicokinetic (ADME) data pertaining to benalaxyl is provided to aid the evaluation of toxicity hazards for human health.
The study summaries are presented in Risk Assessment Report - RAR, Benalaxyl- Volume 3 Annex B.6: Toxicology and metabolism, November, 2018.

Table 10: Summary table of toxicokinetic studies

Method	Results	Remarks	Reference
^{14}C-Benalaxyl, Blood pharmacokinetics, excretion and tissue distribution of radioactivity in the rat after a single oral administration ^{14}C-Benalaxyl Oral (gavage) Sprague – Dawley rats M 2 groups G1, G2 3 rats/group G1 - 10 mg kg bw G2 - 100 mg/kg bw Time: 0.25 (15 min.), 1, 2, 4, 6, 8, 24, 48 hrs and 72 hrs OECD TG 417 GLP: None Acceptable	a preliminary investigation of pharmacokinetics of labeled benalaxyl at two different doses applied by gavage to the rats. Similar pharmacokinetics profiles were obtained for both dose groups. peak blood level = 0.25 - 1 h elimination half-live ÷ 50 hrs ^{14}C-Benalaxyl – quantificable level at 72 hrs (in blood)	no relevant guidelines in conducting the pharmacokinetics and metabolism study	RAR-08_Vol-3 CA_B.6.1.2.1 Reference number: CA 5.1.1/01 Anonymous (1996a)
^{14}C-Benalaxyl, Blood pharmacokinetics, excretion and tissue distribution of radioactivity in the rat after a single oral administration (Final report) ^{14}C-Benalaxyl Oral (gavage)	A tissue distribution, excretion and pharmacokinetics of ^{14}C-Benalaxyl after a single oral administration to male rats at two dose levels ^{14}C-Benalaxyl is rapidly absorbed from the gastrointestinal tract, small absorbtion related with dose, a low peak concentrations and AUCs (blood concentration vs time) elimination half-life = 30 hrs faeces excretion > 90% urinary excretion <10%	a repeat low dose (14 daily doses of unlabelled substance followed by a single administration of radiolabel material) composition of excreted by biliary-faecal route remains	RAR-08_Vol-3 CA_B.6.1.2.2 Reference number: CA 5.1.1/02 Anonymous (1996b)

Method	Results	Remarks	Reference
Sprague Dawley CrlCD (SD)Br M 2 groups (15/G) G1 - 10 mg/kg bw (41 µCi/kg) G2 - 100 mg/kg bw (49 µCi/kg) OECD TG 417 GLP/QA: Yes/Yes Acceptable	24 h – urinary route is majoritary than by biliary-faecal route pick level in LIVER at 0.5 h after administration and high concentration in kidney at 72 h and 168 h after the administration it was present only in liver	unknown; (some metabolites unidentified) No residue definition for human biomonitoring can be defined (unfinalised)	
^{14}C-Benalaxyl, Blood pharmacokinetics, excretion and tissue distribution of radioactivity in the rat after repeated oral administrations ^{14}C-Benalaxyl Oral (gavage) 14 consecutive days 10 mg/kg bw of benalaxyl unradiolabelled and the last one with ^{14}C-Benalaxyl OECD TG 417 GLP/QA: Yes/Yes Acceptable	blood pharmacokinetics, the excretion and tissue distribution of radioactivity after repeated daily administration of benalaxyl in rats (15 consecutive doses, ^{14}C-Benalaxyl with the last dose). a small part of ^{14}C-benalaxyl is rapidly absorbed from the gastrointestinal tract low peak concentrations and AUCs elimination half-life = around 36 hrs >90% excretion in faeces <10% excreted by urinary route Elevated levels of radioactivity were found in the liver at 0.5 h after administration, presuming an extensive metabolism of benalaxyl. Apart from the liver, increased concentrations, in respect to the blood levels, were found also in the intestine wall and kidneys. at 72 h and 168 h after the administration it was present only in liver	B.6.1.2.2, a single oral dose of ^{14}C-Benalaxyl B.6.1.2.3 administration of a single oral dose of radiolabelled material was preceeded by 14 daily doses of unlabeled material (a biliary excretion study (at the both dose levels) Results for blood profiles, excretion pattern and tissue distribution after a single administration of benalaxyl (B.6.1.2.2) were very similar to those obtained in this study (B.6.1.2.3)	RAR-08_Vol-3 CA_B.6.1.2.3 Reference number: CA 5.1.1/03 Anonymous (1996c)
Profiling of radiolabelled metabolites of ^{14}C-Benalaxyl in urine and faeces of male rats after single and repeated oral administration ^{14}C-Benalaxyl Oral (gavage) Sprague-Dawley rats M dose levels: 10 mg/kg bw or 100 mg/kg bw Samples	profile radiolabelled metabolites of benalaxyl in urine and faeces; to determine the rate of formation of the benalaxyl metabolites and to isolate and identify the main metabolites of benalaxyl in urine and faeces. the metabolites are common for the urinary and the faecal metabolic pathway in the both studies Urine: - benalaxyl was absent in urine - metabolite 4 - (0.83%, 0.89% and 1.38% of single low dose, single high dose and multiple doses, respectively) - metabolite 6 - (0.60%, 0.78% and 0.76% of single low dose, single high dose and multiple doses, respectively) - metabolite 7 - (1.02%, 0.995% and 0.82% of single low dose, single high dose and multiple doses, respectively) - metabolite 8 - (0.63%, 1.005% and 0.78% of single low dose, single high dose and multiple doses, respectively) metabolite 12 - unchanged benalaxyl (2.73%, 1.05% and	Study is relevant for pharmacokinetics profile of benalaxyl. the biological samples from study (B.6.1.2.2) – 5 m/ dose one single low dose: 10 mg/kg bw (41) or 100 mg/kg bw (8.5 µCi/kg) and 10 µCi of radiolabelled benalaxyl / rat	RAR-08_Vol-3 CA_B.6.1.2.4 Reference number: CA 5.1.1/04 Anonymous (1997)

Method	Results	Remarks	Reference
Urine: 0-8, 8-24, 24-48, 48-72, 72-96, 96-120, 120-144 and 144-168 hrs Faeces: 0-8 and 8-24 hrs OECD TG 417 GLP/QA: Yes/Yes Acceptable	1.42% of a single low dose, a single high dose and multiple doses, respectively) Faeces: - metabolite 8 - (15.99%, 16.41% and 20.25% of a single low dose, a single high dose and multiple doses, respectively) - metabolite 6 - (7.40%, 9.80% and 9.80% of a single low dose, a single high dose and multiple doses, respectively) - metabolite 7 - (6.56%, 5.80% and 4.73% of a single low dose, a single high dose and multiple doses, respectively) - metabolite 5 - (6.08%, 6.65% and 6.25% of a single low dose, a single high dose and multiple doses, respectively) metabolite 4 - (6.24%, 5.18% and 5.82% of a single low dose, a single high dose and multiple doses, respectively) metabolite 2 - (6.69%, 5.78% and 5.68% of a single low dose, a single high dose and multiple doses, respectively) - metabolite 1 - (8.59%, 8.12% and 9.96% of a single low dose, a single high dose and multiple doses, respectively) - metabolites 3, 9, 10 and 11 were lower than 5% - metabolites 4, 5, 6, 7, 8, 9, 10, 11 and 12 were identified in faeces by GS-MS chromathgraphy techniques - metabolite 1 sum of 9 components: (every < 3.30%) Study results reveals that the metabolic profile was the same following a single oral (low and high) administration or multiple oral administration to rats. The urinary metabolic pathway was identical to the faecal metabolic pathway as the isolated faecal metabolites were the same as urinary metabolites. The only difference was the absence of benalaxyl in urine. Urine was the minor way of radioactivity elimination and therefore the percentages of each metabolite in urine were always very low. Unchanged benalaxyl were found only in faeces.	a repeat low dose (14 daily doses of unlabelled substance followed by a single administration of radiolabel material) Test duration: 168 hrs after dose administration	
[^{14}C]-Benalaxyl Biliary excretion study in the rat Radiolabelled benalaxyl was bile duct-cannulated by oral gavage 2G/4 rats M+F a single administration of ^{14}C benalaxyl: 10 mg/kg bw or 100 mg/kg bw Test period: 8, 24, 48 and 70 hrs Bile collected: 24, 48 and 70 hrs after administration Urine, faeces and cage wash	the percents of benalaxyl radiolabelled excreted in the urine, faeces and bile. total radioactivity in each sample: Low dose: – in bile: a mean of 88.93% and 82.13% (m+f), over 70hrs and 85.58% and 80.30% (m+f) in < 8hrs - in urine: a mean of 3.87% and 7.13% (m+f), after 70hrs - in the faeces: 4.89% and 5.33% (m+f), after 70hrs - in the cage wash: 0.57% and 0.29%, after 70hrs - in tissue of GI tract: 0.01% of dose recovered from the males and 0.02% from the females, after 70hrs High dose: – in bile: a mean of 75.22% and 66.03% of over the 70hrs (m+f) and 69.56% and 60.62% (m+f) < 8 hrs - in urine: a mean of 4.79% and 13.95% (m+ f), after 70hrs - in faeces: 17.56% and 9.18% (m+f), after 70hrs - in the cage wash: 0.18% and 1.41% (m+f) – in tissue of GI tract: a mean of 0.12% of dose recovered from the males and 0.01% from the females ^{14}C-Benalaxyl is absorbed and excreted rapidly, mainly via the enterohepatic circulation (up to 89%) although up to 14%	[^{14}C]-Benalaxyl excretion: <8 hours after dosing - (60 - 80%), in bile in 24h, after dosing (both doses) between 89.45% - in bile 96.80% - urine, faeces and bile (including cage wash) after 70hrs after dosing < 0.2% in carcass	RAR-08_Vol-3 CA_B.6.1.2.5 Reference number: CA 5.1.1/05 Anonymous (2001)

Method	Results	Remarks	Reference
collected: daily up to 70 hrs OECD TG 417 GLP/QA: Yes/Yes Acceptable	may be excreted via renal circulation.		
Absorption, metabolism and excretion of ^{14}C-Galben in albino rats (part 1) ^{14}C- Galben = ^{14}C-Benalaxyl in alpha position of the ester moiety Oral (stomach tube) 8 Albino rats M+F one dose = 100 mg/kg bw of ^{14}C -Galben rats in individual cages - urine, faeces and the various organs ^{14}C-Galben - 1, 2, 4 and 8 days after treatment in blood, organs and carcass 2 rats (m+f) Test method: In house method Guidelines: Guidelines were not available at the time the test was performed GLP: It was not compulsory at that time Acceptable	absorption, distribution, rates and routes of excretion and biotransformation of [^{14}C] - Galben ^{14}C- Galben - rapidly absorbed and completely metabolized excretion after 1, 2, 4 days after administration Metabolites: 2 days after administration - rapidly excreted and almost completely in the faeces and urine 8 days after administration - negligible excretion in urine the higher concentration was measured in the liver - acid and deacylated acid in tissues and organs of rats of both sexes, have been identified among many other metabolites detected in urine and faeces - unknown G6 and G7 and deacylated acid are excreted in higher amounts in urine and faeces - no significant differences between male and female regarding excretion, retention, distribution, as well as the metabolism of ^{14}C – benalaxyl - percentage of radiolabeled was excreted in urine and faeces within 0-48 hours; 97.40% and 97.23% of ^{14}C (m+f) peak elimination of ^{14}C-Galben: 2 - 24 hrs (urine and faeces) Excretion aftter 8 days after administration: ^{14}C-Galben - faeces -76.8% m and 75.18% f - urine - 24.25% m and 22.90% f Excretion 0 - 8 days after administration: ^{14}C-Galben - urine and faeces, approx. 100% (m+f) Distribution and levels of radioactivity in organs and tissues: - 24 hours following test study the major part of the ^{14}C administered remains in the intestine and in its content, in the liver and in the carcass. - 8 days after treatment, only 0.283% (m) and 0.391% (f) of the ^{14}C administered dose remains in the rats, distributed among the organs and tissues Quantitative, the radioactivity as mg/kg is mainly localized in the liver. No significant differences between male and female regarding excretion, retention, distribution, as well as the metabolism of ^{14}C – Benalaxyl have been revealed.	A major metabolites (>5%) Galben-acid and deacylated acid have been identified among the many metabolites detected in urine and faeces G6 and G7 + deacylated acid higher excretion (without supportive studies)	RAR-08_Vol-3 CA_B.6.1.2.6 Reference number: CA 5.1.1/06 Anonymous (1981)
^{14}C-GALBEN metabolism in albino rats (part	to characterize G_6, G_7 and deacylated GALBEN acid, which are the most significant metabolites in urine and faeces Study deals with integrating the results obtained after oral	The right position of both carboxy and	RAR-08_Vol-3 CA_B.6.1.2.7

Method	Results	Remarks	Reference
2) ^{14}C- Galben = ^{14}C-Benalaxyl in alpha position of the ester moiety 8 Albino rats M+F administration by stomach tube a single dose = 100 mg/kg bw ^{14}C-Galben ^{14}C-GALBEN - 1 , 2, 4 and 8 days after treatment in blood, organs and carcass 2 rats (m+f) Test method: In house method Guidelines: Guidelines were not available at the time the test was performed GLP: None Acceptable	administration of ^{14}C – GALBEN and focuses on finding the majority metabolizing liver as the primary target organ. G6, G7 and deacylated GALBEN acid are the most significant metabolites in urine and faeces NMR spectra of G6, G7A (named deacylated acid in Part 1), G7B (previously G7) compounds and the ones of the relevant methyl esters, show the presence in each molecule of the following groups contained in GALBEN: CH3-CH<, Ar-CH2-CO-, -COOCH3, >CH-CH3, Arom (8H). They are quite similar to the synthesised M1 and M2 (G8 and G14) compounds. As suggested for G7A and G7B, additional studies would be required to attribute to the G8 and G14. G6 metabolite, which contains both acidic and alcoholic groups in its molecule according to the NMR characterization, can be formed: a) by oxidizing the aromatic methyl group (at 6 position) of G7A compound; b) by oxidizing a presumed metabolite with two aromatic hydroxymethyl groups to obtain only one carboxylic group.	hydroxymethyl groups in the aromatic ring of G6 metabolite has not been determined. Such compound was isolated and characterized during in vitro studies of GALBEN with rat liver microsomes.	Reference number: CA 5.1.1/07 Anonymous (1983)
In vitro degradation of ^{14}C-GALBEN with rat liver microsomes, supplementary study ^{14}C- Galben = ^{14}C-Benalaxyl in alpha position of the ester moiety ^{14}C-GALBEN to Albino rats by a metabolic scheme from that in vitro test (enzyme was isolated from rat liver microsomes) Test method: In house method Guidelines were not available at the time the test was performed GLP: None Acceptable	A supplementary study for isolating metabolites: benalaxyl when oxidised only by rat liver microsomes and to isolate the metabolites which were found only in traces *in vivo* as result of subsequent reactions. *In vivo* rat liver microsomes to isolate the traces of metabolites M4 as a possible intermediate in the formation of G6. M1 and M2 correspond to G8 and G14 found in urine and/or faeces of rats M1 and M2 percentages decrease progressively being precursors for M4, G7A and G7B M3 is present at low levels G6, G7A and G7B correspond to the already known metabolites isolated and characterized from urine and faeces of rats	Supplementary study for isolating metabolites which confirms the validity of the technique adopted, which permits to isolate also intermediate metabolites (such as M1, M2 and M4). They can be assumed to exist (but cannot be identified) in both urine and faeces	RAR-08_Vol-3 CA_B.6.1.3.1 Reference number: CA 5.1.1/08 Anonymous (1983)

<table>
<tr><th>Method</th><th>Results</th><th>Remarks</th><th>Reference</th></tr>
<tr><td>Metabolic Evaluation of Excreta from Rats Administered a Single Oral Dose of Benalaxyl

Oral route
1 G/ 4 rats
M+F
Vehicle: 0.5% methylcellulose (MC) w/v in distilled water
Dose: 100 mg/kg bw
urine and faeces - 24 hrs prior to dosing
faeces - 24 hrs period
and (0-24 hrs)
urine - 0-8 hrs and 8-24 hrs
OECD TG 417 – OPPTS 870.7485
GLP/QA: Yes/Yes
Acceptable</td><td>to identify and to determine the concentrations of benalaxyl acid, a metabolite of benalaxyl, in faecal and urine samples collected from male and female rats after a single oral administration of benalaxyl.

no mortality
Quantitative identification: The study follows the comparable levels of benalaxyl acid excreted in faeces and urines of the both sexes.

100 mg/kg bw - up to 24h – urinary excretion in M+F

Comparable levels are excreted in the faeces but female urine contains roughly 2 to 3 times the amount of the metabolite than male urine collected up to 24 h after administration</td><td></td><td>RAR-08_Vol-3 CA_B.6.1.3.2

Reference number: CA 5.1.1/09

Anonymous (2015)</td></tr>
<tr><td>An Oral (Gavage) Acute Neurotoxicity Study of Benalaxyl in Rats

Benalaxil
Oral (gavage)
2 G/5 randomnly selected rats
M+F
Dose: 1000 mg/kg bw/G
Approx.. 3-4 hrs following dose administration - F0B and motor activity assessments, Day 0
5 randomly selected rats/sex in cages for approximately 24 hours –urine and feces were collected during</td><td>Presence/absence of benalaxyl acid (metabolite M9) in urine and faeces of rats for up to 24 hours following administration of the test substance.

M9 was found in both faeces and urine of rats treated once by gavage with benalaxyl at 1000 mg/kg bw/d

M9 is a rat metabolite and therefore tested in toxicity studies performed with the parent benalaxyl.

Benalaxyl Acid Concentrations in Rat Urine and Faeces
<table>
<tr><th rowspan="2">Rat Number</th><th rowspan="2">Group</th><th rowspan="2">Gender</th><th>Faeces</th><th>Urine</th></tr>
<tr><th>Concentration (ng/g)</th><th>Concentration (ng/mL)</th></tr>
<tr><td>86875</td><td>4</td><td>M</td><td>8533</td><td>353</td></tr>
<tr><td>86877</td><td>4</td><td>M</td><td>9415</td><td>511</td></tr>
<tr><td>86882</td><td>4</td><td>M</td><td>8377</td><td>380</td></tr>
<tr><td>86903</td><td>4</td><td>M</td><td>9234</td><td>313</td></tr>
<tr><td>86906</td><td>4</td><td>M</td><td>11386</td><td>207</td></tr>
<tr><td colspan="3">Mean value</td><td>9389</td><td>352.8</td></tr>
<tr><td>86923</td><td>4</td><td>F</td><td>15388</td><td>870</td></tr>
<tr><td>86928</td><td>4</td><td>F</td><td>2073</td><td>1058</td></tr>
<tr><td>86946</td><td>4</td><td>F</td><td>5081</td><td>1018</td></tr>
<tr><td>86948</td><td>4</td><td>F</td><td>6905</td><td>850</td></tr>
<tr><td>86956</td><td>4</td><td>F</td><td>13470</td><td>981</td></tr>
<tr><td colspan="3">Mean value</td><td>8583.4</td><td>955</td></tr>
</table></td><td>A neurotoxic potential of benalaxyl was evaluated using a neurotoxicity screening battery (functional observational battery, locomotor activity, and neuropathological assessments).</td><td>RAR-08_Vol-3 CA_B.6.1.3.3

Reference number: CA 5.1.1/10 (see CA 5.7.1/02)

Anonymous (2014a)</td></tr>
</table>

Method	Results	Remarks	Reference
the testing Every cage – no rince (sample collected) Guidelines: OECD TG 424 and OPPTS 870.6200 GLP/QA: Yes/Yes Acceptable			
Stereoselective Determination of Benalaxyl in Plasma by Chiral High-Performance Liquid Chromatography with Diode Array Detector and Application to Pharmacokinetic Study in Rabbit Benalaxyl used for i.v. administration, and dosing volume not stated 12 Japanese White rabbits M Only one dose = 40 mg/kg bw / d by i.v., ear vein Test protocol GLP, GEP, Guidelines (US EPA, OECD) No information on GLP status of OECD GD R (-)- and S (+)-enantiomers were separated and collected by HPLC Acceptable	Studies on ADME concluding that the pharmacokinetics of benalaxyl enantiomers was stereoselective in rabbits. The full relevance will become clear in the context of the results of a full *in vitro* comparative metabolism study. The results suggested that the pharmacokinetics of benalaxyl enantiomers was stereoselective in rabbits after i.v. administration of racemic benalaxyl $p < 0.05$ after i.v. of racemic benalaxyl (40 mg/kg), the maximum concentrations (Cmax) of R (-)- and S (+)-benalaxyl were found at approx. 0.017 h (Tmax) mean t1/2, Cmax and MRT were not statistically different between the two enantiomers, while the mean total plasma clearance (CL) and AUC0→∞ value of S (+)-enantiomer were significantly different from ($P < 0.05$) those of R (-)-enantiomer. The S (+)-/R (-)-enantiomer ratio of the AUC0→∞ after the racemate administration was 1.31. CL of R (-)-enantiomer was more than 1.3-fold higher than that of the S (+)-enantiomer.	More an analytical validation study rather than an in vivo toxicological study. Since only male animals were tested, the information on sex differences is missing.	RAR-08_Vol-3 CA_B.6.1.3.5 Reference number: BIIA5.1 Anonymous (2007)
Stereoselective Metabolism of Benalaxyl in Liver Microsomes from Rat and Rabbit	The metabolism of benalaxyl enantiomers was stereoselective in rat and rabbit liver microsomes, and different in the two species by analytical methods. (-)-R-benalaxyl and (+)-S- benalaxyl in rat liver microsomes t1/2 = 22.35 min racemic benalaxyl	This information might be valuable for further interpretations in case in vivo studies in rats and rabbits conducted	RAR-08_Vol-3 CA_B.6.1.3.6 Reference number: KCA 5.1

Method	Results	Remarks	Reference
Benalaxyl (racemic) indicated as rac-BX Purity: >99% Male Sprague-Dawley rats and Japanese White rabbits Liver: 6 rats +3 rabbits M liver microsomes : 80 µM of benalxyl (rac-BX) and 40 µM of its enantiomers for rat hepatic microsomes and 60 µM of rac-BX and 30 µM of its enantiomers for rabbit hepatic microsomes Test protocol GLP, GEP, Guidelines (US EPA, OECD) No information on GLP status of OECD GD Acceptable	t1/2 = 10.66 min individual benalaxyl enantiomers t1/2 = 5.42 and 4.03 min (-)-R- benalaxyl and (+)-S- benalaxyl in rabbit liver microsomes t1/2 = 11.75 min racemic benalaxyl t1/2 = 15.26 min individual benalaxyl enantiomers t1/2 = 5.66 and 9.63 min no chiral inversion from the (+)-R- benalaxyl to (-)-S-benalaxyl or inversion from (-)-S- benalaxyl to (+)-R-BX in rabbit and rat microsomes. These results suggest metabolism of benalaxyl enantiomers is stereoselective in rat and rabbit liver microsomes and different in the two species. This information might be valuable for further interpretations in case *in vivo* studies in rats and rabbits conducted with benalaxyl show different results or effects levels.	with benalaxyl will notice different results or effects levels. Since only male animals were tested, the information on sex differences is missing. The full relevance will become clear in the context of the results of a full in vitro comparative metabolism study.	Anonymous (2011)
In Vitro Metabolism of [^{14}C] Benalaxyl in Cryopreserved Hepatocytes from Rats, Dogs, and Humans [^{14}C]Benalaxyl (20 µM) Positive control : [4-^{14}C]testosterone Solvent: Ethanol Biological material: Pooled (N>5 - rat (Sprague-Dawley) hepatocytes - dog (Beagle) hepatocytes and pooled human hepatocytes	In cryopreserved rat hepatocytes: - 82% to 25% of total [^{14}C]Benalaxyl - during 1 – 4 hrs - 45% of total [^{14}C]Benalaxyl as 2-hydroxymethyl benalaxyl - after 4 hrs -12% of total [^{14}C]Benalaxyl as 2-hydroxymethyl benalaxyl acid, proposed to be derived by ester – before 4 hrs - less than 10% of [^{14}C]Benalaxyl as dihydroxy benalaxyl acid, hydroxy benalaxyl, a carboxylic acid analogue of benalaxyl (benalaxyl-2-benzoic acid), and glucuronide conjugates of hydroxy benalaxyl and of dihydroxy benalaxyl. In cryopreserved dog hepatocytes: - 8% of [^{14}C]Benalaxyl was attributed to benalaxyl as self - before 4 hrs Benalaxyl was completely metabolized after 4 hours of incubation by glucuronidation of hydroxy/oxidized products were the major compound-related components present in the incubation extracts. - 65% of [^{14}C]Benalaxyl as glucuronides of 2-hydroxymethyl benalaxyl - 20% of [^{14}C]Benalaxyl as glucuronide of dihydroxy benalaxyl	Additional experiment study: - to identify the phase-2 metabolites, glucuronide conjugates The study confirmed that benalaxyl enantiomers were evenly metabolized at the same rate and was not susceptible to stereo-selective metabolism in rat or human hepatocytes.	RAR-08_Vol-3 CA_B.6.1.3.4 Reference number: CA 5.1.1/11 Anonymous (2015)

Method	Results	Remarks	Reference
Incubation time: 1, 2 and 4 hrs Test method: N/A: No standard guideline available GLP/QA: Yes/Yes Acceptable	- 8% of [^{14}C]Benalaxyl as benalaxyl-2-benzoic acid In cryopreserved human hepatocytes: - [^{14}C]Benalaxyl was found to be extensively metabolized after 4 hours of incubation, though not as extensively as in dogs. -14% of [^{14}C]Benalaxyl – after 4hrs, decreasing in time - 25% of [^{14}C]Benalaxyl as 2-Hydroxymethyl benalaxyl was the most significant metabolite present in the extract at all time points – after 4 hrs - 16% of [^{14}C]Benalaxyl as 2-Hydroxylmethyl benalaxyl acid was another major metabolite present in the extracts - in the 4 hrs - 10% of ^{14}C]Benalaxyl as mono- and dihydroxy benalaxyl, benalaxyl-2-benzoic acid and glucuronide conjugates of 2-hydroxymethyl benalaxyl. The metabolism of benalaxyl in rat and human hepatocytes was further investigated using chiral chromatography. After 4 hours of incubation: - the ratio of enantiomers of benalaxyl remained relatively same and consistent with the initial composition of enantiomers as at the beginning of the study in species.		
In Vitro" Percutaneous Absorption of Radiolabelled Benalaxyl in the Concentrate Wettable Powder (WP) Formulation and Two In-Use Dilutions through Human Skin [^{14}C]-Benalaxyl, Lot/Batch: 22284-42-15, radiochemical purity: 99% 4 samples of full-thickness human skin (abdominal) Test methods: OECD TG 428 GLP/QA: Yes/Yes Acceptable	For none of the Test Preparations was absorption considered to be "complete" (as defined in the Guidance Document on Dermal Absorption (EFSA Journal 2011, 9(7):2294), because less than 75% of the absorption occurred within the first half of the study. Test Preparation 1 [^{14}C]-Benalaxyl Concentrate Formulation, 80 g/kg, mixed with saline, ca. 1:1, w/w) The mean total unabsorbed dose, consisting of skin wash, tissue swab and pipette tip at termination of exposure at 8 hrs, donor wash and 24-hour tissue swab, plus the unexposed skin and the radioactivity associated with the stratum corneum was 99.85% of the applied dose at 24 hrs. Since variability between replicates was ≥25% of the mean value: Test Preparation 2 [^{14}C]-Benalaxyl Most Concentrated In-Use Spray Dilution of 1 g/L absorption was incomplete based on the 8-hour data (44.04% of the total absorption occurred within the first 8 hour of the study). The potentially absorbable dose was 4.06% of the applied dose. A standard deviation was added to the overall dermal absorption value to derive a final dermal absorption value of 6.1%, rounded to 6% for the most concentrated in-use spray dilution of 1 g/L. Test Preparation 3 [^{14}C]-Benalaxyl Least Concentrated In-Use Spray Dilution of 0.2 g/L) the absorption was incomplete based on the 8-hour data	According to the EFSA guidance where there is variability between replicates i.e. the standard deviation is > 25% of the mean value, the value of the standard deviation should be added to the overall dermal absorption value The explanation found in the test study presentation in the Dossier was that since there was no 12-hour time point in the study design, the 8-hour data was used to assess whether absorption was complete or incomplete.	RAR-17_Vol-3 CP_B.6.2.1 Reference number: CP 7.3/01 Anonymous (2014)

Method	Results	Remarks	Reference
	(47.24% of the total absorption occurred within the first 8 hour of the study). The potentially absorbable dose was 12.37% of the applied dose.		

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

Toxicokinetic profile of benalaxyl was studied following some old and new studies on rats, mice and dogs following low and/or high dose administration. The data from the acute- and repeated dose toxicity studies indicated that toxicity following exposure to benalaxyl via the dermal route was not of concern compared to oral administration, hence studies on absorption, distribution, metabolism and excretion following exposure via the skin is not required.

Toxicokinetics Section of the Dossier was improved during it renewal period. Pesticides Peer Review Experts' Meeting 182 in September 2018 raised the bridging of toxicokinetics properties between benalaxyl and benalaxyl-M. As an outcome, the pattern of effects and no-observed-adverse-effect-levels (NOAELs) in the available studies with both benalaxyl and benalaxyl-M was concluded. Therefore, the two compounds are of similar toxicity, respectively. Benalaxyl technical used in the toxicity studies is representative of the technical specification proposed for the renewal but not of the original technical specification. Considering both technical specifications, the toxicological relevance of the impurities has not been yet sufficiently assessed, to be concluded upon (data gap). No residue definition for human biomonitoring can be defined (unfinalised). (see B.6.1.2.2).

Oral absorbtion of benalaxyl and/or its hydrolysis products is rapid; its bioavailability is estimated to be 100% more than 90% being excreted within 24 hours, no-related with sex, dose level or regime of administration (single or multiple doses). (see B.6.1.2.3). Inhalatory absorbtion of benalaxyl is low and without a toxicological relevance. A dermal absorbtion study was missing during the first renewal of benalaxyl and the "*In Vitro" Percutaneous Absorption of Radiolabelled Benalaxyl in the Concentrate Wettable Powder (WP) Formulation and Two In-Use Dilutions through Human Skin* was added in 2014, during the renewal process (see B.6.1.2.1). Based on results of the study and relevant variability among replicates for each dose level, the dermal absorption values for benalaxyl exposure in its formulated product were determined to be 0.72%, rounded to 0.7% for the formulation concentrate, 6.1%, rounded to 6% for the most concentrated in-use spray dilution of 1 g/L, and 17.84%, rounded to 18% for the least concentrated in-use spray dilution of 0.2 g/L.

Absorbed benalaxyl is widely distributed within the body, such as 30% of radioactivity can be found in the carcass at time of Tmax (0.5 h) following a single oral dose, but only traces (0.2-0.3%) were found 70 hours after administration, and radioactivity was not quantifiable at 168 hrs.The pattern does not change after repeat-dosing; 40% of radioactivity was found in the carcass at 0.5 h (mainly in stomach, liver and kidneys and, to a lesser extent, intestine wall), radioactivity was quantifiable in liver, intestine wall and kidney, but not in the remaining carcass, at 72 hrs; levels were still quantifiable in liver at 168 hrs. (see B.6.1.2.6 and B.6.1.2.7). Liver is the target organ of benalaxyl. But without an endocrine disruptor EAMS supportive studies reguested during the Experts' meeting Prev 05 at EFSA, this remains questionable.

According to the ADME studies performed by the Applicant in a previous period of renewal or during this period, two dose levels were admitted for toxicokinetics assessment of benalaxyl. *^{14}C-Benalaxyl, Blood pharmacokinetics, excretion and tissue distribution of radioactivity in the rat after a single oral administration* (see B. 6.1.2.1) admitted a peak blood level of radiolabelled benalaxyl at 0.25 - 1 h and after which the levels decreased according to an elimination a half-live about 50 hours, being quantificable at 72 hours after doses administration. About account for the study *^{14}C-Benalaxyl, Blood pharmacokinetics, excretion and tissue distribution of radioactivity in the rat after a single oral administration (Final report)* (see B.6.1.2.2) the results were different and the elimination half-life became to be 30 hours and faeces excretion > 90%, urinary excretion <10% , a pick level in liver at 0.5 h after administration and a high concentration in kidney.

Benalaxyl is present only in liver at 72 h and 168 h after dose administration. At 24 hour, the majoritary route is urinary than bile and faeces. Benalaxyl was extended assessed in terms of setting a half-life in other study, as *^{14}C-Benalaxyl, Blood pharmacokinetics, excretion and tissue distribution of radioactivity in the rat after repeated oral administrations* (see B.6.1.2.3) concluding that a small part of ^{14}C-benalaxyl is rapidly absorbed from the gastrointestinal tract, elimination half-life around 36 hrs and unmodified the rate of excretion >90% in faeces <10% by urinary route. The pick level was in liver at 0.5 h after administration high concentration of radiolabelled benalaxyl was found in kidney and intestines wall and radiolabelled benalaxyl was quantified at 72 h and 168 h after the administration only in liver. These three studies were performed in 1996, in three parts.

A single low (10 mg/kg bw) and one high (100 mg/kg bw) doses, repeated low dose (14 daily doses of unlabelled substance followed by a single administration of radiolabel material), a single oral dose with assessment of biliary excretion study (at both low and high dose levels), determination of toxicokinetic parameters (for both the low and high doses, and after repeated low doses – males only), and tissue distribution and identification of major metabolites (>5%) drew up the targeted assessment.
Benalaxyl is not accumulative in body.

A comparative *in vitro* metabolism study on human, rat and dog hepatocytes (see B.6.1.3.4) was performed in order to investigate a possible enantioselective metabolism of benalaxyl as a racemic compound.
It was interesting that benalaxyl was extensively metabolized in rat and human hepatocytes and was completely metabolized in dog hepatocytes. The metabolite profiles were qualitatively similar in all species tested. An *in vitro* study using rat microsomes was also performed suggesting that metabolism of benalaxyl enantiomers was stereoselective in rat and rabbit liver microsomes, and different in the two species.This information might be valuable for further interpretations in case *in vivo* studies in rats and rabbits conducted with benalaxyl show different results or effects levels. Both BX enantiomers were degraded by rat and rabbit liver microsomes and the degradation was NADPH-dependent. Metabolic rate constants (apparent Km and Vmax) were determined after a 10 min incubation period in rat and rabbit microsomes and Michaelis–Menten plots (see B.6.1.3.6 and B.6.1.3.6).

Excretion is mainly via faeces, instead via urine which is limited (approx. 14%). A biliary excretion study (at low and high dose levels) showed that approximatively 80-90% of administrated radiolabelled benalaxyl is excreted via the bile within 70 hours (60-86% occurring within the first 8 hrs). Metabolites identified in faeces are the same of those found in urine (except, the parent compound - benalaxyl, found in faeces only). The source of the material excreted via faeces is by biliary excretion, and for human risk assessment purposes, the absorption is considered to be complete. (see B.6.1.2.5).
The metabolites are common for the urinary and the faecal metabolic pathway in the both studies (see B.6.1.2.4)
It was considered that the soil metabolite M9 (N-(phenylacetyl)-N-(2,6-xylyl)-D-alanine) would also likely result from the mammalian metabolism of benalaxyl, and for this reason a new ADME study was performed, allowing to ascertain that benalaxyl acid (M9) is detectable in the excreta of male and female rats up to 24 hours after a singular oral administration of 100 mg/kg bw / d of benalaxyl. (see B.6.1.3.2)

The presence of M9 in excreta from rats was also confirmed in another study, following administration of a single dose of benalaxyl at 1000 mg/kg bw/d, although amounts were not proportional to the dose administered when compared against the results of the new ADME study.

Based on the results of these additional investigations, it was confirmed that M9 is a rat metabolite and therefore tested in toxicity studies performed with the parent benalaxyl.

[^{14}C]benalaxyl was extensively metabolized in rat and human hepatocytes and was completely metabolized in dog hepatocytes following incubation for 4 hours. The metabolite profiles were qualitatively similar in all species tested. The major conjugates present in dog hepatocytes were conformational isomers of 2-hydroxymethyl benalaxyl glucuronide and/or 6-hydroxymethyl benalaxyl glucuronide.
In the first two hours of HPLC coupled to a mass spectrometer and a radioactivity detector; rat, dog, human cryopreserved hepatocytes study the metabolite 2-hydroxymethyl benalaxyl and another hydroxy metabolite

were detected in hepatocyte extracts but they were absent in the 4 hour sample, most probably due to further metabolism. (see B.6.1.3.4)

About the metabolite profiles, there is noted the presence of a common major metabolic pathways, such as:

o hydroxylation of the xylene (methyl) moiety to 2-hydroxlmethyl benalaxyl;
o further oxidation to carboxylic acid analogue (benalaxyl-2-benzoic acid);
and
o hydrolysis of the methyl ester function
o minor metabolic pathways were the hydroxylation of phenyl ring and hydrolysis of carboxymethyl group.

Conjugation of 2-hydroxlmethyl benalaxyl was found to produce glucuronide conjugates, as major metabolites, especially in dogs. No major unique metabolite was observed in human hepatocytes.

No enantio-selective metabolism was observed following incubation with rat or human hepatocytes.

All the human metabolites formed were detected in rat. Therefore, it can be said that all potential human metabolites of benalaxyl have been tested in the pivotal toxicology species, thus demonstrating its relevance to derive human toxicological reference values.

10 EVALUATION OF HEALTH HAZARDS

Evaluation of health hazards in line with the CLP Regulation criteria has followed only specific studies for proposed classification existing in finalised RAR, Benalaxyl - Volume 3 Annex B.6: Toxicology and metabolism, November, 2018.

10.1 Acute toxicity - oral route

Table 11: Summary table of animal studies on acute oral toxicity

Method, guideline, deviations if any	**Species, strain, sex, no/group**	**Test substance,**	**Dose levels, duration of exposure**	**Value LD_{50}**	**Reference**
Acute Oral Toxicity Up and Down Procedure in Rats with Benalaxyl Oral (gavage) OECD TG 425 and US EPA OPPTS 870.1100 GLP/QA:Yes/Yes Deviations: None Acceptable	Sprague-Dawley rats derived Albino rat F mulliparous and non-pregnant 1group/5 rats	Benalaxyl tech Lot/Batch: PL13-0055 Purity: 98.4% Vehicle: 0.5% w/v solution of carboxymethyl cellulose	2000 mg/kg bw/d initial dose = 2000 mg/kg bw (1F) study dose: 2000 mg/kg bw/d (4F) - Due to the absence of mortality in first female Duration of exposure = 14 days Body weight: ↑ D7 - D14 (termination) following dosing. Clinical signs: Following administration, two females exhibited nasal discharge and/or reduced faecal volume. A third female was hypoactive and exhibited irregular respiration, oral and ocular discharge, hunched posture, and ano-genital and facial staining. all three females recovered by Day 6 Mortality: No	LD50 > 2000 mg/kg bw/d	RAR-08_Vol-3 CA_B.6.2.1.1 Reference number: CA 5.2.1/01 Anonymous (2013a)

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels, duration of exposure	Value LD_{50}	Reference
			Necropsy: No gross abnormalities were noted for any of the animals.		
Acute Oral Toxicity Study of the M 9834 in the Albino Rat Oral (gavage) Test method: N/A: no standard guideline available. GLP/QA: None Acceptable	Albino rats 2G/5 rats M+F	M 9834	3500 - 5040 mg/kg bw/d Study duration: not mentioned 24 hrs after administration: Mortality: 4500 mg/kg bw/d: 2/5 (m) and 4/5 (f) 3750 mg/kg bw/d: 2/5 (m) and 2/5 (f)	LD50 = 4200 mg/kg bw (3500 - 5040 mg/kg bw, 95% confidence limits probability) Not conclusive for assessment	RAR-08_Vol-3 CA_B.6.2.1.2 Reference number: CA 5.2.1/02 Anonymous (1979)

Table 12: Summary table of human data on acute oral toxicity

There are no relevant human data available.

Table 13: Summary table of other studies relevant for acute oral toxicity

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
An Oral (Gavage) Dose Range-Finding Acute Neurotoxicity Study of Benalaxyl in Rats Oral (gavage) Rats Crl:CD(SD) A single dose to 4 G (G2-G5) Remarks: All criteria for a valid study were met Guidelines: N/A: Dose range-finding study GLP/QA: Yes/Yes Acceptable	Benalaxyl tech. Lot/batch: PL 13-0055 Purity: 98.4%. Vehicle: 0.5% (w/v) methylcellulose in deionized water	Range-finding study previous a neurobehavioral further study. Initial dose levels: 200, 600, and 2000 mg/kg bw/d (Groups 2, 3, and 4, respectively). Group 5 - 400 mg/kg bw/d(additional dose level) Group 1 – control group, received the vehicle in the same condition of study. Group 6 - additional animals at 2000 mg/kg bw/d : 2 M + 2 F (to confirm the previous incidence of convulsions at 2000 mg/kg bw/d) Mortality: In the main study, all animals treated at 2000 mg/kg (1 male and 2 female) were found dead. One male and one female rat were found dead approximately 2 hours following dose administration; the remaining female was found dead approximately 4.5 hours after dose administration. The 2 female in the additional 2000 mg/kg bw/d dosage group (Group 6) were euthanized and discarded at approximately 2 hours following dose administration after observations of clonic convulsions at the 2-hours detailed clinical observation or during cage-side observations.	The number of animals selected for this study was 3/sex/group), reasonable to avoid an unexpected deaths or treatment-related morbidity and/or mortality. Acute neurotoxicity is a supportive study for a classification as Acute Tox. Cat. 4; H302	RAR-08_Vol-3 CA_B.6.7.1.1 Reference number: CA 5.7.1/01 Anonymous (2014c)

		All remaining animals survived to scheduled sacrifice. Body weight: no statistically significant differences were noted. Macroscopic examination: A dark red discoloration of the lungs, partially collapsed lungs, dark red contents of the trachea, and red matting of the skin (nasal, buccal, and ocular areas and forelimbs) were noted for the 2000 mg/kg male in the main group. In addition, red matting of the skin (nasal and buccal areas) was noted for one female in the main 2000 mg/kg bw/d group. Clinical observations: Group 3: one male (approx 2 and/or 4 hrs following dose administration): slightly soiled fur appearance, drooping eyelids, decreased respiratory rate, rales, slightly to moderately impaired mobility, dragging body and low arousal Group 4 - 2000 mg/kg bw/d (1 male and 2 females) were found dead within 5 hours after dose administration: slightly soiled fur appearance, drooping eyelids, decreased respiratory rate, rates, slightly to moderately impaired mobility, dragging body, clonic convulsions, low arousal, and/or circling at 1 female at approx.. 2 and/or 4 hrs after dose administration) and male was found dead prior to the 2-hrs detailed clinical observations Clonic convulsions and red material around the nose and mouth, immediately prior to death female found dead approximately 4.5 hrs: clonic convulsions (noted on 4 separate occasions), tremors, decreased respiration, chromodacryorrhea in both eyes, and red material around the mouth (noted as early as approximately 1.5 hrs following dose administration, between the scheduled detailed clinical observations). female found dead up to 2 hrs following dose administration clinical findings of prostrate and gasping were noted immediately prior to death (prior to the 2-hrs detailed clinical observations one male - 400 and 600 mg/kg bw/d (G3 and G4) and 1 to 2 females in the 200 and 400 (G2 and G3) at approx.. 2, 4, or 8 hrs following dose administration:red depo sits around the eyes, nose, and/or mouth Group 6: additional animals at 2000 mg/kg bw/d : 2 m + 2 f		

		Similar findings to those noted at 2000 mg/kg bw/d in the main phase were confirmed in an additional phase, where both females administered the test substance at 2000 mg/kg bw/d had clonic convulsions at approximately 2 hours following dose administration. The time of peak effect was considered 2 hours following dose administration based on the severity and nature of the findings at this time (lightly soiled fur appearance, drooping eyelids, decreased respiratory rate, impaired mobility, dragging body, clonic convulsions, low arousal, and circling) One male: red deposits on the nose and crusty deposits on the mouth and nose at the 2-hrs detailed clinical observation and slightly soiled fur appearance at the 8-hrs detailed clinical observation while findings for the other male were limited to brown material on the anogenital area at the cage-side observations on study day 1.		
An Oral (Gavage) Acute Neurotoxicity Study of Benalaxyl in Rats Oral (gavage) Rats Sprague-Dawley (Crl:CD (SD)) OECD TG 424 and OPPTS 870.6200 Criteria are met Statistics was performed GLP/QA: Yes/Yes Acceptable	Benalaxyl tech. PL13-0055 Purity: 98.4% Vehicle: 0.5% (w/v) carboxymethyl cellulose aqueous solution	For evaluating the acute neurotoxic potential of benalaxyl technical when administered as a single oral dose to rats Acute neurotoxicity screening battery (FOB): functional observational battery, locomotor activity, and neuropathological assessments. Phase 1 3G (G2 - G4) of 10 m/10f at 200, 400, and 1000 mg/kg bw/d Mortality and clinical observations: At 200 mg/kg and above – a single dose - 1000 mg/kg bw/d (m) and 200, 400, and 1000 mg/kg bw/d (f), ≤ 4.5 hrs following dose administration; the majority of animals that were found dead or euthanized in extremis were noted with clonic convulsions the same with FOB findings at the time of peak effect on study day 0, only. 1000 mg/kg one male was found dead approx 2 hrs following dose administration (clonic convulsions during the continuous 2-hour post-dosing observations) An additional male in this group was found dead approx 4.5 hrs following dose administration (no clinical observations); One and 2 females in the 200 and 1000 mg/kg bw/d groups, respectively, were found dead approx 4 hrs following dose administration: clonic convulsion One female of the 200 mg/kg bw/d group was noted with clonic convulsion and vocalization One female in 400 mg/kg bw/d group was euthanized in extremis approximately 3 hrs	No gross necropsy observations were noted and microscopic examination of tissues was not performed in the unscheduled death animals. The analysed dosing formulations for FOB remains unclear. Study supportive for acute toxicity classification based on mortality up to 2000 mg/kg bw/d. ATE=2000 mg/kg bw/d	RAR-08_Vol-3 CA_ B.6.7.1.2 Reference number CA 5.7.1/01 Anonymous (2014a)

		following dose administration after being noted with increased respiration; this female was also noted with splayed hindlimbs and immobility by the clinical veterinarian. Two of the aforementioned females (that in the 400 mg/kg bw/d group and one in the 1000 mg/kg bw group) were euthanized or found dead prior to completion of the motor activity testing. In addition to the clonic convulsions noted above, one female in the 1000 mg/kg bw/d group was also noted with clonic convulsions approx.. 3 hrs following dose administration on day 0; however, this female survived to the scheduled euthanasia. Control group was survived Phase 2 3G (G1-as a single dose to G3) of 10f at 0, 50, and 100 mg/kg. bw/d females in the 100 mg/kg G3: repetitive movement of the mouth and jaws which correlated with similar home cage FOB findings at the time of peak effect on study day 0 FOB findings were recorded for all animals prior to the initiation of dose administration (pretest), at the time of peak effect (approximately 2 hours post-dosing) on day 0, and on days 7 and 14. In the 100 mg/kg bw/d group, 3 females were noted with repetitive movement of the mouth and jaws and 2 females were noted with salivation on the day of dose administration during the 2-hour continuous post-dosing observations; these findings were considered test substance-related. Control group was survived FOB and motor activity assessment Significantly lower mean rearing counts were noted for females in the 200, 400, and 1000 mg/kg bw/d groups compared to the control group at the time of peak effect on the day of dosing. During Phase 2, at the time of peak effect on the day of dosing one female in the 100 mg/kg bw/d group was noted with repetitive movement of mouth and jaws; this finding was considered test substance-related and corresponded with the clinical findings noted in this group following dose administration. Motor activity assessments revealed higher mean total motor activity values for males at 400 and 1000 mg/kg bw/d and for females at 200, 400, and 1000 mg/kg bw/d at the time of peak effect on the day of dosing, indicating a change in the pattern of habituation in these		

groups.
No gross necropsy observations were noted and microscopic examination of tissues was not performed in the unscheduled death animals. Given the clinical signs and the relationship between dosing and death, the cause of death of these animals was considered to be administration of the test substance.

There were no remarkable macroscopic alterations observed for males and females sacrificed at termination of the study at any dose level.

Brain weights and measurements were unaffected by administration of benalaxyl at any dose level. No test substance-related microscopic lesions were observed in any of the central or peripheral nervous system tissues examined from 5 animals/sex in the control and 1000 mg/kg bw/d groups.

Minimal axonal degeneration was observed sporadically in both control and 1000 mg/kg bw /dgroup animals in the sciatic nerve, peroneal nerve, tibial nerve, lumbar dorsal root fibres, lumbar ventral root fibres, and lumbar spinal nerve.
Summary of histopathology (only findings reported)

Finding	Males		Females	
	0 mg/kg bw/d	1000 mg/kg bw/d	0 mg/kg bw/d	1000 mg/kg bw/d
Lumbar dorsal fibre - examined	5	5	5	5
Degeneration, axonal	1	1	0	1
Lumbar ventral fibre- examined	5	5	5	5
Degeneration, axonal	0	0	1	1
Peroneal nerve – examined	5	5	5	5
Degeneration, axonal	1	1	0	0
Sciatic nerve – examined	5	5	5	5
Degeneration, axonal	2	3	0	0
Spinal node, lumbar	5	5	5	5
Degenerat	1	0	0	0

		ion, axonal		
In Vivo Mammalian Erythrocyte Micronucleus Assay in Rats with Acetyl F4 Sprague-Dawley (Hsd:SD) rats Test methods: OECD TG 474 (2014) and OPPTS 870.5395 (1998), GLP/QA: Yes/Yes Statistics: Yes Acceptable	Acetyl F4 Batch/Lot: 55062-8-22 Purity:99.5% Vehicle: 0.5% w/v solution of carboxymethyl cellulose in deionised water Positive control: Cyclophosphamide, (once in Study Day 2 at 10 mL/kg) 2 daily treatments at 24 hours intervals	dose range finding assay (DRF), the maximum dose tested was 2000 mg/kg/day in 3 rats/sex at 500, 1000 or 2000 mg/kg bw/day of Acetyl F4 Piloerection and lethargy were observed in male and female rats at 2000 mg/kg bw/day Dose Range Finding Assay: No mortality occurred at any dose level during the course of the dose range finding assay. Piloerection and lethargy were observed in male and female rats at 2000 mg/kg bw/d. All other rats appeared normal throughout the observation period. Definitive Micronucleus Assay: No mortality occurred at any dose level during the course of the definitive assay Following the last observation, animals were euthanized and discarded without further examination. Further phase of this study included induced a clastogenic activity and/or disruption of the mitotic apparatus by detecting micronuclei in polychromatic erythrocyte (PCE) cells in rat bone marrow of rats, only male at 500, 1000 and 2000 mg/kg bw/d once a day for 2 consecutive days	Acetyl F4 at doses ≤ 2000 mg/kg bw/day over 2 consecutive days was negative in the micronucleus assay. The maximum dose evaluated for non-toxic materials - 2000 mg/kg bw/d (the limit dose for this assay)	RAR-08_Vol-3 CA_ B.6.8.1.18 Reference number CA 5.8.1/17 Anonymous (2015)

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

One acute oral toxicity study is available for oral acute classification according with CLP Regulation criteria. This is a study carried out in line with OECD TG 425 without deviations and acceptable for this assessment. Study is GLP compliant and is reliable for this scope (see B.6.2.1.1) Acute Oral Toxicity Up and Down Procedure in Rats with Benalaxyl Technical. The conclusion of the study set a half percent of mortality of animals as LD_{50} greater greater than 2000 mg/kg bw in female rats, which excludes the possibility for a classification as toxic by oral route in acute exposure of benalaxyl according to the Regulation (EC) nr. 1272/2008 (CLP Regulation).

An initial dose of 2000 mg/kg bw/d was administered to one healthy female rat by oral gavage which survived to the experiment and four additional females were exposed to fulfill the criteria of the 14 days of study. Females were selected for the test because they are frequently more sensitive to the toxicity of test compounds than males. The most significant findings were the rate of surviving in all animals and gain of body weight during the study. Following administration, two females exhibited nasal discharge and/or reduced faecal volume. A third female was hypoactive and exhibited irregular respiration, oral and ocular discharge, hunched posture, and ano-genital and facial staining.
However, the three females recovered by Day 6, and along with other animals, appeared active and healthy for the remainder of the study. No gross abnormalities in pathological examination of animals.

However, to fulfill the evaluation of any possible acute toxic effect by oral route, an acute neurotoxicity study (see B.6.7.1.2 An Oral (Gavage) Acute Neurotoxicity Study of Benalaxyl in Rats), preceded by the relevant

RF study (see B.6.7.1.1 An Oral (Gavage) Dose Range-Finding Acute Neurotoxicity Study of Benalaxyl in Rats) has been performed during the renewal of approval of benalaxyl in AIR3.
Some clinical and histological findings were in contradiction with the results of main acute oral study performed by the Applicant, respectively those clonic convulsions and death which were not noted for any animals receiving benalaxyl at dosage levels up to 2000 mg/kg bw/d, which is in contrast to what was observed in these required acute neurotoxicity study.
The dose levels were larger than in the main study and the relevance of exposure effects on benalaxyl was improved.
In a DRF (dose-range finding) study there were administred the following dose level: 200, 600, and 2000 mg/kg bw/d (Groups 2, 3, and 4, respectively) for phase 1. However, due to excessive toxicity and lethality at 2000 mg/kg bw/d, dosing at this level was discontinued after 1 male and 2 females were dosed. An additional dosage level, 400 mg/kg bw/d (Group 5) respectively, was added to the study.
All animals treated at 2000 mg/kg bw/d during the main study phase (1 male and 2 females) were found dead within 5 hours of dosing.
Clinical findings, including clonic convulsions, tremors, decreased respiration, chromodacryorrhea, gasping, and/or red material around the nose and/or mouth, were noted for these animals between the scheduled detailed clinical observations, generally immediately prior to death.
During the main phase, detailed clinical observations included slightly soiled fur appearance, drooping eyelids, decreased respiratory rate, rates, slightly to moderately impaired mobility, dragging body, clonic convulsions, low arousal, and/or circling, noted for 1 male in the 600 mg/kg bw/d group and 1 female in the 2000 mg/kg bw/d group at approximately 2 and/or 4 hours following dose administration.
In addition, red deposits around the eyes, nose, and/or mouth were noted for a single male in the 400 and 600 mg/kg groups and 1 to 2 females in the 200, 400, and 2000 mg/kg bw/d groups at approximately 2, 4, or 8 hours following dose administration.
The time of peak effect was considered 2 hours following dose administration based on the severity and nature of the findings noted at this time.
The results of the additional phase dosed at 2000 mg/kg bw/d corroborated the findings in the main phase.
Both females receiving 2000 mg/kg bw/d had clonic convulsions at approximately 2 hours following dose administration, in addition to wiping of the mouth on the cage floor and/or walls, repetitive movement of the mouth and jaws, tremors, vocalisation, and/or salivation. These females were subsequently euthanised due to the findings of clonic convulsions.
Findings for the 2 males receiving 2000 mg/kg bw/d included red deposits on the nose, crusty deposits on the mouth and nose, slightly soiled fur appearance, and/or brown material on the anogenital area. These males survived to the scheduled euthanasia.
In the main study, all animals treated at 2000 mg/kg bw/d (1 male and 2 female) were found dead. One male and one female rat were found dead approximately 2 hours following dose administration; the remaining female was found dead approximately 4.5 hours after dose administration.
The 2 female in the additional 2000 mg/kg bw/d dosage group (Group 6) were euthanized and discarded at approximately 2 hours following dose administration after observations of clonic convulsions at the 2-hours detailed clinical observation or during cage-side observations.
Macroscopic findings of dark red discoloration of the lungs, partially collapsed lungs, dark red contents of the trachea, and red matting of the skin (nasal, buccal, and ocular areas and forelimbs) were noted for the 2000 mg/kg bw/d male in the main group. In addition, red matting of the skin (nasal and buccal areas) was noted for one female in the main 2000 mg/kg bw/d group.

In the second acute neurotoxicity study, based on dose-respone in the first DRF study it was selected a maximum dose level of 1000 mg/kg in in view of avoiding the rate of mortality of rats and allowing a properly FOB and motor activity assessments.
Unfortunately, from Phase 1 of the acute neurotoxicity study, on a single dose of benalaxyl resulted in mortality and/or moribundity for males at 1000 mg/kg and for females at 200, 400, and 1000 mg/kg within approximately 4.5 hours following dose administration; the majority of animals that were found dead or euthanized in extremis were noted with clonic convulsions.

In addition, test substance-related FOB findings (lower mean rearing counts) for females at 200, 400, and 1000 mg/kg and/or higher mean total motor activity values for males at 400 and 1000 mg/kg at the time of peak effect on day 0.
Based on these results, 200 mg/kg was considered to be the no-observed-adverse-effect level (NOAEL) for acute neurotoxicity in male rats.
For Phase 2, females in the 100 mg/kg group were noted with clinical findings of repetitive movement of the mouth and jaws which correlated with similar home cage FOB findings at the time of peak effect on study day 0 only. No test substance-related effects were noted for females in the 50 mg/kg group. Therefore, the NOAEL for acute neurotoxicity in female rats was considered to be 50 mg/kg.
There were no test substance-related macroscopic or microscopic findings, or effects on brain weights or brain dimensions for any treated group.

Phase 1				
Dose level (mg/kg bw)	**Males**	**Time of death**	**Clinical sign(s)**	**Observation time point(s)**
200	0 / 10	N/A	-	-
400	0 / 10	N/A	-	-
1000	2 / 10	Found dead at 2 h post dosing	Clonic convusions	Continuous 2 h post dose observation
		Found dead at 4.5 h post dosing	No clinical signs	N/A
Dose level (mg/kg bw)	**Females**	**Time of death**	**Clinical sign(s)**	**Observation time point(s)**
50	0 / 10	N/A	-	-
100	0 / 10	N/A	3 animals: repetitive movement of mouth and jaws	Continuous 2 h post dose observation
			2 animals: salivation	Continuous 2 h post dose observation
200	1 / 10	Found dead at 4 h post dosing	Clonic convusions, Vocalisation, Vocalisation upon handling	Continuous 2 h post dose observation
400	1 / 10	Euhanised in extremis at 3 h post dosing	Increased respiration, (+ splayed hindlimbs and immobility)	Continuous 2 h post dose observation
1000	2 / 10	Both found dead at 4 h post dosing	Clonic convusions	Continuous 2 h post dose observation
		N/A (animal survived)	Clonic convusions	3 h post dose

Motor activity assessments revealed higher mean total motor activity values for males at 400 and 1000 mg/kg and for females at 200, 400, and 1000 mg/kg at the time of peak effect on the day of dosing, indicating a change in the pattern of habituation in these groups.

Results of necropsy were essential to consider mortality as a reason foe an acute toxicity classification.

Summary of histopathology (only findings reported)

Finding	**Males**		**Females**	
	0 mg/kg	**1000 mg/kg**	**0 mg/kg**	**1000 mg/kg**
Lumbar dorsal fibre - examined	5	5	5	5

Degeneration, axonal	1	1	0	1
Lumbar ventral fibre-examined	5	5	5	5
Degeneration, axonal	0	0	1	1
Peroneal nerve – examined	5	5	5	5
Degeneration, axonal	1	1	0	0
Sciatic nerve – examined	5	5	5	5
Degeneration, axonal	2	3	0	0
Spinal node, lumbar	5	5	5	5
Degeneration, axonal	1	0	0	0

Brain weights and measurements were unaffected by administration of benalaxyl at any dose level. No test substance-related microscopic lesions were observed in any of the central or peripheral nervous system tissues examined from 5 animals/sex in the control and 1000 mg/kg groups.
Minimal axonal degeneration was observed sporadically in both control and 1000 mg/kg group animals in the sciatic nerve, peroneal nerve, tibial nerve, lumbar dorsal root fibres, lumbar ventral root fibres, and lumbar spinal nerve.

An adittional study could be considered a DRF (dose-range finding study), having as test substance one of main metabolites of benalaxyl as Acetyl F4. (see B.6.8.1.18 *In Vivo* Mammalian Erythrocyte Micronucleus Assay in Rats with Acetyl F4).
A DRF was performed to assess test substance toxicity and determine the maximum tolerated dose (MTD) or maximum feasible dose (MFD) for the micronucleus assay.
The MTD is defined as the dose that induces some signs of toxicity but is not expected to produce mortality within two days after administration, or severe and prolonged clinical signs of toxicity. The MFD is defined by 1) physical properties that limit the dose formulation concentration, 2) limitations on volume that can be administered, 3) bioavailability of compound or 4) bone marrow toxicity such that PCE proportions <20% of vehicle.

The maximum dose evaluated for non-toxic materials - 2000 mg/kg bw/d (the limit dose for this assay).

In the DRF assay, 3 animals/sex were exposed to 500, 1000 or 2000 mg/kg bw of Acetyl F4. Piloerection was observed at 2000 mg/kg/day in male and female rats. Following the last observation, animals were euthanized by exposure to CO2 and discarded without further examination. No mortality or differences in clinical observations were seen between the sexes, therefore only male rats were used in the definitive assay.
No mortality occurred at any dose level during the course of the dose range finding assay. Piloerection and lethargy were observed in male and female rats at 2000 mg/kg bw/d. All other rats appeared normal throughout the observation period.
An increasing of the liver weight was mentioned at 1000 mg/kg bw/d in both animals.

Human data:
As for most pesticides, no information is available on effects in humans due to exposure to the active ingredient, benalaxyl, itself. According to the Applicant, the Company FMC, USA, the Occupational medical surveillance of employees in manufacturing did not reveal indications of adverse effects but there are no more recent data.

10.1.2 Comparison with the CLP criteria

According to the Regulation (EC) No 1272/2008 (CLP), a classification as Acute Tox. 4 (the lowest classification) is required for substances with oral LD50 of 300-2000 mg/kg bw. The LD_{50} for oral toxicity doe not excede 2000 mg/kg bw and thus benalaxyl fulfils the classification criteria for acute oral toxicity cat.4.

However, it should be noted that Regulation EC No 1272/2008 (CLP), section 3.8.1 states that:

"Acute toxicity refers to lethality and STOT-SE to non-lethal effects. However, care should be taken not to assign both classes for the same toxic effect, essentially giving a "double classification", even where the criteria for both classes are fulfilled. In such case the most appropriate class should be assigned."

There is, already, a proposed classification for acute toxicity Cat. 4 on the basis of the LD_{50} studies with cut-off values of 300-2000 mg/kg bw. The observed mortality is presumably caused by the neurotoxic effects. According to the CLP criteria Guidance on the Application of the CLP,Version 5.0, July 2017, rate of mortalities in rats observed within 72 hours after the first treatment can be considered an acute effect. Mortality seems to be attributed to multiple exposures in the An Oral (Gavage) Dose Range-Finding Acute Neurotoxicity Study of Benalaxyl in Rats RAR-08_Vol-3 CA_B.6.7.1.1.

Based on the available data summarised above, it was demonstrated that the acute neurotoxic effects can lead to mortality at dose levels that are below the classification criteria for acute toxicity Cat. 4. (300-2000 mg/kg bw).

It was reported that acute neurotoxic effects occurred immediately after dosing. Hence, these effects are considered relevant for classification. Some of these effects are indicative of neurotoxicity. At necropsy no test substance-related changes were noted in surviving animals which died at the end of the observation period. In the acute neurotoxicity study, high mortality was observed up to 2000 mg/kg bw per day.

In conclusion, the most sensitive species for assessing acute oral toxicity are rats. The lowest LD50 value in the rat acute tox studies (2000 mg/kg bw for both males and females) shall be used as the basis for classification.

The acute oral LD_{50} in the rat of 2000 mg/kg bw meets the criteria for Category 4 ($300 < ATE (LD_{50}) \leq 2000$ mg/kg bw).

The available animal studies clearly indicate that benalaxyl is acutely toxic via the oral route in rat and would support a classification in Category 4. A derived LD50 from acute neurotoxicity studies would deliver a value of ATE of 2000 mg/kg bw, which is also in line with the ATE for oral Acute Tox 4 according to table 3.1.2, Annex I of the CLP regulation.

10.1.3 Conclusion on classification and labelling for acute oral toxicity

Acute Tox. 4; H302

ATE = 2000 mg/kg bw

RAC evaluation of acute oral toxicity

Summary of the Dossier Submitter's proposal

Benalaxyl does not currently have a harmonized classification for acute toxicity. No mortality was observed at 2000 mg/kg bw in a standard acute oral toxicity study in rats (Anonymous 2013a; 5.2.1/01). However, lethality occurred at and below 2000 mg/kg bw in an acute neurotoxicity study and a respective range-finding study (Anonymous 2014c, 5.7.1/01; Anonymous 2014a, 5.7/02). Based on the mortality in the latter two studies the DS proposed classification with Acute Tox. 4; H302 and an ATE of 2000 mg/kg bw.

Comments received during consultation

Comments were received from 1 Member State Competent Authority (MSCA) and a manufacturer. The manufacturer agreed with the DS's proposal. The commenting MSCA considered the DS's proposal of Acute Tox. 4 and STOT SE 2 (nervous system) to

represent a double classification. They preferred STOT SE 2 only, pointing out the lack of mortality in the standard acute toxicity study.

Assessment and comparison with the classification criteria

The studies relevant for the assessment of acute oral toxicity are summarized in the table below. In addition to the acute toxicity and acute neurotoxicity studies presented by the DS, the table also includes two other gavage studies in rats: a prenatal developmental (PNDT) toxicity study and a 5-week tolerability study.

Overview of oral gavage studies relevant for acute toxicity assessment		
Species; year; reference number	**Method** (for further details see the CLH report)	**Mortality or severe toxicity**
Rat 20135.2.1/01	Acute oral toxicity, up-and-down procedure (OECD TG 425) Strain: Sprague-Dawley derived Vehicle: aqueous carboxymethyl cellulose 5 females, 2000 mg/kg bw	No mortality at 2000 mg/kg bw 1 animal showed hypoactivity and irregular respiration; no significant clinical signs in the remaining 4 animals
Rat 1979 5.2.1/02	Acute oral toxicity Pre-guideline, limited reporting Dose levels: 3750 and 4500 mg/kg bw 5/sex/group	LD_{50} = 4200 mg/kg bw Mortality at 3750 mg/kg bw: 4/10 Mortality at 4500 mg/kg bw: 6/10
Rat 2014 5.7.1/01	Acute oral neurotoxicity, dose range-finding study Strain: Sprague-Dawley (Crl:CD(SD)) Vehicle: aqueous methylcellulose Dose levels (main phase): 0, 200, 400, 600, 2000 mg/kg bw 3/sex/dose (except 2000 mg/kg bw: only 1 male and 2 females, dosing discontinued due to excessive toxicity) Additional phase (to confirm clonic convulsions and lethality): 2000 mg/kg bw, 2/sex Scheduled sacrifice 1 day after dosing	2000 mg/kg bw, main phase: all 3 animals died within 4.5 hours; clonic convulsions in 2 animals 2000 mg/kg bw, additional phase: both females euthanized due to clonic convulsions; both males survived (no convulsions in males) 600 mg/kg bw: 1 male clonic convulsions; no significant clinical signs in the remaining 5 animals
Rat 2014 5.7.1/02	Acute oral neurotoxicity (OECD TG 424) Strain: Sprague-Dawley (Crl:CD(SD)) Vehicle: aqueous methylcellulose Dose levels (Phase 1): 0, 200, 400, 1000 mg/kg bw; 10/sex/group Dose levels (Phase 2): 0, 50, 100 mg/kg bw; 10 females/group Scheduled sacrifice 15 days after dosing	1000 mg/kg bw: 4 animals found dead (2 m, 2 f) within 4.5 hours, 3 of them (1 m, 2 f) clonic convulsions; 1 female clonic convulsions and survived; no significant clinical signs in the remaining 15 animals 400 mg/kg bw: 1 female increased respiration, splayed hindlimbs and immobility, euthanized in extremis 3 h post-dosing; no significant clinical signs in the remaining 19 animals 200 mg/kg bw: 1 female clonic convulsions and found dead 4 h post-dosing; no significant clinical signs in the remaining 19 animals
Rat 2015 5.6.2/02	Prenatal developmental toxicity (OECD 414) Strain: Sprague-Dawley (Crl:CD(SD))	450 mg/kg bw (administered to 10 animals): 3 animals found dead within 6 hours after the first dose

	Vehicle: aqueous methylcellulose Dose levels (Phase 1): 0, 15, 50, 150 mg/kg bw/d Dose levels (Phase 2 – due to lack of maternal toxicity in Phase 1): 0, 450/300 mg/kg bw/d Dosing GD 6-19 25/group	The dose was then reduced to 300 mg/kg bw/d. 300 mg/kg bw/d: no mortality, no significant clinical sings
Rat 1982 5.3.1/01	5-week repeated dose, non-guideline Strain: Wistar (BOR:WISW) Vehicle: Traganth 0.5% Dose levels: 0, 10, 100, 800 mg/kg bw/d The top dose was gradually increased: after the 1st week to 1000 mg/kg bw/d, after the 2nd week to 1500 mg/kg bw/d, after the 3rd week to 2500 mg/kg bw/d, after the 4th week to 3500 mg/kg bw/d, in the middle of the 5th week to 4000 mg/kg bw/d 10/sex/group + 2-week recovery at 0 and 800/4000 mg/kg bw/d, 5/sex/group	800/4000 mg/kg bw/d: no mortality, no clinical signs

The GLP- and OECD guideline-compliant acute oral toxicity study (2013; 5.2.1/01) reported no mortality at 2000 mg/kg bw. Hypoactivity and irregular respiration were observed in 1 out of 5 animals.

No mortality or clinical signs were reported up to 4000 mg/kg bw/d in a non-guideline rat repeated dose study (1982; 5.3.1/01).

In contrast, all dosed animals (1 male, 2 females) died at 2000 mg/kg bw in the main phase of the range-finding acute neurotoxicity study (2014; 5.7.1/01). Two of them showed clonic convulsions. Since the standard acute toxicity study (2013; 5.2.1/01) conducted shortly before by a different laboratory had not found any mortality at this dose, 2 additional males and 2 females were dosed at 2000 mg/kg bw in the range-finding neurotoxicity study to confirm the severe toxicity. Both additional females had to be sacrificed *in extremis* due to clonic convulsions, whereas the males survived without convulsions.

A top dose of 1000 mg/kg bw was then selected for the main acute neurotoxicity study (2014; 5.7.1/02). At doses of 1000, 400 and 200 mg/kg bw, 4, 1 and 1 out of 20 animals per group died, respectively.

A single dose of 450 mg/kg bw was lethal to 3 out of 10 pregnant rats in a PNDT study (2015; 5.6.2/02) from the same laboratory as the acute neurotoxicity studies. No overt toxicity was observed after reduction of the top dose to 300 mg/kg bw/d. RAC notes that acute toxicity classification should preferably be based on standard acute toxicity studies using non-pregnant animals (Guidance on the application of the CLP criteria, 3.1.3.3.5, in combination with OECD TG 420), so this study is considered only supplementary in the assessment of acute toxicity.

RAC does not see any obvious explanation for the difference in sensitivity between the standard acute oral toxicity study and the acute oral neurotoxicity studies, besides presumably a different breeder. All these studies are recent, conducted under GLP, and

do not show deficiencies. The result of the standard acute toxicity study (i.e. no mortality at 2000 mg/kg bw) is in line with an older gavage study reporting no mortality up to 4000 mg/kg bw/d. Lethality below 2000 mg/kg bw was not limited to a single study either, as it occurred in the rat PNDT study from the same laboratory. According to the CLP guidance (Guidance on the application of the CLP criteria, 3.1.2.3.2), the lowest ATE from a suitable study should be taken forward for classification.

Although the acute neurotoxicity studies were not standard acute toxicity studies, they used young adult animals (6-8 weeks old), females were non-pregnant and the top dose was 2000 mg/kg bw (equal to the limit dose for acute toxicity studies). The post-observation period was 2 weeks only in the main study (testing up to 1000 mg/kg bw), but the peak of effects occurred within a couple of hours, so the short 1-day observation period in the range-finding study is unlikely to compromise the mortality rates in this case. Therefore, RAC agrees with the DS that the acute neurotoxicity studies provide reliable information on acute toxicity of benalaxyl and can be used for classification of acute toxicity.

The range-finding study reported a 100% mortality of females (4/4) and 33% mortality of males (1/3) at 2000 mg/kg bw. Females appeared to be somewhat more sensitive than males. The next lower dose of 1000 mg/kg bw resulted in a 20% mortality in both sexes (males 2/10, females 2/10). The male LD_{50} appears to lie around 2000 mg/kg bw, whereas the female LD_{50} somewhere between 1000 (20% mortality) and 2000 mg/kg bw (100% mortality). A dose causing 50% mortality in females cannot be determined from these data as one of the mortality values is 100%. In the absence of a reliable LD_{50} estimate for females, a conservative ATE of 1000 mg/kg bw is proposed by RAC.

In conclusion, RAC considers that benalaxyl warrants to be classified as **Acute Tox. 4; H302** with an **ATE** of **1000 mg/kg bw** based on mortality of female rats in acute oral neurotoxicity studies.

10.2 Acute toxicity - dermal route

Not evaluated in this report.

10.3 Acute toxicity - inhalation route

Not evaluated in this report.

10.4 Skin corrosion/irritation

Not evaluated in this report.

10.5 Serious eye damage/eye irritation

Not evaluated in this report.

10.6 Respiratory sensitisation

Not evaluated in this report.

10.7 Skin sensitisation

Not evaluated in this report.

10.8 Germ cell mutagenicity

Not evaluated in this dossier in term of a proposal for classification. The genotoxic potential of benalaxyl has been investigated in a battery of *in vitro* and *in vivo* studies.However, a short summary of the RAR regarding mutagenicity is presented, since it may be of importance for the classification of carcinogenicity

Table 14: Summary table of mutagenicity/genotoxicity tests in vitro

Method, guideline, deviations, if any	Test substance	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
Bacterial Reverse Mutation Assay with Benalaxyl Technical OECD TG 471 and US EPA OPPTS 870.5100 GLP/QA: Yes/Yes. No statistics Deviations:No Acceptable	Ben Tech: PL13-0055 Purity: 98.4% Vehicle: DMSO S9 Mix: 10% v/v S9 fraction, 4 mM β-nicotinamide-adenine dinucleotide phosphate, 5 mM glucose-6-phosphate, 33 mM KCl, 8 mM MgCl2, and 100 mM sodium phosphate buffer (pH 7.4). S9 was prepared from male Sprague-Dawley rats that were i.p. with Aroclor™ 1254	S. typhimurium tester strains: TA98, TA100, TA1535 and TA1537 in ± S9 activation E. coli tester strain: WP2 uvrA in ±S9 activtion. Preliminary cytotoxicity(range-finding) assay:1.5, 5.0, 15, 50, 150, 500, 1500 and 5000 µg/plate (± S9 Mix) Mutation assays: 50, 150, 500, 1500 and 5000 µg/plate (± S9 Mix)	Initial Toxicity-Mutation Assay - no positive mutagenic responses with any of the tester strains in either ± S9 activation.	RAR-08_Vol-3 CA_B.6.4.1.1 Reference number: CA 5.4.1/01 Anonymous (2014a)
Reverse mutation assay "Ames test" using Salmonella typhimurium and Escherichia coli OECD TG 471, EC Method B13/14 and US EPA OPPTS 870.5100 GLP/QA: Yes/Yes Acceptable	Ben tech: BENAL 102/09 Purity: 98.65% Vehicle: DMSO S9 Mix contained: 5 mL of S9 fraction, 1 mL of 1.65 M KCl/0.4 M MgCl2, 2.5 mL of 0.1 M glucose-6-phosphate, 2 mL of 0.1 M NADP,	S. typhimurium tester strains: TA98, TA100, TA1535 and TA1537 in ±S9 activation E. coli tester strain: WP2uvrA in ±S9 activation 1st experiment (plate incorporation): 50, 150, 500, 1500 and 5000 µg/plate in ± S9 activation 2nd experiment (pre-incubation): 5, 15, 50, 150, 500, 1500 and 5000 µg/plate (- S9 Mix); 50, 150, 500, 1500 and 5000 µg/plate (+ S9 Mix)	1st experiment - Small but statistically significant increases in the frequency of the revertant colonies were observed in WP2uvrA- at 5000 µg/plate and in TA98 at 1500 µg/plate in the presence of metabolic activation in the 1st experiment only. 2nd experiment – Increasing 2 times the concurrent solvent control, had counts that were within the historical control range for each strain and were not reproducible over 2	RAR-08_Vol-3 CA_B.6.4.1.2 Reference number: CA 5.4.1/02 Anonymous: (2010)

Method, guideline, deviations, if any	Test substance	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
	25 mL of 0.2 M sodium phosphate buffer (pH 7.4) and 14.5 mL sterile distilled water. S9 was prepared in-house from the liver of male rats weighing approx. 250 g. Each rat orally received three consecutive daily doses of phenobarbitone/ β-naphthoflavone (80/100 mg/kg bw/day) prior to S9 preparation on day 4.		separate experiments. Low biological relevance.	
Microbiological mutagenesis study on CRA 109 (M 9834); Genetic mutation test in Salmonella typhimurium (AMES) Test method: No guideline was available. The test complies with EC method B14. Remarks: Ames method (1975) Guidelines and GLP/QA: GLP was not implemented at the time the study was performed. Statistics: No Evaluation criteria: Not indicated. Acceptable	Benalaxyl:M 9834; CRA 109 Purity: 98% Lot/Batch: 5532/86 Vehicle: DMSO S9 Mix contained (per mL): S9 equivalent to 50 mg of fresh rat liver, $MgCl_2$ 8 µmol, KCl 33 µmol, Glucose-6-phosphate 5 µmol, NADP 4 µmol, and phosphate buffer (pH 7.4) 100 µmol. S9 was prepared in-house from the liver of male rats weighing approx. 200 g. Rats received a single i.p. injection of Aroclor 1254 in peanut oil at 500	S. typhimurium tester strains: TA1535, TA1537, TA1538, TA98 and TA100 in ± S9 activation 1st experiment: 2, 20, 200, 500 and 2000 µg/plate (± S9 Mix); 2nd experiment: 200, 400, 800 and 1600 µg/plate (± S9 Mix)	Citotoxicity (Preliminary Toxicity Test): Benalaxyl has presented a strong toxic effect at 2000 µg/plate in the 1st experiment, and at 1600 µg/plate in the 2nd experiment. At these both concentrations, precipitation of the test substance was also observed. The effect was less evident in the presence of metabolic activation. Benalaxyl was non-mutagenic ±S9 metabolic activation, while the positive controls were highly mutagenic.	RAR-08_Vol-3 CA_B.6.4.1.3 Reference number: CA 5.4.1/03 Anonymous (1979a)

Method, guideline, deviations, if any	Test substance	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
	mg/kg bw/d			
Salmonella typhimurium reverse mutation assay with BENALAXYL Test method: OECD TG 471, and Commission Directive 2000/32/EC, B 13/14, May 2000 GLP/QA: Yes/Yes Acceptable	BenalaxylFCF/T /162-99 (ex lotto 4) Purity: 96.49% Vehicle: DMSO S9 Mix contained KCl 33 µM, MgCl2 8 µM, glucose-6-phosphate 5 µM, NADP 5 µM, in sodium ortho phosphate buffer (pH 7.4) 100 µM. The S9 was prepared in-house from the liver of male rats weighing approx. 220-320 g. Rats received a single i.p. injection of Aroclor 1254 in peanut oil at 500 mg/kg bw/d. S9 was 15% v/v in the cultures.	S. typhimurium tester strains: TA1535, TA1537, TA98, TA100 and ± S9 activation 1st experiment (plate incorporation): 33, 100, 333, 1000, 2500 and 5000 µg/plate (± S9 Mix) 2nd experiment (pre-incubation): 33, 100, 333, 1000, 2500 and 5000 µg/plate (± S9 Mix)	Cytotoxicity (Preliminary Toxicity Test): benalaxyl was non-toxic to the strains of bacteria use (TA98 and TA100). In presence of metabolic activation the range of negative or solvent controls was not quite reached in TA1537 and TA100 (2nd experiment). Benalaxyl did not induce gene mutations by base pair changes or frameshifts in the genome of the strains used.	RAR-08_Vol-3 CA_B.6.4.1.4 Reference number: CA 5.4.1/04 Anonymous (2002a)
Microbiological study of mutagenesis on CRA 109 (M 9834): DNA damage and repair test (Mitotic gene conversion in Saccharomyces cerevisiae D4) Test method: Main deviations from OECD TG 480 were the use of 4 different concentrations of CRA 109, instead of 5; growing stage (stationary or growing) of cells used was not reported; and the test was not repeated using stationary	Benalaxyl:M 9834; CRA 109 Lot/Batch: 5532/86 Purity: 98% Vehicle: DMSO S9 Mix contained (per mL): S9 equivalent to 75 mg of fresh rat liver, MgCl2 8 µmol, KCl 33 µmol, Glucose-6-phosphate 5 µmol, NADP 4 µmol, and phosphate buffer (pH 7.4) 100 µmol.	Saccharomyces cerevisiae strain D4 1.2 x 108 cells/ml. Dose level: 8, 40, 200 and 1000 µg/mL (± S9 Mix)	Benalaxyl: M 9834; CRA 109 and its possible metabolites, obtained with liver microsomes of rats induced with Aroclor, did not increase the frequency of gene conversion for the marker adenine and tryptophan after 16 hours contact with the microorganism. Both positive controls - MMS - Methyl methanesulfonate (200 µg/mL) without S9 mix and CPA - Cyclophosphamide (1500 µg/mL) with S9 mix induced significant increases in gene conversion frequency.	RAR-08_Vol-3 CA_B.6.4.1.5 Reference number: CA 5.4.1/05 Anonymous (1979b)

Method, guideline, deviations, if any	Test substance	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
phase cells to confirm negative results. Guidelines and GLP/QA: N/A: No Guidelines or GLP were compulsory at the time the study was performed. Statistics: No Evaluation criteria: Not indicated Acceptable	S9 was prepared in-house from the liver of male rats weighing 200-250 g. Rats received a single i.p. injection of Aroclor 1254 in peanut oil at 500 mg/kg bw/d			
Microbiological Study of Mutagenesis on CRA 109 (M 9834): In vitro Gene Mutation test in Schizosaccharomyces pombe P1 Test method: In-house method was used that complied with principles of OECD Guidelines 480 (OECD guideline 480 was deleted with effective date 2 April 2014 Test performance: Not reported Guidelines and GLP/QA: No GLP or Guidelines were compulsory at the time the test was performed. Acceptable	Benalaxyl:M 9834; CRA 109 Purity: 98% Vehicle: DMSO S9 Mix prepared from male Icem:CER (SPF Caw) rats, treated with a single i.p. injection of Aroclor 1254	Schizosaccharomyces pombe P1 (SP 198, ade6-60/rad10-198, h-), 1.2 x 108 cells/ml. 0.32, 0.16, 8, 40, 200 and 1000 µg/mL repeated with concentrations of 160, 80, 40 and 20 µg/ml without metabolic activation (1 replication), and concentrations of 800, 400, 200 and 100 µg/ml with metabolic activation (2 replications).	Benalaxyl was toxic at 80 µg/ml and above – S9 activation and at concentration of 1000 µg/ml +S9 activation. It did causes increasing in the frequency of mutation, ± S9 activation, compared to the negative controls. 1st experiment of mutagenic activity of CRA 109 on Schizosaccharomyces pombe P1 after 16 hours contact at 35º C 2nd experiment of mutagenic activity of CRA 109 on Schizosaccharomyces pombe P1 after 16 hours contact at 35º C without metabolic activation 2nd and 3nd experiments of mutagenic activity of CRA 109 on Schizosaccharomyces pombe P1 after 16 hours contact at 35º C with metabolic activation CRA 109 was therefore found to be non-mutagenic in this test while the positive standards were highly mutagenic.	RAR-08_Vol-3 CA_B.6.4.1.6 Reference number: CA 5.4.1/06 Anonymous (1980)
In vitro chromosome aberration test in Chinese Hamster Ovary (CHO) cells with benalaxyl	Benalaxyl: FCF/T/162-99 (ex lotto 4) Purity: 96.49%	Chinese Hamster Ovary (CHO) cells 5 x 10^4 – 9 x 10^4 Preliminary 22.3, 44.5, 89.1, 178.1, 356.3, 712.5, 1425 and 2850	1st experiment - Preliminary toxicity test CLEAR TOXIC EFFECT: After 4 hours of treatment	RAR-08_Vol-3 CA_B.6.4.1.7 Reference number:

Method, guideline, deviations, if any	**Test substance**	**Relevant information about the study including rationale for dose selection (as applicable)**	**Observations**	**Reference**
Test method: OECD TG 473 and EC Method B 10 Guidelines: OECD TG 473 (1997) and EC Method B.10. Compared to current OECD 473 (29 July 2016), exposures without metabolic activation were longer than required (i.e. 24 and 48 h) and only 200 metaphases (100 x culture) were scored per concentration Statistic: Fisher's exact test was used for determining statistical significance. Analytical determinations: Not performed. GLP/QA: Yes/Yes Acceptable	Vehicle: Acetone Non-activation (-S9): Ethyl methanesulfonate (EMS, purity > 98%; 75 – 250 µg/mL) Activation (+S9): Cyclophosphamide (CPA, purity 98%; 1 µg/mL) S9 Mix contained KCl 33 µM, $MgCl_2$ 8 µM, glucose-6-phosphate 5 µM, NADP 4 µM, in sodium ortho phosphate buffer (pH 7.4) 100 µM. S9 was prepared in-house from the liver of male rats weighing approx. 220-320 g, orally received 3 consecutive daily doses of phenobarbitone/ β-naphthoflavone (80/100 mg/kg bw/day) prior to S9 preparation on day 4.	µg/mL (-S9) cytotoxicity (range - finding) assay (mM) (±S9 Mix): 22.3, 44.5, 89.1, 178.1, 356.3, 712.5, 1425 and 2850 µg/mL (≅9 mM) (± S9 Mix)	with 98.1 µg/mL and above in the absence of S9 mix were observed after and with 178.1 µg/mL and above in the presence of S9 mix. STRONG TOXIC EFFECT: 24 hrs continuous treatment with 44.5 µg/mL and above in the absence of S9 mix. 2nd experiment - Cytogenetic assays: STRONG TOXIC EFFECT: indicated by reduced cell numbers and/or mitotic indices below 50% of control were observed in all parts. Higher concentrations were not evaluable for cytogenetic damage due to strong test item induced toxicity with strongly reduced mitotic indices. In detail, in the absence of S9 mix reduced cell numbers were observed after 4 hrs treatments with 75 µg/mL (54% of control) and after 46 hrs continuous treatments with 37.5 µg/mL (59% of control). Additionally, reduced cell numbers were obtained in the presence of S9 mix at 24 hrs preparation intervals with 100 µg/mL (16% of control). The mitotic indices were clearly reduced in the absence of S9 mix after 24 hrs continuous treatment with 25 µg/mL (47% of control).	CA 5.4.1/07 Anonymous (2002a)
In vitro study of the Induction of Chromosome Aberrations by Compound M 9834 in Human Lymphocyte Cultures Test method: In-house method was used which complied to a great extent with	Benalaxyl: M 9834 (No information: Batch number and purity) Vehicle: DMSO Non-activation (-S9): Mitomycin C (1.3 µg/mL) Activation	Human Lymphocyte Cultures – HLC (from man) - fresh 3.3, 10, 33 and 100 µg/mL	Benalaxyl was found to be toxic to lymphocyte cultures, especially in the absence of metabolic activation. - number of metaphases equal to the control (every dose) Benalaxyl (M 9834) did not induce any statistically significant increases of chromosome aberrations in	RAR-08_Vol-3 CA_B.6.4.1.8 Reference number: CA 5.4.1/08 Anonymous (1980)

Method, guideline, deviations, if any	Test substance	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
EC method B10. Guidelines: In-house method was used which complied to a great extent with EC method B10. GLP/QA: N/A: GLP was not implemented at the time the study was performed and no guideline was available Deviations: Main deviations were that specifications of active substance (purity) were not reported. Evaluation criteria: Only metaphases with a number of chromosomes "specific" for the species used, were considered. Possible aberrations were classified as follows: fragments; gaps; breaks; and exchanges. Test performance: Not indicated. Acceptable	(+S9): Phenacetin (1.28 µg/mL) S9 Mix consisted of S9, plus KCl 33, $MgCl_2$, glucose-6-phosphate, and NADP. No further details are provided. S9 was prepared in-house from the liver of male rats weighing approx. 150-200 g, received a single i.p. injection of Aroclor 1254		human lymphocyte cultures, up to 100 µg/mL, both in the ±S9 metabolic activation. The study is negative but should be considered as supplemental on the basis of missing information on batch number and purity.	
Evaluation of Galben in the Primary Rat Hepatocyte; Unscheduled DNA Synthesis Assay Test method: Test method was based on the procedure described by Williams (1977, 1980) and complied to a great extent with OECD Guideline 482. OECD 482 was deleted in April, 2014. Deviations: Any kind of procedure to block	Galben Th (= benalaxyl) Lot/Batch: FCF/T/1354 Purity: 94% Vehicle: DMSO 2-Positive: acetylaminofluorene (2-AAF; 0.05 µg/mL)	Hepatocytes – liver (adult male Fisher 344 rat) Dose level: 0.5, 1, 2.5, 5, 10, 25, 50 and 100 µg/mL	Benalaxyl (Galben) was found to be insoluble at a concentration of 10 mg/mL in culture medium; cloudy suspensions of material were observed for concentrations down to 100 µg/mL and complete solubility appeared to be maintained at 50 µg/mL and lower. Treatments with 1000 and 250 µg/mL were completely lethal to the cells, and 100 µg/mL caused excessive toxicity. At 50 µg/mL, the survival increased sharply to the level observed for the solvent control cultures and	RAR-08_Vol-3 CA_B.6.4.1.9 Reference number: CA 5.4.1/09 Anonymous (1983)

Method, guideline, deviations, if any	Test substance	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
entry of cells into S-phase, the results were evaluated in function of "evaluation criteria" and not by means of a statistical analysis, and no independent experiment was performed to confirm the results. Remarks: The positive control nuclear labelling is not used as a reference point to estimate mutagenic risk associated with the UDS activity of the test material; it is used only to demonstrate that methodology was adequate. Statistics: No GLP/QA: Yes/Yes Acceptable			no morphological evidence of toxicity was observed. Galben Th (= benalaxyl), Lot/Batch: FCF/T/1354 with a purity of 94% was inefficient in this test. Only a statement of Quality Assurance Unit (reference 21 CFR 58.35 (b)(7)) was included in the report, which claimed that the test was carried out according to GLP	
In vitro Mammalian Cell Gene Mutation Test (L5178Y/TK+/- Mouse Lymphoma Assay) with Benalaxyl Technical Test method: OECD TG 476 and OPPTS 870.5300 Remarks: Verification of a clear positive response was not required (OECD TG 476). For negative results without activation, an extended treatment assay was performed in which cultures were continuously exposed to the test substance for 24 hours without S9 activation.	Ben Tech PL13-0055 Purity: 98.4% Vehicle: DMSO Positive controls: Non-activation (-S9): MMS (99.9% pure) Activation (+S9): DMBA (99% pure) S9 was prepared from male Sprague-Dawley rats that were i.p. with Aroclor™ 1254 (200 mg/mL in corn oil) at a dose of	L5178Y/TK+/ cultured MLC – mouse lymphoma cells ($TK^{+/-}$ assay), cultured mouse lymphoma L5178Y cells were treated with benalaxyl technical in DMSO: 0.781, 1.56, 3.13, 6.25, 12.5, 25, 50, 75, 100 and 150 µg/mL (4-hours treatment with S9 mix prepared from Aroclor treated rats), 3.13, 6.25, 12.5, 25, 50, 75, 100 and 150 µg/mL (4-hours treatment without S9), and 1.56, 3.13, 6.25, 12.5, 25, 50, 75 and 100 µg/mL (24-hours treatment without S9), selected on the basis of the preliminary test.	Visible precipitate was observed at the concentration of 150 µg/mL at the beginning and end of the 4-hours treatment ±S9. Relative suspension growth (RSG) of 26 to 93%, 41 to 101% and 32 to 112%, respectively, and subsequently cells cloned culture at: The cloned cultures had relative total growth (RTG) of 23 to 114% (4-hours treatment with S9), 45 to 116% (4-hours treatment without S9) and 30 to 108% (24-hours treatment without S9). Cultures treated at concentrations ≥6.25 µg/mL with S9 mix had average induced mutant frequencies (IMF) ≥90 mutants/106 clonable cells; these	RAR-08_Vol-3 CA_B.6.4.1.10 Reference number: CA 5.4.1/10 Anonymous (2014a)

Method, guideline, deviations, if any	Test substance	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
Statistics: No Analytical determination: No Deviations: No deviations from the protocol or assay-method SOPs Acceptable	500 mg/kg bw/d,		increases in IMF demonstrated dose-response relationship. Benalaxyl produced mostly small colonies indicating clastogenic effects rather than mutagenic (IMF no increased under either treatment condition without S9 mix). Benalaxyl was positive in the presence of metabolic activation in this gene mutation assay in mammalian cells and induced mainly small colonies, but was negative without metabolic activation. The induction of small colony mutants is usually associated with chemicals that induce gross chromosome aberrations and not gene mutation. Slight increase in mutant frequencies was noted with increasing cytotoxicity. The majority of experts' meeting PRAS 182 – sept, 2018 agreed to consider this study negative.	

Table 15: Summary table of mutagenicity/genotoxicity tests in mammalian somatic or germ cells *in vivo*

Method, guideline, deviations if any	Test substance,	Relevant information about the study (as applicable)	Observations	Reference

Method, guideline, deviations if any	**Test substance,**	**Relevant information about the study (as applicable)**	**Observations**	**Reference**
In vivo Gene Mutation in Chinese Hamster V79 Cells; Test Substance: Galben TH Test method: In-house method was used that complied to a great extent with OECD TG 476. GLP/QA: N/A: GLP was not implemented at the time the study was performed and no guideline was available. Acceptable	Galben Th (= benalaxyl) FCF/T/1354 Purity: 94% Vehicle: Ethanol Non-activation (-S9): Ethyl methanesulfonate (EMS) Activation (+S9): Dimethylnitrosamine (DMNA) S9 Mix contained (for 10 mL): S9 homogenate 3 mL, $MgCl_2$ 50 mM, 0.5 mL, KCl 330 mM, 0.5 mL, glucose-6-phosphate 15 mg/mL minimal medium, 1 mL, NADP 15 mg/mL minimal medium, 0.5 mL, NADPH 15 mg/mL minimal medium, 0.5 mL, DMEM (minimal medoum), 2 mL, Hepes 20 mM, 2 mL.	cultures of Chinese hamster V79 Locus examined: HGPRT Preliminary Assay: 0, 10^{-3} M, 10^{-4} M, 10^{-5} M, 10^{-6} M, 10^{-7} M and 10^{-8} M Cytotoxicity(range-finding) assay: 3 x 10^{-5} M, 1 x 10^{-5} M, 3 x 10^{-6} M, 1 x 10^{-6} M Mutation assay: 3 x 10^{-7} M transferase transgene (xprt).	Preliminary cytotoxicity assay Benalaxyl was not soluble in the medium at the top dose level of 10^{-3} M, while at the dose of 10^{-4} M (in the absence of S9 mix), it was toxic to the cells (no survival). The same concentration of 10^{-4} M with metabolic activation resulted in 56% survival of cells. Mutation assays – mutation frecquency not significant increase in the ±S9 activation. Positive control – significant increases. Benalaxyl not induce gene mutation in cultured Chinese hamster V79 cells. Fibroblast colonies of Chinese hamster V79 cells have ability to metabolise a toxic purine analogue (6-thioguanine – 6-TG) by the enzyme hypoxanthine-guanine-phosphoribosyl-transferase (HGPRT) into nucleotides (selecting the HGPRT-deficient cells through recessive mutation). Since the gene which codes for the enzyme HGPRT is located on the X chromosome, of which only one copy is present in male cells, a single mutation is sufficient to evidence this resistance.	RAR-08_Vol-3 CA_B.6.4.1.11 Reference number: CA 5.4.1/11 Anonymous (1983)
Benalaxyl Technical is not genotoxic Test method: N/A: evaluation of available data Guidelines and GLP/QA: No/N/A Acceptable. International Workshop on Genotoxicity Testing (IWGT; Thybaud et al., 2007) and European Food Safety Authority (EFSA,	Benalaxyl tech.	Ten in vitro genotoxicity and three in vivo genotoxicity assays have been conducted over 35 years.	All tests were negative for genotoxicity except gene mutation assay on L5178 TK+/‘ mouse lymphoma Additional TK data were provided and reveal a significant bone marrow exposure at Tmax.	RAR-08_Vol-3 CA_B.6.4.1.12 Reference number: CA 5.4.1/12 Anonymous (2014)

Method, guideline, deviations if any	**Test substance,**	**Relevant information about the study (as applicable)**	**Observations**	**Reference**
2011).				
In Vivo Micronucleus Assay in Rats using Benalaxyl Technical All dose formulation - 10 mL/kg by i.p. (CP by oral gavage) Test method: OECD TG 474 and OPPTS 870.5395 Remarks: All criteria for a valid study were met. Guidelines and GLP/QA: Yes/Yes Statistics: Statistical significance was determined using the binomial distribution (Kastenbaum-Bowman tables). Acceptable	Benalaxyl tech. Lot/Batch: PL13-0055 Purity: 98.4% Vehicle control: 0.5% methylcellulose (400 cPs) aqueous solution in deionized water Control: 0.5% methylcellulose aqueus solution (negative) and positive (cyclophosphamide – CP) Dose Range - finding toxicity tests: 500, 600 and 700 mg/kg (1st study) Toxicity test: 100, 200, 300 and 400 mg/kg (2nd study) Micronucleus assay: 68.75, 137.5 and 275 mg/kg 68.75, 137.5 and 27	Bone marrow cells [polychromatic erythrocytes (2000 PCEs/animal)] The test animal: Rats, strain Sprague-Dawley (Hsd:SD 3/sex/dose - DRF study 5 males (Groups 2,3 and 5, i.e. low, mid dose and positive control, respectively) 10 males (Group 1 and 4, control and high dose).	Range finding (toxicity tests) 1st DRF study: - mortality 2/3 m and 1/3 f at 500 mg/kg bw/d 1/3 m and 3/3 f at 600 mg/kg bw/d 2/3 m and 2/3 f at 700 mg/kg bw/d - clinical signs: Piloerection/lethargy/prostration at 600 mg/kg bw/d (m) and at 500 mg/kg bw/d (f) and 700 mg/kg bw/d (f) Lethargy and piloerection in at 500 mg/kg bw/d (m) and 700 mg/kg bw/d (m) Piloerection and prostration at 600 mg/kg bw/d (f) 2nd DRF study - mortality 1/3 m and 1/3 f at 300 mg/kg bw/d 2/3 m and 1/3 f at 400 mg/kg bw/d -clinical signs: Lethargy and piloerection at 400 mg/kg (m + f) at 300 mg/kg bw/d (m) Lethargy, piloerection and prostration at 300 mg/kg bw/d (f) MTD = 275 mg/kg bw/d Micronucleus assay - No mortality occurred at any dose level during the course of the definitive assay. -Clinical signs: Lethargy, piloerection and prostration at 275 mg/kg. bw/d -Citotoxicity: No appreciable reductions in the PCEs/EC ratio in the test substance groups compared to the	RAR-08_Vol-3 CA_B.6.4.2.1 Reference number: CA 5.4.2/01 Anonymous (2014)

Method, guideline, deviations if any	**Test substance,**	**Relevant information about the study (as applicable)**	**Observations**	**Reference**
			vehicle control group. No statistically significant increase in the incidence of MnPCEs in the test-substance treated groups was observed relative to the negative control group ($p > 0.05$, Kastenbaum-Bowman tables). The positive control induced a statistically significant increase in the incidence of mnPCEs ($p < 0.05$, Kastenbaum-Bowman tables). The number of MnPCEs in the vehicle control groups did not exceed the historical control range. Benalaxyl tech no induce a significant increase in the incidence of micronucleated polychromatic erythrocytes. It was negative or non-clastogenic in the in vivo Micronucleus assay in male rats. Administration of Benalaxyl tech at doses up to and including a dose of 275 mg/kg bw/dwas concluded to be negative in the micronucleus assay.	
Plasma and Bone Marrow Concentrations of [^{14}C]-Benalaxyl in Male Sprague Dawley Rats Following a Single Intraperitoneal Administration of [^{14}C]-Benalaxyl Test method: OECD TG 474 Guidelines GLP/QA: Yes/Yes Statistics: Statistical significance was performed. Acceptable	Radiolabelled: [14C]-UL-Dimethylphenyl-Benalaxyl Lot/Batch: 80534-04-28 Radiochemical Purity: 99% Specific Activity: 0.19 µCi/mg (61 mCi/mmol) Chemical purity: 99% The test material – Non-Radiolabelled: Benalaxyl Lot/Batch: G333:87 Purity: 99% 4 male Sprague Dawley rats 275 mg/kg bw/d (200 µCi/kg; 10 mL/kg) Vehicle: 0.5% (w/v) methylcellulose (400 cps) in deionized water Blood and bone marrow were collected at 0.5 h post-dose (tmax, as determined in ADME	Species: Rat Strain: Sprague-Dawley Age: 6-7 weeks Weight at dosing: M	Results: [^{14}C]-Benalaxyl was present in both the plasma (32505 ng equiv/g) and bone marrow) at 275 mg/kg bw/d and at 0.5 h post-dose.	RAR-08_Vol-3 CA_B.6.4.2.1-01bis Reference number: CA 5.4.2/01bis Anonymous (2018a)

Method, guideline, deviations if any	**Test substance,**	**Relevant information about the study (as applicable)**	**Observations**	**Reference**
	studies) and processed.			
Micronucleus induction in bone marrow cells of rats treated by intraperitoneal route with the test article Benalaxyl Tech Test method: OECD TG 474 and EC method B.12 Guidelines and GLP/QA: Yes/Yes Acceptable	Benalaxyl technical Lot/Batch: FCF/T/95-91/1997 Purity: 96.68 ± 0.95% Vehicle: 0.5% (w/v) methylcellulose aqueous solution Control materials: Negative: 0.5% methylcellulose aqueous solution Positive: Cyclophosphamide (CP Dose Level: (a) Dose Range-finding: 600 and 700 mg/kg toxicity tests: (b) Micronucleus assay: 125, 250 and 500 mg/kg bw/d	Rat Strain: Sprague-Dawley (Crl:SD) 9 weeks (main study) Males and females: 218-356 g (main study) Number of animals: 3/sex/dose in the DRF studies 5/sex/dose in the main study	injection at doses up to and including a dose of 500 mg/kg bw/d was concluded to be Results: 125, 250 and 500 mg/kg bw/d: - not induce statistically significant increase in the frequency of micronucleated cells in the bone marrow 24 and 48 hours from the administration 500 mg/kg bw/d: no significant change in the PCE/NCE ratio was observed, systemic exposure was demonstrated by the death of one male and 4 females treated	RAR-08_Vol-3 CA_B.6.4.2.2 Reference number: CA 5.4.2/02 Anonymous (2000)
In vivo Study of the Induction of Chromosome Aberrations in the Chinese Hamster by Compound M9834 Administred Orally Test method: Study did not complied with EC method B11 for the following reasons: only 2 animals/sex/group were used (instead of 5/sex/group); Remarks: The study was conducted and in 2000 on micronucleus (B.6.4.2.2) and in 2014 (B.6.4.2.1) Guidelines and GLP/QA: GLP were not compulsory at the time the test was performed. Statistics: Yes Deviations: Deviations from EC method B11. Acceptable as additional information taking into consideration deviations from currently accepted Guidelines.	Benalaxyl technical (M 9834) Oral (gavage) twice in 24 hours Chinese hamsters groups: 2 m and 2 f Dose levels were set on the basis of available data, with the highest dose level close to the LD_{50}. Six hours after the second treatment, all animals received an i.p. injection of colchicine at the dose of 6 mg/kg bw/d (10 ml/kg). Vehicle: 0.5% methyl cellulose aqueous solution Negative (vehicle, 10 mL/kg) Positive (mitomycin C (MMC) 10 ml/kg solution in sterile physiological saline (i.p. injection) Dose level: 1000, 2000 and 4000 mg/kg bw/d M 9834 suspended in methocel	3 animals died after repetition of dosage; only one sampling time was performed (7 hours after last treatment) instead of two sampling time at 6 and 24 hours; and specifications of active substance (purity) were not reported	At 2000 mg/kg bw/d -2 animals and one treated with MMC died after the second treatment. It was observed that MMC induced statistically significant increases of chromosome aberrations both including and excluding gaps. 4000 mg/kg bw/d -no clastogenic activity This study is not reliable on to fulfil requirements regarding in vivo genotoxicity testing.	RAR-08_Vol-3 CA_B.6.4.2.3 Reference number: CA 5.4.2/03 Anonymous (1980)

Table 16: Summary table of human data relevant for germ cell mutagenicity

No study available.

10.8.1 Short summary and overall relevance of the provided information on germ cell mutagenicity

The genotoxicity of benalaxyl has been adequately investigated in standard tests. In all of these assays, benalaxyl was examined at dose levels up to those causing clear toxicity in the test system or insolubility

The potential of benalaxyl to induce mutagenic effects was investigated in 10 in vitro and 3 in vivo tests developed over 35 years. Nine of ten in vitro and all three in vivo studies were negative for genotoxicity. They adequately cover all genotoxicity endpoints such as structural and numerical chromosomal alterations and induction of gene mutations.

All tests were negative or within some uncertains for genotoxicity except the forward gene mutation assay (Anonimous, 2014) on the L5178 TK+/‘ mouse lymphoma assay test system. In this study, the primary focus were dose-related positive results obtained following four hours exposure in the presence of metabolic activation.The increased mutant frequency was related mainly to small colonies, thus suggesting a clastogenic rather than a mutagenic event. Therefore, the result of Gene mutation in L5178Y cells (TK+/- Mouse Lymphoma Assay) ±S9 mix is a precipitate observed at 150 µg/mL at the beginning and end of the 4-h treatment launching the ipothesis that ±S9 mix the induction of small colony mutants is usually associated with chemicals that induce gross chromosome aberrations and not gene mutation).

Therefore, the result of Gene mutation in L5178Y cells (TK+/- Mouse Lymphoma Assay) ±S9 mix is a precipitate observed at 150 µg/mL at the beginning and end of the 4-h treatment launching the ipothesis that ±S9 mix the induction of small colony mutants is usually associated with chemicals that induce gross chromosome aberrations and not gene mutation)

It has been conducted and a supportive study on toxicokinetic (TK) to quantify the Plasma and Bone Marrow Concentrations of [14C]-Benalaxyl in Male Sprague Dawley Rats Following a Single Intraperitoneal Administration of [14C]-Benalaxyl (Anonymous, 2018a). Additional TK data were provided and showed significant bone marrow exposure at Tmax. For a single intraperitoneal administration to rats at 275 mg/kg bw per day, a presence of the radiolabelled and non-radiolabelled benalaxy l14C]-benalaxyl was determined in plasma and bone marrow – after 0.5 h post-dose administration, as plasma (32505 ng equiv/g) and bone marrow (34664 ng equiv/g), respectively.

That both micronucleus studies were confirmed to be negative on the basis of these TK data provided.

The results are unconclusive for a suitable classification.

10.8.2 Comparison with the CLP criteria

Not relevant.

Conclusion on classification and labelling for germ cell mutagenicity

Not relevant.

10.9 Carcinogenicity

Long-term study for carcinogenicity has been investigated in rats, mice and dogs and a detailed results are comprehensive listed in the tables below.

Table 17: Summary table of animal studies on carcinogenicity

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
Short-term range finding study of Evaluation of Chronic Toxicity and Oncogenic Potential of Galben (CAS No. 71626-11-4) in Swiss Mice (Oral Dosing Study) The 13-week mouse study was considered as a Dose range-finding study, not suitable for the derivation of a NOAEL Oral (dietary) Method: In house method Swiss mice 20/sex/group (6) Weekly: - Measurements of individual body weight, food and water consumption - Observations for group behaviour, mortality, and toxic signs, and lesions at clinical examination Remarks: This is a range-finding for setting dose levels to be used in the main carcinogenicity study. No ophthalmological, haematological or biochemical examinations were performed. GLP/QA:N/A: RF study Acceptable	Benalaxyl: any information Dose levels: 0, 500, 1000, 2000, 3000 and 5000 ppm (0, 80.25, 160.5, 321, 481.5, 802.5 mg/kg bw/day for males and 0, 90.75, 181.5, 363, 544.5, 907.5 mg/kg bw/day for females) After 40 days of treatment, 10 surviving animals/sex/group were sacrificed; terminal sacrifice was conducted at the end of 90 days of treatment.	The 90-day study in mice was conducted manly as tolerability test, in order to set dose levels for the main carcinogenicity study. Given that no hematology or clinical chemistry was performed, changes in liver weight that was used for setting the high dose level in the carcinogenicity study. Dose levels tested: 5000 ppm - marked toxic effect in liver weight. 3000 ppm was selected as the highest dose level for the long-term study. The NOEL was 500 ppm due to equivocal increased liver weight at 1000 ppm in females. Proposed NOAEL = 2000 ppm equivalent to 320.6 mg/kg bw/day (m) and 363.0 mg/kg bw/day (f) based increased liver weight at 1000 ppm, but not set based on equivocal and not associated effects on liver with histopathological changes. Results: No mortality at all dose levels. Body weight, water and food consumption were not affected by treatment Clinical observation did not reveal any abnormality that could be related to treatment, apart from an excessive waste of food caused by mice treated with 5000 ppm. Clinical chemistry: 10000 and 12000 ppm at week 5: ↑ cholesterol (m + f) This observation was confirmed for animal treated at 10000 ppm at week 13 while the parameter had returned to normal values after the recovery period in those treated at 12000 ppm. 1000 and 10000 ppm: ↓ ucose (f) urea in females at 100 and 10000 ppm: ↓ urea (f) 100 and 10000 ppm: ↓ ATP (m) 10000 ppm: ↓SGOT (m) and at all treated group (f) 100 and 10000 ppm: ↓ SGPT (m) 100, 10000 and 12000 ppm: ↓ LDH (f) 100 and 10000 ppm: ↑ total proteins (f) 10000 ppm: ↑alpha$_2$-globulins (m + f) 100, 10000 and 12000 ppm: chlorine (m). Urinalysis There were alterations in urinalysis parameters.	RAR-08_Vol-3 CA_B.6.3.2.1 Reference number: CA 5.3.2/01 Anonymous (1985)

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
		Histopathological examination: brain, thymus with mediastinal lymph nodes, lungs, liver, spleen, pancreas, kidneys, adrenals, stomach, duodenum, colon, rectum, urinary bladder, uterus, ovaries, testes with epididymis. 1000 and 10000 ppm: ↑ absolute and relative liver weights (m) 10000 and 12000 ppm: ↑ realative weight (f) 10000 ppm: ↑ relative kidney weight (m + f) 100 ppm: ↑ the relative pituitary weight (m) 10000 ppm: ↓ of absolute spleen weight (m) 100 ppm: ↓ relative spleen weight (f) 10000 ppm: diffuse steatosis in the liver (m + f) more severe in males The same lesion was observed sporadically in animals from the other groups in which, however, consisted of mild perilobular steatosis. 10000 ppm: Sporadic cases of necrosis, limited to a few myocardial fibres, were also observed in the heart (m) 1000, 3000 and 5000 ppm (at 2000 ppm the increase was only significant at the 40-day sacrifice) - Liver weight was significantly and dose-related increased in females treated, and in males treated with 5000 ppm Necropsy: lobulation of liver was evident in several males at 1000 and 10000 ppm, associated in some cases, with rounded edges (This finding was also present sporadically in animals of the other groups). In females at 10000 ppm the liver appeared darker than normal; females appeared more affected than males.	
Lifetime oral dosing studies in rats: combined oncogenicity and chronic toxicity of GALBEN technical (M 9834) Oral (dietary) Test method: Test method complied with EPA proposed guidelines for registering pesticides in the US (Fed. Reg. 43, No. 163, 1978). 65 Sprague-Dawley	Galben technical M9834 (= benalaxyl) Lot/Batch: FCF/T/1213 Detailed impurities profile included in the report Purity: 96.8% Vehicle and/or positive control: Certified NIH No positive control – not required Dose levels: 0, 4, 10 and 1000 ppm,	No significant differences in bwt/bw gain between the treated and control groups; No consistent differences in food and water consumption were observed. Survival - 47 - 61% of animals of each group died during the study. All animals which died, killed when moribund or killed at termination were necropsied. Only few statistically significant differences were found between the control and test animals (increase in percentage of eosinophils in females at 1000 ppm at week 52; increase in erythrocyte counts in females at 4 and 100 ppm at 18 months; and increased reticulocyte count in females at 4 ppm at 24 months). Since all values were within the normal range for this age and strain of rats, and no dose-response relationship was noted, these differences were not considered treatment-related.	RAR-08_Vol-3 CA_B.6.5.1.1 Reference number: CA 5.5 /01 Anonymous (1985)AnonymusAnonymusAnonymus

<table>
<tr><th>Method, guideline, deviations if any, species, strain, sex, no/group</th><th>Test substance, dose levels duration of exposure</th><th>Results</th><th>Reference</th></tr>
<tr>
<td>rats/sex

Strain: Crl:CD(SD)

Guidelines and GLP/QA: Yes/Yes: The study was carried out according to GLP (21 CFR, Part 58)

Acceptable</td>
<td>equivalent to 0, 4.42 mg/kg bw/day in males + 5.64 mg/kg bw/day in females

104 consecutive weeks - 24 months

The dose levels were selected on the basis of the results obtained in a 13-week oral subchronic toxicity study -
CA 5.3.2/01
Anonymous: (1985)</td>
<td>After 12 months:

Histopathology was performed on all rats. The brain, heart, kidneys, liver, ovaries, testes and thymus (not weighed after 12 months) were weighed.

After 24 months:

Histopathology was performed on all rats.

Corneal opacities were observed in animals of all groups and were attributed to occurrence of sialodacryoadenitis (SDA) virus infection, which had no effect on the integrity of this study

Haematological parameters – a few statistically differences: (within the normal range for this age and strain of rats, and no dose-response)

1000 ppm at week 52: ↑ eosinophils (f)

4 and 100 ppm at 18 months: ↑ erythrocyte counts (f)

4 ppm at 24 months: ↑ reticulocyte count (f)

Clinical chemistry:
<table>
<tr><th rowspan="3">Parameter</th><th colspan="8">Sex and dose level (ppm)</th></tr>
<tr><th colspan="4">Male</th><th colspan="4">Female</th></tr>
<tr><th>0</th><th>4</th><th>100</th><th>1000</th><th>0</th><th>4</th><th>100</th><th>1000</th></tr>
<tr><td colspan="9">6 months</td></tr>
<tr><td>LDH (IU/L)</td><td>367</td><td>356</td><td>368</td><td>453</td><td>27</td><td>337</td><td>312</td><td>242</td></tr>
<tr><td>Potassium (meq/L)</td><td>4.5</td><td>4.7</td><td>4.8</td><td>4.7</td><td>4.3</td><td>4.2</td><td>4.2</td><td>4.1</td></tr>
<tr><td colspan="9">12 months</td></tr>
<tr><td>LDH (IU/L)</td><td>1052</td><td>1132</td><td>946</td><td>1168</td><td>589</td><td>731</td><td>696</td><td>746</td></tr>
<tr><td>Potassium (meq/L)</td><td>5.1</td><td>5.3</td><td>5.0</td><td>4.8</td><td>4.1</td><td>4.2</td><td>4.1</td><td>4.1</td></tr>
<tr><td colspan="9">18 months</td></tr>
<tr><td>LDH (IU/L)</td><td>272</td><td>309</td><td>364</td><td>495*</td><td>303</td><td>334</td><td>261</td><td>329</td></tr>
<tr><td>Potassium (meq/L)</td><td>4.1</td><td>4.4</td><td>4.4</td><td>4.6*</td><td>3.8</td><td>3.5</td><td>4.0</td><td>4.3</td></tr>
<tr><td colspan="9">24 months</td></tr>
<tr><td>LDH (IU/L)</td><td>1038</td><td>1050</td><td>1363</td><td>1312</td><td>1171</td><td>1074</td><td>950</td><td>1097</td></tr>
<tr><td>Potassium (meq/L)</td><td>4.8</td><td>4.8</td><td>4.8</td><td>5.1</td><td>4.0</td><td>4.2</td><td>4.0</td><td>4.1</td></tr>
</table>
*: p≤0.05
18 months:

dose-related

1000 ppm statistical significance ↑ LDH (m) only at in comparison to controls.

1000 ppm ↑serum potassium levels in comparison to controls

No biologically significant differences among groups were noted at urinalysis.

Ocular abnormalities noted in clinical observation were, in the opinion of the veterinary ophthalmologist, not</td>
<td></td>
</tr>
</table>

<table>
<tr><th>Method, guideline, deviations if any, species, strain, sex, no/group</th><th>Test substance, dose levels duration of exposure</th><th>Results</th><th>Reference</th></tr>
<tr><td></td><td></td><td>
related to administration of benelaxyl due to the sporadic and unilateral nature.

1000 ppm at 24 months: increases in relative heart weight (10%) (m)

2 and 100 ppm at 24 months: decreases absolute ovary weights of female rats at were significant statistic

Macroscopic observations: at interim kill - milk cysts (galactoceles) in mammary glands, which were observed in females of all groups, including the control.

100 ppm: ↑ hyperkeratosis of oesophagus (m)

1000 ppm: ↑ chronic (lymphocytic) inflammation and/or fibrosis, involving ↑ the Harderian (m)

100 ppm: ↑ submucosal lymphoid hyperplasia in the large intestine (m)

100 ppm: ↑ dilated, fluid-filled sinusoids (m)

4 and 100 ppm: ↑ macrophages in cervical lymph nodes (m)

4 ppm: ↑ mesenteric lymph nodes congestion (f)

4 and 100 ppm: ↓ glandular/lobular hyperplasia in mammary glands (m)

100 ppm: ↓ chronic prostatitis (m)

100 ppm: ↓ haemosiderosis in the spleen (m)

4 ppm: ↓ haemosiderosis in the spleen (f)

4 ppm: ↑ reticuloendotelial cell hyperplasia in the spleen (m)

4 ppm: ↓ erythroid hypoplasia (f).

1000 ppm: ↑ relative heart weight (m)

4 and 100 ppm: ↓absolute ovary weights (f)

Neoplasms: after 12 months.

19 in total in all groups, (including the controls) were observed microscopically:
<table>
<tr><td rowspan="2">Organ & tumour (or lesion)</td><td colspan="8">Sex and dose level (ppm)</td></tr>
<tr><td colspan="4">Male</td><td colspan="4">Female</td></tr>
<tr><td>0</td><td>4</td><td>100</td><td>1000</td><td>0</td><td>4</td><td>100</td><td>1000</td></tr>
<tr><td>Adrenals – No. examined</td><td>54</td><td>51</td><td>55</td><td>53</td><td>54</td><td>55</td><td>54</td><td>55</td></tr>
<tr><td>Medulla, carcinoma</td><td>9</td><td>6</td><td>7</td><td>8</td><td>4</td><td>4</td><td>1</td><td>1</td></tr>
<tr><td>(Medullary hyperplasia)</td><td>1</td><td>6</td><td>4</td><td>0</td><td>1</td><td>1</td><td>0</td><td>2</td></tr>
<tr><td>Brain – No. examined</td><td>54</td><td>52</td><td>55</td><td>54</td><td>54</td><td>55</td><td>54</td><td>55</td></tr>
<tr><td>Astrocytoma</td><td>0</td><td>1</td><td>1</td><td>2</td><td>0</td><td>0</td><td>0</td><td>0</td></tr>
<tr><td>Ependymoma</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>1</td></tr>
<tr><td>Liver – No. examined</td><td>54</td><td>52</td><td>55</td><td>54</td><td>54</td><td>55</td><td>54</td><td>55</td></tr>
</table>
</td><td></td></tr>
</table>

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference

Adenoma, neoplastic nodule	0	0	0	0	0	0	1	3
Heaptocellular carcinoma	1	2	1	0	1	3	1	1
Reticulum cell sarcoma (multiple organ)	0	2	0	1	3	3	1	0
Pancreas – No. examined	54	52	55	54	53	55	54	54
Islet cell adenoma	5	6	6	4	1	3	0	1
Islet cell adenocarcinoma	0	1	1	1	0	0	0	0
Parathyroids – No. examined	50	45	38	53	47	46	49	52
Adenoma	1	2	4	0	0	1	1	0
Pituitary – No. examined	53	50	54	53	54	51	51	53
Adenoma	22	18	25	20	42	40	41	47
Adenocarcinoma	0	0	1	0	0	0	1	0
Thyroid – No. examined	53	51	55	54	53	51	54	55
Parafollicular cell tumour (C-cell)	5	5	12	3	5	8	2	8
Follicular adenoma	2	0	0	1	0	0	1	0
Follicular adenocarcinoma	0	1	2	0	0	0	0	1

- Adrenal's medullary tumours -3 tumours in males at 100 ppm, which showed distant metastases to lungs and/or liver

- Brain: Intracerebral astrocytomas and ependymoma

- Liver: A total of 16 primary hepatocellular tumours were observed, representing a population frequency of 3%, which is compatible with the frequency of spontaneous hepatocellular neoplasms. Although the incidence of hepatocellular neoplasm was found to be greater in females at 1000 ppm, the difference was not statistically significant

- Mammary glands: Fibroadenomas were extremely frequent among all female groups (range: 38–52 %); adenocarcinomas ranged 4 to 9% in all groups

- Reticulum cell sarcoma was the most frequent malignant neoplasm showing multiple organ involvement in ten rats (2.3%)

- Pancreas: Islet cell adenomas were more common in males and noted with similar frequency among all male groups (range 7 to 11%).

- Parathyroid: Solitary distinct nodules within the

<table>
<tr><th>Method, guideline, deviations if any, species, strain, sex, no/group</th><th>Test substance, dose levels duration of exposure</th><th>Results</th><th>Reference</th></tr>
<tr><td></td><td></td><td>
parathyroids were all classified as adenomas, as no distinction was possible between “nodular” hyperplasia and adenoma; however taking in consideration the incidence, the effect was not attributed to administration.

- Adenomas of the pituitary were extremely frequent in all female groups (range 78 to 89%) and not uncommon among males (range 36 – 46%).

- Thyroid: parafollicular cell hyperplasia and tumours in males and females

<table>
<tr><td rowspan="3">Parameter</td><td colspan="8">Sex and dose level (ppm)</td></tr>
<tr><td colspan="4">Male</td><td colspan="4">Female</td></tr>
<tr><td>0</td><td>4</td><td>100</td><td>1000</td><td>0</td><td>4</td><td>100</td><td>1000</td></tr>
<tr><td>Total No. of animals with primary tumours / Total primary tumours</td><td>40 / 62</td><td>36 / 60</td><td>46 / 73</td><td>39 /56</td><td>52 / 106</td><td>51 / 99</td><td>47 / 84</td><td>52 / 94</td></tr>
<tr><td>No. of tumours / animal bearing tumours</td><td>1.55</td><td>1.67</td><td>1.59</td><td>1.44</td><td>2.04</td><td>1.94</td><td>1.79</td><td>1.81</td></tr>
<tr><td>Total No. of animals with metastatic tumours / Total metastatic tumours</td><td>2 / 2</td><td>3 / 3</td><td>0 / 0</td><td>5 / 5</td><td>6 / 6</td><td>9 / 9</td><td>2 / 3</td><td>3 / 3</td></tr>
<tr><td>No. of metastatic tumours / animal bearing tumours</td><td>0.05</td><td>0.08</td><td>0.00</td><td>0.13</td><td>0.12</td><td>0.18</td><td>0.06</td><td>0.06</td></tr>
</table>

The systemic toxicity was indicated by statistically significant increased LDH and K at the top dose in males (at 18 months but not at the end of the study, K was also increased at 24 months but without statistical significance), and by an increased heart weight without concurrent histopathological findings.

No findings in the heart were reported in other studies with Benalaxyl.

The heart weight together with LDH and K changes cannot be dismissed and all agreed at 100 ppm (4.42/5.64 mg/kg bw per day for m and f, respectively).

Astrocytoma was considered the most critical effect.

No HCD were available for the performing laboratory. Incidences of astrocytoma were available, but information on severity was missing.
</td><td></td></tr>
</table>

<table>
<tr><th>Method, guideline, deviations if any, species, strain, sex, no/group</th><th>Test substance, dose levels duration of exposure</th><th>Results</th><th>Reference</th></tr>
<tr><td></td><td></td><td>Carcinogen potential at 100 ppm (4.42/5.64 mg/kg bw per day for male and female, respectively) with the increased incidence at 1000 ppm in males and the absence of dose response at lower doses.

Based on the occurrence of astrocytomas in the rat study, a classification as Carc. Cat 2 is appropriate.</td><td></td></tr>
<tr><td>Evaluation of chronic toxicity and oncogenic potential of GALBEN (CAS No. 71626-11-4) in Swiss mice (Oral Dosing Study)

Oral (dietary)

Test method: In-house test method and EPA (44 FR 27334, 1979)

60 Swiss mice

78 consecutive weeks

Measurements of individual body weight, food and water consumption were performed weekly for the first 13 weeks, and then bi-weekly.

Observations for group behaviour and mortality were done 3 times a day (twice daily on holidays and weekends); individual behaviour, pharmacologic and toxic signs, and lesions detectable at clinical examination, were recorded once weekly.

Haematological examinations were performed before the beginning of treatment, and at 52 and 78 weeks (terminal sacrifice) on 10 animals/sex/group.

Clinical chemistry was conducted at week 78 in 10</td><td>Galben (= Benalaxyl)

Lot/Batch: Not indicated

Purity: 94%

Vehicle as a powdered diet / No positive control – not required

Dose level:

0, 250, 1000 and 3000 ppm (44.93, 180.87 and 558.87 mg/kg bw/day for males and to 42.93, 174.26 and521.73 mg/kg bw/day for females) for 78 consecutive weeks.

The highest concentration was chosen on the basis of the preliminary 90-day range-finding study, in which this concentration produced detectable toxic changes (see CA 5.3.2/01).</td><td>Body weight gains appeared to be affected in treated males from the week 33 to the end of the study. Differences from control ranged from 5 to 10% but the effect was more evident at 250 ppm and there was no dose-relationship.

No consistent differences in food and water consumption were observed.

1000 and 3000 ppm: ↓ Survival (m) related to amyloidosis

At all doses: ↓ bw gain (m)

3000 ppm: ↑absolute and relative liver weight (f)

Clinical chemistry:

52 weeks ↓ red blood cell count at in female mice of all treated groups

76 weeks no significant difference was observed. Analysis of organ weight data showed statistically significant increases in absolute and relative liver weight ($p < 0.01$) in females treated at 3000 ppm.

Pathological observations:

Liver weights:
<table>
<tr><td rowspan="2">Organ</td><td colspan="8">Sex and dose level (ppm)</td></tr>
<tr><td colspan="4">Male</td><td colspan="4">Female</td></tr>
<tr><td>0</td><td>250</td><td>1000</td><td>3000</td><td>0</td><td>250</td><td>1000</td><td>3000</td></tr>
<tr><td>Last recorded body weight (g) (week 77)</td><td>48.2 ± 6.2</td><td>44.7 ± 5.4</td><td>45.9 ± 4.0</td><td>42.4 ± 4.5</td><td>39.5 ± 4.9</td><td>40.0 ± 4.9</td><td>40.9 ± 8.8</td><td>38.8 ± 3.2</td></tr>
<tr><td>Liver</td><td colspan="8"></td></tr>
<tr><td>Absolute (g)</td><td>2.891 ± 1.109</td><td>3.042 ± 1.153</td><td>3.242 ± 1.401</td><td>3.209 ± 1.558</td><td>2.268 ± 0.436</td><td>2.269 ± 0.454</td><td>2.451 ± 0.665</td><td>2.902 ± 0.782 **</td></tr>
<tr><td>Relative (%)</td><td>6.646 ± 2.195</td><td>7.073 ± 1.873</td><td>7.862 ± 3.198</td><td>7.664 ± 3.248</td><td>6.036 ± 0.967</td><td>5.900 ± 1.079</td><td>6.522 ± 1.785</td><td>7.644 ± 1.814**</td></tr>
</table>
**: p≤0.01

Non – neoplastic:

Amyloidosis was observed in the liver at ≥250 ppm in males

HCD for amyloidosis in the liver were provided and all</td><td>RAR-08_Vol-3 CA_B.6.5.2.1

Reference number: CA 5.5 /02

Anonymous (1985)</td></tr>
</table>

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
animals/sex/group. A complete necropsy was performed for each animal that spontaneously died or was sacrificed. Weights of brain, pituitary, thyroid, thymus with mediastinal lymph nodes, lungs, heart, liver, spleen, kidneys, adrenals, ovaries and testes were recorded. Histopathological examination was carried out on all tissues and organs taken at necropsy in the highest dose and control group. All gross lesions, brain, Zymbal glands, thymus and mediastinal lymph nodes, lungs, liver, spleen, kidneys, adrenals, stomach (fore and glandular), urinary bladder, prostate, uterus, ovaries, testes and epididymis were also roiutinely examined in the other groups. Guidelines and GLP/QA: N/A: Yes/Yes. Statistics: Statistical analysis was performed by means of analysis of variance (ANOVA), Tukey test, Yates' correct test (Snedecor and Cochran, 1967), Mantel test or Fisher's exact test, the effect of different doses was estimated according to Cochran-Armitage tests for linear trends		the data were within those HCD. Amyloidosis was also reported in the kidneys, spleen and adrenal glands. Urinary bladder tumours (transitional bladder tumour) were observed in males at 3000 ppm (3 tumours out of 60 animals). This dose exceed the MTD. Neoplastic: 3/60 mice - type of bladder transitional cell tumour was observed in males treated at 3000 ppm (3 tumours/60 animals). Such tumours were not observed in females or with the lower dose levels. Two of these tumours were detected only at microscopic examination and were at a very early stage, and one was observed at gross examination at necropsy. Respective latency time were 45, 54, and for the tumour observed at necropsy, 68 weeks from the start of the treatment.	

<table>
<tr><th>Method, guideline, deviations if any, species, strain, sex, no/group</th><th>Test substance, dose levels duration of exposure</th><th>Results</th><th>Reference</th></tr>
<tr><td>in proportions and frequencies (1955).

Acceptable</td><td></td><td></td><td></td></tr>
<tr><td>Carcinogenicity study in the mouse: Data on historical controls – Swiss mice

Attachment to the Final Report Evaluation of chronic toxicity and oncogenic potential of GALBEN® (CAS No. 71626-11-4) in Swiss mice (Oral Dosing Study)

Guidelines and GLP/QA: N/A

Acceptability: The older historical controls (from 1977 to 1979) were not reliable on, due to the lack of information about the protocol/techniques of preservation/microscopic examination as well as time of sacrifice of surviving animals.

Historical control carcinogenicity data were available.

The data set consisted of 6 studies (in addition to the study with benalaxyl) performed at the testing facility between 1977 and 1981.</td><td>GALBEN® (CAS No. 71626-11-4)

GALBEN (=Benalaxyl)</td><td>It was concluded that two positive results obtained in controls of 1981 were still lower than results obtained in the high dose group of BT 5004 study, and were of little weight of evidence about the frequency of that kind of tumours in untreated animals.

There were marked differences among the general tumour incidence in the various experiments.
<table>
<tr><th></th><th colspan="7">Study No. (Year)</th><th>Range (%)</th></tr>
<tr><th></th><th>BT 606 (1977)</th><th>BT 702 (1977)</th><th>BT 303 (1979)</th><th>BT 305 (1979)</th><th>BT 5002(1) (1981)</th><th>BT 5004(2) (1981</th><th>BT 5006(3) (1981)</th><th></th></tr>
<tr><td>M</td><td>0/60</td><td>0/100</td><td>0/100</td><td>0/90</td><td>1/60 (1.7%)</td><td>0/60</td><td>0/50</td><td>0-1.7</td></tr>
<tr><td>F</td><td>0/60</td><td>0/100</td><td>0/100</td><td>0/90</td><td>0/60</td><td>0/60</td><td>2/50 (4%)</td><td>0-4.0</td></tr>
<tr><td>M + F</td><td>0/120</td><td>0/200</td><td>0/200</td><td>0/180</td><td>1/120 (0.8%)</td><td>0/120</td><td>2/100 (2%)</td><td>0-2.0</td></tr>
</table>
(1) Surviving animals were sacrificed at 80 weeks;
(2) Surviving animals were sacrificed at 78 weeks (benalaxyl study)
(3) Surviving animals were sacrificed at 104 weeks</td><td>RAR-08_Vol-3 CA_B.6.5.2.2

Reference number: CA 5.5 /03

Anonymous (2000)</td></tr>
<tr><td>BT 5004: Evaluation of chronic toxicity and oncogenic potential of Galben (CAS No. 71626-11-4) in Swiss mice (oral dosing study). Pathology Peer Review of Urinary</td><td>Galben (CAS No. 71626-11-4)

Galben (=Benalaxyl)</td><td>A pathology peer review (PPR) was conducted in 2001 on sections of urinary bladder tumours from 3 male Swiss mice used in the oncogenicity study CA 5.5 /03

It was considered the study pathologists diagnosis of “transitional cell carcinoma” for the 3 lesions in question to be incorrect and made a diagnosis for the 3 lesions of “submucosal mesenchymal tumour” of the mouse urinary bladder.</td><td>RAR-08_Vol-3 CA_B.6.5.2.3

Reference number: CA 5.5 /04

Anonymous (2001a)</td></tr>
</table>

<table>
<tr><th>Method, guideline, deviations if any, species, strain, sex, no/group</th><th>Test substance, dose levels duration of exposure</th><th>Results</th><th>Reference</th></tr>
<tr><td>Bladder Tumours

Guidelines: N/A

GLP/QA: Yes/Yes

Acceptable</td><td></td><td>The results of the study revealed that the tumors observed are therefore not relevant to the human risk assessment.</td><td></td></tr>
<tr><td>BT 5004: Evaluation of chronic toxicity and oncogenic potential of Galben® (CAS No. 71626-11-4) in Swiss mice (oral dosing study)

Report of the Pathology Working Group on Urinary Bladder Tumours

Guidelines and GLP/QA: N/A

Acceptable</td><td>Galben

Galben (=Benalaxyl)

CAS No. 71626-11-4)</td><td>The 3 sections of urinary bladder from animals Number 82, 98 and 100, by the study pathologists (SP) and the PPR Reference number: CA 5.5 /04 (2001a) were examined by the PWG panel. With the exception of the chairman, none ofthe members of the PWG had prior knowledge of the study pathologists or the PPR diagnoses.

The slides label contained no information regarding dosage levels or the treatment group to which the animals had been assigned. After the slides had been examined, the panel members were asked to comment on the biological significance of the lesions.

The results of the PWG confirmed the conclusion of the PPR. None of the 3 urinary bladder lesions were considered to be transitional cell carcinomas. There was unanimous agreement that all 3 were submucosal mesenchymal tumours of the mouse urinary bladder as described by Halliwell (1998). None of the participants considered “transitional cell carcinoma” as a possible diagnosis.

This lesion has been reported in the literature for many years under a variety of neoplastic and non-neoplastic diagnostic terms including: vegetative changes, submucosal atypical cellular masses, smooth muscle tumours, leiomyosarcoma, atypical haemangiosarcoma, decidual-like reaction or submucosal granuloma.

Controversy as to aetiology, pathogenesis, biology and classification of the lesion still exists including whether or not the lesion should be classified as a tumour.

Assuming that the lesion is neoplastic, its non-epithelial nature is important since the vast majority of spontaneous and chemically induced mouse and human urinary bladder tumours are of epithelial origin.</td><td>RAR-08_Vol-3 CA_B.6.5.2.4

Reference number: CA 5.5 /05

Anonymous (2001b)</td></tr>
<tr><td>Study of the toxicity of repeated oral administration of product M 9834 (GALBEN) to Beagle dogs at the dosage levels of 10, 200 and 800 ppm for 52 weeks

Guidelines Test method was not specified in the report</td><td>Galben (=Benalaxyl)

FCF/T/1198

Purity: 92.5% - (Detailed impurities profile included in the report)</td><td>Dose level:
<table><tr><td>Dose level (ppm)</td><td>0</td><td>10</td><td>200</td><td>800</td></tr><tr><td>Males (mg/kg/day)</td><td>0</td><td>0.32</td><td>6.5</td><td>25.2</td></tr><tr><td>Females (mg/kg/day)</td><td>0</td><td>0.33</td><td>7.0</td><td>27.8</td></tr></table>
Histological findings were atrophy of the seminiferous tubules of the testes in 2 males at 800 ppm

Bw and food consumption appear as normal

Benalaxyl dispersed in dog food (in calcium carbonate)</td><td>RAR-08_Vol-3 CA_B.6.3.2.5

Reference number: CA 5.3.2 /05

Anonymous (1982)</td></tr>
</table>

<table>
<tr><th>Method, guideline, deviations if any, species, strain, sex, no/group</th><th>Test substance, dose levels duration of exposure</th><th>Results</th><th>Reference</th></tr>
<tr><td>(in house method), but detailed test procedure was included in report (along with RBM Standard Operating Procedures) and complied to a great extent with OECD Guideline 409

GLP/QA: Yes/Yes

The study was claimed to be carried out according to GLP (Fed. Reg., Vol. 43, n.º 247, 1978 – Part 58) by RBM Management. The report also includes a QA statement.

Statistics: Yes

Standard laboratory dog diet (detailed composition included in the report) / No positive control – not required</td><td></td><td>at 0 (basic diet), 10, 200 and 800 ppm was administered daily, 7 days a week, to groups of 6 males and 6 females Beagle dog/group for 52 weeks.

All animals survived to the scheduled necropsy.

No clinical observations or changes in behaviour, ophthalmic examination findings, changes in body weights or food consumption, or alterations in urinalysis parameters.

No test substance-related alterations in haematological and clinical chemistry parameters.

Macroscopic examination and organ weight determinations did not reveal any abnormalities.

There was no adverse effect attributable to treatment with benalaxyl in symptomatology, body weight, haematology, blood chemistry, urinalysis, faeces, organ weight or macroscopic examinations.

Haematology and coagulation parameters

alterations of MCHC, ↑n all treated groups at week 6 and ↓ at 10 and 800 ppm at week 26; ↓ ESR at 10 and 800 ppm at week 10; ↑of leukocytes at 10 ppm at week 15, and ↓ prothrombin time at 200 and 800 ppm at week 26.

↑ total and direct bilirubin at 800 ppm

↑pseudocholinesterase at 200 ppm at week 10

↓ alpha$_2$-globulins at 200 ppm

↑ gamma globulins at 200 and 800 ppm at week

↑ glucose in all treated groups

↑alkaline phosphatase at 800 ppm at week 21

↑ glucose at 200 ppm

↓ potassium at 10 ppm at week 26

↓ albumin and A/G ratio at 200 and 800 ppm

↑ globulins and sodium at 800 ppm

↑ beta- and gamma globulins at 200 ppm at week 38
↓LDH and globulins at 800 ppm

↑ proteins at 200 ppm

↑ albumin, A/G ratio and sodium at 800 ppm

↓ alpha$_2$-globulins in all treated groups at week 52

Urinalysis and occult blood in faeces

<table>
<tr><td rowspan="2">Organ</td><td colspan="4">Dose level</td></tr>
<tr><td>0 ppm</td><td>10 ppm</td><td>200 ppm</td><td>800 ppm</td></tr>
<tr><td>Final weight, fasted (kg)</td><td>10.98</td><td>10.20</td><td>10.46</td><td>10.42</td></tr>
<tr><td>Liver (g)</td><td>299.09</td><td>283.29</td><td>281.98</td><td>282.04</td></tr>
</table>
</td><td></td></tr>
</table>

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference

Relative to bw %	2.78	2.81	2.75	2.74
Right testis (g)	4.53	5.68	4.53	5.72
Relative to bw %	0.04	0.05*	0.04	0.05*
Left testis (g)	4.82	5.56	4.63	5.99
Relative to bw %	0.04	0.05	0.04	0.06*
Prostate (g)	8.06	9.45	8.38	7.16
Relative to bw %	0.07	0.08	0.07	0.07
Right ovary (mg)	500.67	583.50	712.00	630.00
Relative to bw per 1000	5.28	6.06	7.35	6.89
Left ovary (mg)	644.33	607.67	695.17	466.67
Relative to bw per 1000	6.65	6.39	7.31	5.11
*: p<0.05				

Parameter	Dose level			
	0 ppm	10 ppm	200 ppm	800 ppm
Spleen				
Haemorrhagic infarcts	3 / 25 / 2.00	2 / 16.67 / 2.00	-	2 / 16.67 / 2.00
Haematic stasis	3 / 25 / 2.00	3 / 25 / 2.00	4 / 33.33 / 1.75	-
Hyperplasia	-	-	-	1 / 8.33 / 1.00
Lungs				
Chronic interstitial pneumonia	11 / 91.67 / 1.00	7 / 58.33 / 1.00	8 / 66.67 / 1.00	8 / 66.67 / 1.00
Peribronchial lymphoid hyperplasia	-	1 / 8.33 / 1.00	-	-
Thymus				
Cysts	-	-	-	2 / 16.67 / 2.00
Heart				
Suppurative phlogosis	-	1 / 8.33 / 1.00	-	-
Liver				
Foci of parvicellular phlogosis	1 / 8.33 / 2.00		1 / 8.33 / 2.00	-
Kidneys				
ORO: fatty degeneration	10 / 83.33 / 1.90	10 / 83.33 / 2.00	8 / 66.67 / 1.63	10 / 83.33 / 1.60
Foci of parvicellular phlogosis	1 / 8.33 / 2.00	1 / 8.33 / 2.00	3 / 25 / 2.00	4 / 33.33 / 1.75
Deposits of calcium salts	5 / 41.67 / 2.00	1 / 8.33 / 2.00	5 / 41.67 / 2.00	4 / 33.33 / 2.00
Hyperaemia	-	1 / 8.33 / 1.00	2 / 16.67 / 1.50	1 / 8.33 / 1.00
Ileum				
Abscess focus	-	1 / 8.33 / 2.00	-	-
Endometrium				
Hyperplasia due to oestrus cycle	2 / 25 / 2.00	2 / 16.67 / 2.00	6 / 50 / 2.00	2 / 25 / 2.00
Ovaries				
Presence of corpora lutea	3 / 25 / 2.00	4 / 33.33 / 2.00	4 / 33.33 / 2.00	4 / 33.33 / 2.00

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		<table><tr><td>Presence of mature follicles</td><td>-</td><td>1 / 8.33 / 2.00</td><td>1 / 8.33 / 2.00</td><td>-</td></tr><tr><td>Prostate</td></tr><tr><td>Foci of parvicellular phlogosis</td><td>-</td><td>1 / 8.33 / 2.00</td><td>-</td><td>1 / 8.33 / 2.00</td></tr><tr><td>Testes</td></tr><tr><td>Atrophy</td><td>-</td><td>-</td><td>-</td><td>2 / 16.67 / 1.50</td></tr><tr><td>Epididymis</td></tr><tr><td>Foci of inflammatory infiltration</td><td>-</td><td>-</td><td>-</td><td>1 / 8.33 / 2.00</td></tr><tr><td>Pituitary</td></tr><tr><td>Congenital cyst</td><td>2 / 16.67 / 2.00</td><td>4 / 33.33 / 2.00</td><td>4 / 33.33 / 2.00</td><td>1 / 8.33 / 2.00</td></tr><tr><td>Thyroid</td></tr><tr><td>Foci of parvicellular phlogosis</td><td>-</td><td>-</td><td>-</td><td>1 / 8.33 / 2.00</td></tr><tr><td>Dilatation</td><td>-</td><td>-</td><td>-</td><td>1 / 8.33 / 1.00</td></tr><tr><td>Mammary gland</td></tr><tr><td>Hyperplasia due to oestrus phase</td><td>-</td><td>4 / 33.33 / 1.25</td><td>3 / 25 / 2.00</td><td>2 / 25 / 2.00</td></tr></table> Results reveal a real activity of benalaxyl in dogs tissues, even without a significant statistic relevance	

Table 18: Summary table of human data on carcinogenicity

There are no relevant data in humans. Epidemiological data are very weak and without following some specific parameters of benalaxyl relevant for carcinogenicity, neurotoxicity and so on.

Table 19: Summary table of other studies relevant for carcinogenicity

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference

<table>
<tr>
<td>A 90-Day Dietary Combined Toxicity and Neurotoxicity Study of Benalaxyl in Rats
90 consecutive days

Test animals: Rats, Crl:CD(SD
10 rats/sex/G
Guidelines: OPPTS 8700.3100 and 870.6200, OECD 408 and OECD 424
GLP/QA: Yes/Yes
Acceptable</td>
<td>Benalaxyl
Lot/Batch: PL13-0055
Purity: 98.4%
Vehicle: acetone/
Positive control – not required.

Dose level:
100, 1000, and 10000 ppm:
6, 62, and 677 mg/kg/day (m)
7, 74, and 745 mg/kg/day (f)</td>
<td>The immunotoxic potential was evaluated by measuring organ weights and histopathology endpoints of critical immune organ systems including the spleen, thymus, and l ymph nodes.

Hepatocellular hypertrophy was characterized by expansion of the hepatocellular cytoplasm. The distribution of the change was predominantly centrilobular and extended to a more generalised pattern with increased severity of the change.

Follicular cell hypertrophy in the thyroid glands was characterized by expansion of the cytoplasm of the thyroid follicular cells and a decrease in the amount of follicular colloid.</td>
<td>1000 ppm group, a significantly higher mean body weight gain was noted for males during study days 63-70 and a significantly lower mean body weight gain was noted for females during study days 70-77 compared to the control group.

body weights and body weight gains for males and females in the 10000 ppm group were considered test substance-related.

Differences were significant during study days 0-7 and 21-28 for males and during study days 35-42 and 0-90 for females.

Organ weight data showed test substance-related and dose-related higher mean liver weights (absolute, relative to final body weight, and relative to brain weight) in the 1000 and 10000 ppm group males and females.

The liver weight differences (mean absolute, relative to final body weight, and relative to brain weight) were significantly higher in the 10000 ppm group males (absolute: 33.2%; relative to final body weight: 40.9%; relative to brain weight: 37.7%) and females (absolute: 40.3%; relative to final body weight: 54.6%; relative to brain weight: 40.8%).

1000 ppm group:

significantly higher for liver weight relative to final body weight in the 1000 ppm group females (relative to final body weight: 11.2%).

100 ppm group:

1 male was found dead - day 14; no remarkable clinical or macroscopic findings were noted without a microscopic examination, for this male and a cause of death could not be determined.
1 female from the same group was euthanized in extremis on study day 80; at necropsy, the cause of death for this female was determined to be malignant lymphoma. - gross observations included a firm lobulated mass on thymus, enlarged spleen and mediastinal and renal lymph nodes, and depressed areas in the kidneys were performed.
In the 10000 ppm group:
1 female - Swollen liver was noted at necropsy. This observation corresponded with the microscopic finding of moderate hepatocellular hypertrophy.

Although the liver weight differences in the 1000 ppm group were considered to be test substance-related due to the presence of a clear exposure-dependent response, the changes were considered to be adaptive rather than a toxic response because of minimal magnitude, and likely the result of CYP2B induction.

1000 ppm is the targeted dose level based on decreased BW(G), clinical chemistry and increased liver weight with hepatocellular hypertrophy

<table>
<tr><td>Observation</td><td>0 ppm</td><td>100</td><td>1000</td><td>10000</td></tr>
</table>
</td>
<td>RAR-08_Vol-3 CA_B.6..2.3

Reference number: CA 5.3.2 /03

Anonymous (2014b)</td>
</tr>
</table>

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<td></td>
<td>
<table>
<tr><td></td><td></td><td>ppm</td><td>ppm</td><td>ppm</td></tr>
<tr><td colspan="5">Males</td></tr>
<tr><td>Liver</td><td>10</td><td>10</td><td>10</td><td>10</td></tr>
<tr><td>Hepatocellular hypertrophy</td><td>0</td><td>0</td><td>0</td><td>9</td></tr>
<tr><td>Minimal</td><td>-</td><td>-</td><td>-</td><td>2</td></tr>
<tr><td>Mild</td><td>-</td><td>-</td><td>-</td><td>3</td></tr>
<tr><td>Moderate</td><td>-</td><td>-</td><td>-</td><td>4</td></tr>
<tr><td>Thyroid gland</td><td>10</td><td>10</td><td>10</td><td>10</td></tr>
<tr><td>Follicular cell hypertrophy</td><td>2</td><td>1</td><td>2</td><td>9</td></tr>
<tr><td>Mild</td><td>2</td><td>1</td><td>2</td><td>7</td></tr>
<tr><td>Moderate</td><td>0</td><td>0</td><td>0</td><td>2</td></tr>
<tr><td colspan="5">Females</td></tr>
<tr><td>Liver</td><td>10</td><td>10</td><td>10</td><td>10</td></tr>
<tr><td>Hepatocellular hypertrophy</td><td>0</td><td>0</td><td>3</td><td>10</td></tr>
<tr><td>Minimal</td><td>-</td><td>-</td><td>3</td><td>1</td></tr>
<tr><td>Mild</td><td>-</td><td>-</td><td>0</td><td>7</td></tr>
<tr><td>Moderate</td><td>-</td><td>-</td><td>0</td><td>2</td></tr>
<tr><td>Thyroid gland</td><td>10</td><td>10</td><td>10</td><td>10</td></tr>
<tr><td>Follicular cell hypertrophy</td><td>1</td><td>2</td><td>2</td><td>7</td></tr>
<tr><td>Mild</td><td>1</td><td>2</td><td>2</td><td>7</td></tr>
<tr><td>Moderate</td><td>0</td><td>0</td><td>0</td><td>0</td></tr>
</table>
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<tr>
<td>An Oral (Gavage) Prenatal Developmental Toxicity Study of Benalaxyl in Rats

gavage

Test animals: rats Sprague-Dawley Crl:CD (SD)

Test method: OECD Guideline 414 and OPPTS 870.3700

Statistics: WTDMS™

Guidelines and GLP/QA: Yes/Yes

Acceptable</td>
<td>Benalaxyl technical, Lot/Batch: PL 13-0055, Purity: 98.4%.

Vehicle: 0.5% (w/v) carboxymethyl cellulose aqueous solution.</td>
<td>Benalaxyl was administered orally by gavage to 3 (Phase 1) and 1 (Phase 2) groups of 25 bred female Crl:CD(SD) rats once daily from gestation days 6 through 19.

control group: 25 bred females received the vehicle Phase 1: 15, 50, and 150 mg/kg/day

Due to the lack of toxicity during the first phase, a second phase (Phase 2) was added to see maternal toxicity and/or developmental toxicity.

Phase 2: the vehicle or test substance was administered orally by gavage to 2 groups</td>
<td>Phase 2: 3 of 10 females dosed at 450 mg/kg/day on the first gestation day 6 were found dead between approximately 4 hours 24 minutes and 5 hours 56 minutes following administration of the first dose corresponding to the T_{max} of approximately 30 minutes with a half-life of 36 hours using ^{14}C-benalaxyl (CA 5.1.1/02).

Clinical findings noted for these females prior to death included hypoactivity, laboured respiration, soft faeces, clear material around the mouth, and/or clear discharge from the eyes.

No remarkable macroscopic findings were noted for any of these females at necropsy; therefore, the cause of the deaths could not be determined.

The dosage level for this group was reduced to 300 mg/kg/day beginning on gestation day 6 or 7 and continuing throughout the duration of the study. And +3 f were added

10/25 females received a single dose of 450 mg/kg/day, surviving females received 300 mg/kg/day for the remainder of the dosing period and 18 females received 300 mg/kg/day over the entire regimen.

Test substance-related effects in the 450/300 mg/kg/day group:

a mean maternal body weight loss

lower mean food consumption

lower mean serum alkaline phosphatase

higher mean serum cholesterol (9.3%) and alanine</td>
<td>RAR-08_Vol-3 CA_ B.6.6.2.2

Reference number: CA 5.6.2/02

Anonymous (2015)</td>
</tr>
</table>

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<tr>
<td></td>
<td></td>
<td>(Groups 1-2) of 25 bred female Crl:CD(SD) rats once daily from gestation days 6 through 19 at dosage levels of 0 and 450 mg/kg/day, respectively.</td>
<td>aminotransferase (9.8%)

higher mean liver weights

enlarged liver, and centrilobular to midzonal hepatocellular hypertrophy

<table>
<tr><td>Parameter</td><td>0 mg/kg/day</td><td>450/300 mg/kg/day</td></tr>
<tr><td>Alkaline phosphatase (U/L)</td><td>173</td><td>134* / -22.5</td></tr>
<tr><td>Alanine aminotransferase (U/L)</td><td>61</td><td>67** / 9.8</td></tr>
<tr><td>Glucose (mg/dL)</td><td>94</td><td>97* / 3.2</td></tr>
<tr><td>Cholesterol (mg/dL)</td><td>75</td><td>85* / 9.3</td></tr>
<tr><td>Chloride (mEq/L)</td><td>101</td><td>100** / -1.0</td></tr>
<tr><td>Sodium (mEq/L)</td><td>140</td><td>138** / -1.4</td></tr>
</table>

Necropsy on gestation day 20 for 450/300 mg/kg/day (f):

1 female: enlarged liver, which correlated with hepatocellular hypertrophy

All females: higher liver weights (17.8% and 17.2%, absolute and relative body weight)- significant compared with control.

12/25 f: centrilobular to midzonal hepatocellular hypertrophy

Foetus - 450/300 mg/kg/day

no external malformations or developmental variations

visceral developmental variations were limited to renal papilla(e) - Woo and Hoar grade 1) was noted for 3 and 14 foetuses in the control and 450/300 mg/kg/day groups

no distended ureter(s) and accessory lobules in the liver

Overall total proportion of variations per litter in the 450/300 mg/kg/day group was significant</td>
<td></td>
</tr>
<tr>
<td>Benalaxyl: Assessment of Endocrine Disrupting Potential

Guidelines: N/A: review of existing data

GLP/QA: N/A

Acceptable</td>
<td>Benalaxyl</td>
<td>A sum of assessments related to supposition about lack of ED effects</td>
<td>Thyroid findings

Thyroid histopathology seen in the 5-week rat study was limited to the highest dose level (initially 800 mg/kg bw/d and escalated gradually to 4500 mg/kg bw/d). Findings occurred in the absence of any effects on thyroid weight, but were associated with marked (up to 80%) increases in liver weight.

Similar effects on the thyroid were not observed in the 90-day rat study at the highest dietary concentration of 10000 ppm (equivalent to mean intakes of 637 and 784 mg/kg bw/d in males and females respectively) and there is no evidence for an effect on the thyroid in the rat combined chronic toxicity/carcinogenicity study.

Effects are therefore seen in one study, and at a gavage dose level clearly exceeding the limit dose.

A mechanistic study (CA 5.3.1/02) demonstrated that the administration of benalaxyl to the rat for 28 days at dietary concentrations of 1000, 5000 and 10000 ppm caused an increase in liver weight, microsomal protein and cytochrome P450 content; and a marked induction of hepatic CYP2B and UDP-glucuronyltransferase (UDP-</td>
<td>RAR-08_Vol-3 CA_ B.6.8.3.1

Reference number: CA 5.8.3

Anonymous (2014)</td>
</tr>
</table>

			GT) activities which is the rate-limiting step in biliary excretion of thyroxine (T4). Thyroid follicular cell hypertrophy and hepatocellular hypertrophy. The increased excretion of T4 and resulting lower plasma T4 levels feedback to the pituitary gland and cause increased plasma thyroid stimulating hormone (TSH) levels (Capen, 2008). The sustained increase in plasma TSH level may result in thyroid follicular cell hypertrophy, followed by thyroid hyperplasia and/or neoplasia with longer term exposure. The lack of T4-binding globulin (TBG) and shorter half-life of T4 in rats compared to humans is responsible for the species differences in thyroid hypertrophy, hyperplasia, and/or neoplasia with microsomal enzyme induction in rats versus humans. Testicular findings Atrophy of the seminiferous tubules was observed in the 12-month dog study at the highest dietary concentration of 800 ppm (equivalent to 25 mg/kg bw/d). 12-month dog study, an association (in one dog) with body weight effects and the absence of similar findings in the 90-day dog study, findings are not considered to be clearly related to treatment with benalaxyl. Prostate findings Significantly lower mean prostate weights seen in some treated groups in the 90-day dog study	
5 weeks cumulative toxicity study joined by a two-week recovery period with "GALBEN TH" by oral application on rats Test method: In-house method Guidelines and GLP/QA: N/A: No GLP	Galben TH: Not indicated Rat Wistar (BOR:WISW (SPF/TNO)) Vehicle: 0.5% Traganth 10/sex after 1 week = 1000 mg/kg bw/day 10/sex after 2 week =1500 mg/kg bw/day 10/sex after 3 week =2500 mg/kg bw/day 10/sex after 4 week =3500 mg/kg bw/day 15/sex after 4 ½ week = 4000 mg/kg	No histopathology was conducted at the end of the recovery period. Histopathological examination revealed changes in the thyroid, which was not re-examined at the end of the recovery period.	A slightly but signficant prologation of prothrombin time was noted in females at 800/4000 mg/kg/day at the end of the 5-week treatment period; in addition, a slight increase of cholesterol, total protein, albumin and a reduction of SGOT were observed. Males also showed SGOT and alkaline phosphatase decreases. Liver weights were significantly increased compared to controls in animals of both sexes at 800/4000 mg/kg/day (males: +51% and females: +80%), and in animals of both sexes treated at 100 mg/kg/day (males: +17% and females: +13%). Tested doses had no effect on mortality, clinical signs, feed intake, urinalysis and body weight Histopathology revealed slightly increased incidences of fatty infiltration of single hepatocytes and/or slight diffuse small droplet fatty infiltration in the liver of the high dose animals compared to controls however, no degeneration of the liver cells was observed. Changes in the thyroid, i.e. thyroid activation, at 800/4000 mg/kg/day were also observed. Liver weights of animals treated at 100 mg/kg/day and at 800/4000 mg/kg/day were dose-related significantly increased compared to controls, being highly significant at 800/4000 mg/kg/day (+ 51% in males and + 80% in females). slight lymphocytic-histiocytic infiltrations in the liver, lung, heart, kidney, urinary bladder, intestine,	RAR-08_Vol-3 CA_ B.6.8.3.1 Reference number: CA 5.3.1/01 Anonymous (1982)

<table>
<tr>
<td></td>
<td>bw/day

This dose level is presented as 800/4000 mg/kg/day</td>
<td></td>
<td>epididymis, and prostate which, together with lymphoid hyperplasia in mesenteric lymph nodes

Other findings occurring in both control and test group included infiltration of the uterine stroma by eosinophils, and congestion of the spleen, which was considered by the authors of study, to be related to the sacrifice of the animals.
<table>
<tr><td rowspan="3">Organ & Lesion</td><td colspan="4">Sex and dose level (mg/kg/day)</td></tr>
<tr><td colspan="2">Male</td><td colspan="2">Female</td></tr>
<tr><td>0</td><td>800 / 4000</td><td>0</td><td>800 / 4000</td></tr>
<tr><td colspan="5">Liver - Fatty infiltration</td></tr>
<tr><td>Single hepatocytes</td><td>6/10</td><td>6/10</td><td>8/10</td><td>5/10</td></tr>
<tr><td>Diffuse small droplet - slight</td><td>0/10</td><td>3/10</td><td>2/10</td><td>4/10</td></tr>
<tr><td colspan="5">Thyroid - Hyperplasic elevation of the follicular epithelium</td></tr>
<tr><td>Slight</td><td>1/10</td><td>3/10</td><td>0/10</td><td>2/10</td></tr>
<tr><td>Moderate</td><td>0/10</td><td>3/10</td><td>0/10</td><td>0/10</td></tr>
<tr><td colspan="5">Thyroid</td></tr>
<tr><td>tendency to formation of microfollicular structure^</td><td>0/10</td><td>2/10</td><td>0/10</td><td>0/10</td></tr>
</table>
</td>
<td></td>
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</table>

10.9.1 Short summary and overall relevance of the provided information on carcinogenicity

Table 20: 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
Rat	19 tumours Not relevant HCD	Liver, brain, mammary glands, pancreas, and pituitary gland.	some significant macroscopic observations and atrophy or hypertrophy were present in organs and tissues	Follicular cell hyperplasia and hypertrophy/ Follicular cell adenocarcinomas	both sexes and presence of non-neoplastic tumours have had an occurrence significant for carcinogenicity.	MTD can cause effects such as cell death (necrosis) with associated regenerative hyperplasia, which can lead to tumour development as a secondary consequence unrelated to the intrinsic potential of the substance itself to cause tumours at lower less toxic doses	Oral	Hypertrophy of the thyroid and the TSH response is counteracted by T4 supplementation supporting effects being due to a negative feedback mechanism. induction of cytochrome P450 and related drug metabolising enzymes including an increase of UDP-GT
Mice	3 lesions in male mice		cell tumours of the	No increased the latency time in	Amyloidosis was frequent,	Deposition of amyloid was	Oral	hepatocellular adenomas result

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
	treated at the highest dose level of 3000 ppm		urinary bladder	treated group	and widely distributed among all groups, mainly in male..	found in the adrenals, kidney, liver, spleen, salivary glands, stomach and intestine, and Zymbal glands. In male mice who died spontaneously a clear correlation was found between the occurrence of amyloidosis and treatment; although at a lesser extent, the association of amyloidosis and treatment was still evident in males sacrificed at termination.		from induction of the cytochrome P450 drug metabolising system phenobarbital-like mode of action lacking human relevance
Dog	incidence of seminiferous tubules atrophy	no	No data		No data	No data	Oral	No data

Summary table of studies relevant for carcinogenicity were fulfilled with the most significant data. All the detailed studies are presented in Annex 1 of this CLH Report.

A 90-day study in mice (see B.6.3.2.1 Short-term range finding study of Evaluation of Chronic Toxicity and Oncogenic Potential of Galben (CAS No. 71626-11-4) in Swiss Mice (Oral Dosing Study)) was conducted manly as tolerability test and as a DRF study for a further carcinogenicity study. Given that no hematology or clinical chemistry was performed, the summary already contains key information i.e. changes in liver weight that was used for setting the high dose level in the carcinogenicity study. The top dose level of 5000 ppm (corresponding to 802.5 mg/kg bw/day for males and 907.5 mg/kg bw/day for females) was inadequate to be used as the highest dose level for oncogenicity and chronic toxicity study in mice due to the marked toxic effect shown in liver weight, females appeared more affected than males, dose-related.effect. Differences observed in adrenal and kidney weight were transient, inconsistent, and limited to one sex; because of their random distribution, they were considered of no toxicological significance. 3000 ppm was selected as the highest dose level for the long-term study og carcinogenicity.

Pathological examinations revealed 3 lesions in male mice treated at the highest dose level of 3000 ppm were originally diagnosed as "transitional cell tumour of the urinary bladder", a type of tumours considered by the study pathologist rather uncommon. However, pathology peer reviews of the original slides determined these to be "submucosal mesenchymal tumour" of the mouse urinary bladder, a lesion non-epithelial in origin, unique to the mouse urinary bladder, and with no counterpart in any other species including humans and therefore of no relevance for risk assessment in humans.

Distribution of regressive changes (amyloidosis) after 78 weeks of treatment with benalaxyl

Dose level (ppm)	No. of animals / sex	Liver				Spleen		Kidneys		Adrenal glands	
		Amyloidosis		Other degenerative changes							
		No.	%	No.	%	No.	%	No.	%	No.	%
0	60 / M	8	13.3	4	6.7	9	15.0	10	16.7	3	5.0
	60 / F	1	1.7	7	11.7	1	1.7	0	-	1	1.7
Total (120 M & F)		9	7.5	11	9.2	10	8.3	10	8.3	4	3.3
250	60 / M	12	20.0	3	5.0	22**	36.7	12	20.0	3	5.0
	60 / F	1	1.7	7	11.7	0	-	1	1.7	0	-
Total (120 M & F)		13	10.8	10	8.3	22	18.3	13	10.8	3	2.5
1000	60 / M	19*	31.7	4	6.7	29**	48.3	28**	46.7	4	6.7
	60 / F	6	10.0	13	21.7	6	10.0	8*	13.3	3	5.0
Total (120 M & F)		25	20.8	17	14.2	35	29.2	36	30.0	7	5.8
3000	60 / M	12	20.0	1	1.7	26**	43.3	21**	35.0	8	13.3
	60 / F	1	1.7	6	10.0	0	-	2	3.3	0	-
Total (120 M & F)		13	10.8	7	5.8	26	21.7	23	19.2	8	6.7

*: $p \leq 0.05$; **: $p \leq 0.01$

Categorical Analysis of Amyloidosis and Survival in Male Mice

Dose level (ppm)	Category	Animals found dead	Surviving Animals (terminal sacrifice)	Σx^2	P**
0	Affected*	9	5	3.26	n.s.
	Non-affected	17	29		
	Total	26	34		
250	Affected*	16	8	5.38	< 0.05
	Non-affected	13	23		
	Total	29	31		
1000	Affected*	32	5	10.44	< 0.01
	Non-affected	11	12		
	Total	43	17		
3000	Affected*	30	7	1.05	n.s.
	Non-affected	16	7		
	Total	46	14		

*: Number of affected animals per group; amyloidosis, any organ; **: Tail probability range of adjacent value of chi-square; n.s.: not significant

The dose level of 250 ppm (corresponding to 44.4 and 42.6 mg/kg bw/day in males and females respectively) is associated with the highest incidence of primary amyloidosis in a biologically relevant way of benalaxyl. Amyloidosis was observed in liver and in multiple tissues (in males). A NOAEL for carcinogenicity could not be set because of the low survival rate at the two high doses tested.

About account for the carcinogenicity it could be noted the results from study range-findind study CA_B.6.3.2.1. (A 90-day study in mice) reveales the dose level as tolerability test and also set dose levels for the main carcinogenicity study. Missings in testing procedure of no hematology or clinical chemistry data, changes in liver weight that were used only for setting the high dose level in the carcinogenicity study (5000 ppm - marked toxic effect in liver weight and 3000 ppm was selected as the highest dose level for the long-term study; an equivocal increased liver weight was observed at 1000 ppm, only in females.

A mechanistic study (see B.6.3.1.2 IR6141 and Benalaxyl: Study by dietary administration to male CD rats for 28 days to compare hepatic parameters) showed that the administration of benalaxyl to the rat for 28 days at dietary concentrations of 1000, 5000 and 10000 ppm (corresponding to 120, 600 and 1200 mg/kg bw/day) caused an increase in liver weight, microsomal protein and cytochrome P450 content; and a marked induction of hepatic CYP2B and UDP-glucuronyltransferase (UDP-GT) activities.

Thyroid follicular cell hypertrophy in this study was considered to be secondary to hepatic microsomal enzyme induction, as evidenced by hepatocellular hypertrophy which is the liver's typical response to hepatic enzyme induction (Anonymous, 2010). A high level of T4 excretion and as a feedback a lower plasma T4 levels in the pituitary gland increased plasma thyroid stimulating hormone (TSH) levels conducting to the thyroid follicular cell hypertrophy, followed by thyroid hyperplasia and/or neoplasia with longer term exposure (Anonymous, 2008).

The lack of T4-binding globulin (TBG) and shorter half-life of T4 in rats compared to humans is responsible for the species differences in thyroid hypertrophy, hyperplasia, and/or neoplasia with microsomal enzyme induction in rats versus humans. Thyroid follicular hyperplasia in the rat is a homeostatic response to the enhanced hepatic metabolism and excretion of thyroid hormone (T4) following the induction of CYP2B and uridine diphosphate glucuronyltransferase UPD-GT activity, and a consequent increase in TSH secretion (Anonymus, 2002).

This mechanism is not generally thought to be of relevance to humans due to physiological and biochemical differences between the rat and humans (IPCS, 2007).

About possibility of a second target organ, some studies developed are not conclusive to support this hypothesis and liver is the main target organ in three species (mice, rats, dogs):

In the study B.6.3.1.1 5 weeks cumulative toxicity study joined by a two-week recovery period with "GALBEN TH" by oral application on rats reveals the liver weights significantly increased compared to controls in animals of both sexes at 800/4000 mg/kg bw/day (males: +51% and females: +80%), and in animals of both sexes treated at 100 mg/kg bw/day (males: +17% and females: +13%).

Histopathology noted a slightly increased incidences of fatty infiltration of single hepatocytes and/or slight diffuse small droplet fatty infiltration in the liver of the high dose animals compared to controls however, no degeneration of the liver cells was observed. Changes in the thyroid, i.e. thyroid activation, at 800/4000 mg/kg bw/day were also observed. Histopathological examination revealed changes in the thyroid, which was not re-examined at the end of the recovery period.

Similar effects on the thyroid were not observed in the 90-day rat study at the highest dietary concentration of 10000 ppm (equivalent to mean intakes of 637 and 784 mg/kg bw/d in males and females respectively) and there is no evidence for an effect on the thyroid in the rat combined chronic toxicity/carcinogenicity study. Effects are therefore seen in one study, and at a gavage dose level clearly exceeding the limit dose (see B.6.3.2.3 A 90-Day Dietary Combined Toxicity and Neurotoxicity Study of Benalaxyl in Rats)

In the 1000 ppm group, the differences were only significantly higher for liver weight relative to final body weight in the 1000 ppm group females (relative to final body weight: 11.2%).

Although the liver weight differences in the 1000 ppm group were considered to be test substance-related due to the presence of a clear exposure-dependent response, the changes were considered to be adaptive rather than a toxic response because of minimal magnitude, and likely the result of CYP2B induction.

Test substance-related findings in the general toxicity phase animals primarily involved the liver and thyroid glands and included higher mean gamma glutamyltransferase values, gross observation of a swollen liver (1

female), and histologic evidence of hepatocellular hypertrophy and thyroid follicular cell hypertrophy at 10000 ppm; hepatocellular hypertrophy in females and higher mean liver weights were also recorded at ≥1000 ppm. (see B.6.3.2.3 A 90-Day Dietary Combined Toxicity and Neurotoxicity Study of Benalaxyl in Rats). Hepatocellular hypertrophy by expansion of the hepatocellular cytoplasm, centrilobular and extended to a more generalised pattern with increased severity of the change. Follicular cell hypertrophy in the thyroid glands by expansion of the cytoplasm of the thyroid follicular cells and a decrease in the amount of follicular colloid. Significantly lower mean brain weight at 1000 and 10000 ppm group males however, but not noted in the neurotoxicity phase females or general toxicity phase males or females.

The dose level of 1000 ppm was significant than control in albumin and total protein levels, in males only. Relative liver weights were increased by less than 15% in both males and females, and liver histopathology revealed a minimal hepatocellular hypertrophy in 3 females only. Thyroid follicular hypertrophy incidence and the same degree as controls in males and females with one exception (10-fold lower dose level than control).

Test substance-related increasing of the liver weights (25.7% in absolute and 26.1% relative to body and brain weights, respectively) were noted in males from 75/200 mg/kg bw/day group. A few statistically significant differences were observed between control and the treated groups. Also, a lowered prostate weights (absolute and relative to body and brain weights, ranging from 60.3% to 62.0%) in the 7.5 mg/kg bw/day group males, lowered prostate weight relative to final body weight (39.3%) in the 75/200 mg/kgbw/day group males, and lowered spleen weight relative to final body weight (36.4%) in the 25 mg/kgbw/day group females. (see B.6.3.2.4 A 90-Day Oral (Capsule) Toxicity Study of Benalaxyl in Beagle Dogs).

Target organ is liver based on highest degree of liver weight gain and lobulation observed at necropsy. (see B.6.3.2.2 13-week oral subacute toxicity study of the product M 9834 (GALBEN) administered to Charles River CD (SD) BR rats in the diet, at the doses of 10, 100, 1000, 10000 and 12000 ppm) and a NOAEL by oral (dietary) study for benalaxyl was set at 1000 ppm (58.9 mg/kgbw/day in males and 71.5 mg/kgbw/day in females).

Hematological observation Some statistically significant differences were observed when the control and the treated groups were compared, a lower mean HGB (-6.2%), MCH (-4.0%), and MCHC (-2.1%) values in the 10000 ppm group males, and lower mean MCV (-5.7%) and MCH (-6.0%) values in the 10000 ppm group females, respectively. However, the changes in males did not show a clear dose-response (MCH and MCHC), were not associated with other alterations in red blood cell parameters, were of minimal magnitude, and the group mean values and all individual animal values (with the exception of 1 individual animal value each for haemoglobin and MCHC) were within the HC database range of study means and reference range, respectively. Similarly, the changes in females were not associated with other alterations in red blood cell parameters, were of minimal magnitude, and the group mean values and all individual animal values (with the exception of 1 individual animal MCH value) were within the HC database range of study means and reference range, respectively. Significantly lower mean prothrombin time (-9.0%) was also noted in the 10000 ppm group females; however, this change was in a direction of no known toxicological importance. Also, a higher mean activated thromboplastin time (+13.0%) was noted in the 100 and 10000 ppm group females. The liver weight differences (mean absolute, relative to final body weight, and relative to brain weight) were significantly higher in the 10000 ppm group males (absolute: 33.2%; relative to final body weight: 40.9%; relative to brain weight: 37.7%) and females (absolute: 40.3%; relative to final body weight: 54.6%; relative to brain weight: 40.8%). (see B.6.3.2.3 A 90-Day Dietary Combined Toxicity and Neurotoxicity Study of Benalaxyl in Rats).

Selected haematological parameters (mean ± SD (% difference to control))

Parameter	**Sex and dose level (ppm)**			
	Male			
	0	**100**	**1000**	**10000**
	n=10	**n=10**	**n=10**	**n=10**
Week 13				
HGB (g/dL)	16.2 ± 0.81	16.2 ± 0.90 (0.0)	16.5 ± 0.98 (1.9)	15.2 ± 0.57* (-6.2)

Parameter	Sex and dose level (ppm)			
	Male			
	0	100	1000	10000
	n=10	n=10	n=10	n=10
MCV (fL)	52.7 ± 1.39	51.9 ± 1.51 (-1.5)	52.7 ± 1.41 (0.0)	51.6 ± 1.22 (-2.1)
MCH (pg)	17.6 ± 0.40	17.1 ± 0.60 (-2.8)	17.5 ± 0.58 (-0.6)	16.9 ± 0.50* (-4.0)
MCHC (g/dL)	33.4 ± 0.39	32.9 ± 0.53 (-1.5)	33.2 ± 0.53 (-0.6)	32.7 ± 0.70* (-2.1)
PT (seconds)	15.9 ± 0.76	16.6 ± 1.07 (4.4)	16.9 ± 1.98 (6.3)	17.7 ± 2.61 (11.3)
APTT (seconds)	14.6 ± 1.25	14.8 ± 1.56 (1.4)	14.0 ± 1.99 (-4.1)	14.9 ± 1.12 (2.1)
Parameter	Female			
Week 13				
HGB (g/dL)	15.7 ± 0.83	15.8 ± 1.02 (0.6)	16.1 ± 0.80 (2.5)	15.2 ± 0.85 (-3.2)
MCV (fL)	54.3 ± 1.28	54.4 ± 1.33 (0.2)	54.2 ± 1.72 (-0.2)	51.2 ± 1.34** (-5.7)
MCH (pg)	18.3 ± 0.55	18.3 ± 0.43 (0.0)	18.2 ± 0.53 (-0.5)	17.2 ± 0.44** (-6.0)
MCHC (g/dL)	33.6 ± 0.55	33.6 ± 0.55 (0.0)	33.5 ± 0.48 (-0.3)	33.6 ± 0.36 (0.0)
PT (seconds)	15.5 ± 0.73	15.4 ± 0.78 (-0.6)	15.4 ± 0.68 (-0.6)	14.1 ± 0.56** (-9.0)
APTT (seconds)	11.5 ± 0.72	12.6 ± 0.74* (9.6)	12.1 ± 1.25 (5.2)	13.0 ± 0.61** (13.0)

*: $p<0.05$; **: $p<0.01$

Only few statistically significant differences were found between the control and test animals (increase in percentage of eosinophils in females at 1000 ppm at week 52; increase in erythrocyte counts in females at 4 and 100 ppm at 18 months; and increased reticulocyte count in females at 4 ppm at 24 months). Since all values were within the normal range for this age and strain of rats, and no dose-response relationship was noted, these differences were not considered treatment-related. (see B.6.5.1.1 Lifetime oral dosing studies in rats: combined oncogenicity and chronic toxicity of GALBEN technical (M 9834))

Selected haematology parameters (mean values)

Parameter	Sex and dose level (ppm)							
	Male				Female			
	0	4	100	1000	0	4	100	1000
3 months								
RBC (10^6/µL)	8.87	8.66	8.49	8.68	7.96	8.04	7.75	7.96
WBC (10^3/µL)	11.0	11.9	12.4	11.2	10.0	8.7	8.1	8.3
Eosinophils (%)	1	1	1	1	1	2	1	1
6 months								
RBC (10^6/µL)	8.51	8.60	8.47	8.44	7.62	7.69	7.54	7.58
WBC (10^3/µL)	12.9	11.6	12.9	13.1	7.4	6.9	7.6	7.0
Eosinophils (%)	<1	1	1	<1	1	1	<1	<1
12 months								
RBC (10^6/µL)	8.93	8.45	8.30	8.57	7.61	7.87	7.69	7.74
WBC (10^3/µL)	10.7	11.8	10.8	9.4	9.7	5.8	6.7	6.3
Eosinophils (%)	1	3	1	1	1	1	2	3*
18 months								
RBC (10^6/µL)	7.62	8.18	8.05	8.08	6.91	7.64*	7.41*	7.32

Parameter	Sex and dose level (ppm)							
	Male				Female			
	0	4	100	1000	0	4	100	1000
WBC (10^3/µL)	11.9	14.1	14.2	13.4	8.6	9.7	7.3	7.2
Eosinophils (%)	2	1	2	1	1	2	1	1
24 months								
RBC (10^6/µL)	7.07	7.56	7.60	6.78	7.28	6.57	7.24	6.94
Reticulocyte (%)	0.5	1.2	0.4	3.5	0.5	1.5*	0.6	0.5
WBC (10^3/µL)	12.9	15.5	12.4	14.5	8.2	7.0	8.3	8.6
Eosinophils (%)	1	1	1	1	1	1	1	1

*: $p \leq 0.05$

B.6.3.1.1 5 weeks cumulative toxicity study joined by a two-week recovery period with "GALBEN TH" by oral application on rats – Treatment with benalaxyl and 800/4000 mg/kgbw/day caused reversible changes in haematological and clinical chemistry parameters, and a significant increase in liver weight (+51% in males, and +80% in females, also reversible).

At the end of the treatment period, prothrombin time was increased in females at 800/4000 mg/kg/day. This change recovered after 2 weeks without treatment.

Clinical observations Significant elevated levels of ALP (i.e. 1.9- and 2.4-fold) were noted in females from 75/200 mg/kgbw/day group during the weeks 8 and 13, respectively (200 mg/kgbw/day was administered during these period), and in males (i.e.1.8-fold) during the week 13 compared to controls. In males, the differences were within the HC data reference range of the testing facility. Only two females exhibited increasing ALP values (1.6- and 2.4-fold, respectively) falling outside of the testing facility HC data reference range in weeks 8 and 13 (see B.6.3.2.4 A 90-Day Oral (Capsule) Toxicity Study of Benalaxyl in Beagle Dogs).

Lower mean serum alkaline phosphatase (ALP) values and higher mean serum cholesterol values were noted in all test substance-treated groups when compared to the control group. ALP values were significantly lower in the 15 and 150 mg/kgbw/day group females (by 22.4% and 21.4%, respectively). There was no apparent dose-response relationship and the direction of change was considered to be toxicologically irrelevant, and therefore non-adverse.

B.6.6.2.2 An Oral (Gavage) Prenatal Developmental Toxicity Study of Benalaxyl in Rats – Phase 1 – Serum cholesterol values were higher in the 15, 50, and 150 mg/kgbw/day group females by 10.8%, 10.8%, and 14.9%, respectively, attaining statistical significance at 150 mg/kgbw/day.

Phase 2 Coagulation was not affected by treatment. Lower serum alkaline phosphatase (ALP) and higher serum cholesterol and serum alanine aminotransferase (ALT) values were noted in the 450/300 mg/kg/day group females. Serum ALP values were significantly lower (22.5%) in the 450/300 mg/kgbw/day group females. The direction of change was considered to be toxicologically irrelevant, and therefore non-adverse.

A significantly higher (9.3%) serum cholesterol value was noted in the 450/300 mg/kgbw/day group females compared to the control group; the change was however of minimal magnitude, correlated with higher liver weights, and was considered an adaptive change. A higher (9.8%) ALT value was noted in the 450/300 mg/kgbw/day group females. The change was of minimal magnitude, correlated with higher liver weights, and hepatocellular hypertrophy, and all individual values were within the HC range; on this basis it was considered to be adaptive and not adverse. Other differences attaining statistical significance (higher (3.2%) serum glucose value, lower serum chloride (1.0%) and sodium (1.4%) values) were of minimal magnitude, or within HC range, and were considered toxicologically irrelevant.

B.6.3.1.1 5 weeks cumulative toxicity study joined by a two-week recovery period with "GALBEN TH" by oral application on rats – At the end of the test period, cholesterol values were increased in females at 800/4000 mg/kgbw/day; albumin and total protein values were also significantly increased, but considered within normal range; SGOT and alkaline phosphatase values were significantly reduced in males and females at 800/4000 mg/kgbw/day. Most changes recovered at the end of the recovery period.

Summary of significant changes at clinical chemistry (mean values)

Parameter	Sex and dose level (mg/kgbw/day)							
	Male				Female			
	0	10	100	800 / 4000	0	10	100	800 / 4000
	After 5 weeks of treatment							
Cholesterol (mmol/L)	2.00	1.93	2.12	2.32	2.00	2.32	2.50	3.10*
	After 2 weeks of recovery							
	2.66			1.74	1.82			1.98
Ref val	1.5-2.6							
Albumin (µmol/L)	After 5 weeks of treatment							
	518.8	542.1	527.6	544.8	536.2	542.1	527.6	585.6*
	After 2 weeks of recovery							
	510.2			501.7	481.2			195.4
Ref val	420-650							
Total protein (g/L)	After 5 weeks of treatment							
	60.2	61.4	61.8	63.4	60.8	61.4	62.1	67.4*
	After 2 weeks of recovery							
	57.2			57.1	53.4			56.8
Ref val	55-75							
SGOT (U/L)	After 5 weeks of treatment							
	149.4	124.0	126.2	81.4*	131.4	117.4	134.6	80.2**
	After 2 weeks of recovery							
	159.6			165.4	157.0			128.6*
Ref val	Up to 200							
Alkaline phosphatase	After 5 weeks of treatment							
	353.1	351.2	341.1	262.4*	284.8	294.7	237.6	152.2**
	After 2 weeks of recovery							
	158.8			156.2	125.0			106.8
Ref val	Young animals up to 500; adult animals up to 250							

Ref val: Normal reference value (as included in the report); *: $p<0.05$; **: $p<0.01$

Histological observations Test substance-related increases of liver weights (25.7% in absolute and 26.1% relative to body and brain weights, respectively) were noted in males from 75/200 mg/kgbw/day group. Few statistically significant differences were observed between control and the treated groups. These have included lowered prostate weights (absolute and relative to body and brain weights, ranging from 60.3% to 62.0%) in the 7.5 mg/kgbw/day group males, lowered prostate weight relative to final body weight (39.3%) in the 75/200 mg/kgbw/day group males, and lowered spleen weight relative to final body weight (36.4%) in the 25 mg/kgbw/day group females. (see B.6.3.2.4 A 90-Day Oral (Capsule) Toxicity Study of Benalaxyl in Beagle Dogs).

Histophatology observations included hyperkeratosis of oesophagus (increased in males at 100 ppm), chronic (lymphocytic) inflammation and/or fibrosis involving the Harderian glands increased in males at 1000 ppm), submucosal lymphoid hyperplasia in the large intestine (increased in males at 100 ppm), dilated, fluid-filledsinusoids (increased in males at 100 ppm), increased macrophages in cervical lymph nodes (reduced in males at 4 and 100 ppm), mesenteric lymph nodes congestion (increased in females at 4 ppm), glandular/lobular hyperplasia in mammary glands (decreased in males at 4 and 100 ppm), chronic prostatitis (decreased in males at 100 ppm), haemosiderosis in the spleen (decreased in males at 100 ppm and in females at 4 ppm), reticuloendotelial cell hyperplasia in the spleen (increased in males at 4 ppm), and erythroid hypoplasia (decreased in females at 4 ppm). A weak chain of the study was a lack of in-depth investigations about account for the increased relative heart weight in males at 1000 ppm, or the decreased absolute ovary weights in females at 4 and 100 ppm.(see B.6.5.1.1 Lifetime oral dosing studies in rats: combined oncogenicity and chronic toxicity of GALBEN technical (M 9834))

Selected organ weight data (mean values)

Organ	Sex and dose level (ppm)

	Male				**Female**			
	0	**4**	**100**	**1000**	**0**	**4**	**100**	**1000**
12 months								
Terminal body weight (g)	651	669	659	633	339	365	340	375
Liver								
Absolute (g)	18.3	20.0	17.3	18.1	10.2	10.3	9.2	10.5
Relative (%)	2.8	3.0	2.7	2.9	3.0	2.8	2.7	2.8
Heart								
Absolute (g)	1.9	1.8	1.7	1.8	1.1	1.1	1.0	1.1
Relative (%)	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Testes								
Absolute (g)	3.3	3.6	3.5	3.6				
Relative (%)	0.5	0.5	0.5	0.6				
Ovaries								
Absolute (mg)					94	112	91	99
Relative (%)					2.8	3.1	2.8	2.7
24 months								
Terminal body weight (g)	734	683	745	689	517	501	527	530
Liver								
Absolute (g)	22	21	21	20	15.9	14.7	15.2	17.1
Relative (%)	3.1	3.2	2.8	2.9	3.2	3.0	2.9	3.3
Heart								
Absolute (g)	2.2	2.1	2.1	2.4	1.5	1.5	1.5	1.5
Relative (%)	0.3	0.3	0.3	0.4*	0.3	0.3	0.3	0.3
Testes								
Absolute (g)	3.5	3.2	3.7	3.3				
Relative (%)	0.5	0.5	0.5	0.5				
Ovaries								
Absolute (mg)					160	122*	120*	132
Relative (%)					3.3	2.5	2.4	2.6

*: $p \leq 0.05$

In study B.6.3.1.1 5 weeks cumulative toxicity study joined by a two-week recovery period with "GALBEN TH" by oral application on rats – Liver weights of animals treated at 100 mg/kg bw/day and at 800/4000 mg/kg bw/day were dose-related significantly increased compared to controls, being highly significant at 800/4000 mg/kg bw/day (+ 51% in males and + 80% in females).

At the end of the recovery period, liver weight of animals previously treated at 800/4000 mg/kg bw/day were comparable to controls.

Summary of liver weight (mean values)

Liver weights	**Sex and dose level (mg/kg bw/day)**							
	Male				**Female**			
	0	**10**	**100**	**800 / 4000**	**0**	**10**	**100**	**800 / 4000**
	After 5 weeks of treatment							
Absolute (g)	9.777	11.787	11.398*	14.885 **	6.521	4.042	7.638*	12.273 **
Adjusted (g)	10.092	10.763	11.756*	15.237 **	6.803	6.758	7.680*	12.232 **
	After 2 weeks of recovery							
Absolute (g)	9.765			9.800	6.613			7.603
Adjusted (g)	9.773			9.792	6.684			7.532

*: $p<0.05$; **: $p<0.01$

After 12 months of exposure, some neoplasms (19 in total in all groups, including the controls) were observed microscopically

Selected neoplastic findings at terminal sacrifice (affected animals)

Organ & tumour (or lesion)	Sex and dose level (ppm)							
	Male				Female			
	0	4	100	1000	0	4	100	1000
Adrenals – No. examined	54	51	55	53	54	55	54	55
Medulla, carcinoma	9	6	7	8	4	4	1	1
(Medullary hyperplasia)	1	6	4	0	1	1	0	2
Brain – No. examined	54	52	55	54	54	55	54	55
Astrocytoma	0	1	1	2	0	0	0	0
Ependymoma	0	0	0	0	0	0	0	1
Liver – No. examined	54	52	55	54	54	55	54	55
Adenoma, neoplastic nodule	0	0	0	0	0	0	1	3
Heaptocellular carcinoma	1	2	1	0	1	3	1	1
Reticulum cell sarcoma (multiple organ)	0	2	0	1	3	3	1	0
Pancreas – No. examined	54	52	55	54	53	55	54	54
Islet cell adenoma	5	6	6	4	1	3	0	1
Islet cell adenocarcinoma	0	1	1	1	0	0	0	0
Parathyroids – No. examined	50	45	38	53	47	46	49	52
Adenoma	1	2	4	0	0	1	1	0
Pituitary – No. examined	53	50	54	53	54	51	51	53
Adenoma	22	18	25	20	42	40	41	47
Adenocarcinoma	0	0	1	0	0	0	1	0
Thyroid – No. examined	53	51	55	54	53	51	54	55
Parafollicular cell tumour (C-cell)	5	5	12	3	5	8	2	8
Follicular adenoma	2	0	0	1	0	0	1	0
Follicular adenocarcinoma	0	1	2	0	0	0	0	1

Neoplastic findings overview at terminal sacrifice

Parameter	Sex and dose level (ppm)							
	Male				Female			
	0	4	100	1000	0	4	100	1000
Total No. of animals with primary tumours / Total primary tumours	40 / 62	36 / 60	46 / 73	39 /56	52 / 106	51 / 99	47 / 84	52 / 94
No. of tumours / animal bearing tumours	1.55	1.67	1.59	1.44	2.04	1.94	1.79	1.81
Total No. of animals with metastatic tumours / Total metastatic tumours	2 / 2	3 / 3	0 / 0	5 / 5	6 / 6	9 / 9	2 / 3	3 / 3
No. of metastatic tumours / animal bearing tumours	0.05	0.08	0.00	0.13	0.12	0.18	0.06	0.06

In study B.6.3.2.3 A 90-Day Dietary Combined Toxicity and Neurotoxicity Study of Benalaxyl in Rats - Hepatocellular hypertrophy was characterized by expansion of the hepatocellular cytoplasm.

The distribution of the change was predominantly centrilobular and extended to a more generalised pattern with increased severity of the change.

Follicular cell hypertrophy in the thyroid glands was characterized by expansion of the cytoplasm of the thyroid follicular cells and a decrease in the amount of follicular colloid.

There were no remarkable differences in ovarian primordial follicle counts between the 0 (control; mean ± SD = 85.4 ± 26.01, n=9) and 10000 ppm group females (mean ± SD = 75.0 ± 34.25, n=10).

Summary of treatment-related microscopic findings

Observation	0 ppm	100 ppm	1000 ppm	10000 ppm
Males				
Liver	10	10	10	10
Hepatocellular hypertrophy	0	0	0	9
Minimal	-	-	-	2
Mild	-	-	-	3
Moderate	-	-	-	4
Thyroid gland	10	10	10	10
Follicular cell hypertrophy	2	1	2	9
Mild	2	1	2	7
Moderate	0	0	0	2
Females				
Liver	10	10	10	10
Hepatocellular hypertrophy	0	0	3	10
Minimal	-	-	3	1
Mild	-	-	0	7
Moderate	-	-	0	2
Thyroid gland	10	10	10	10
Follicular cell hypertrophy	1	2	2	7
Mild	1	2	2	7
Moderate	0	0	0	0

In study B.6.3.1.1 5 weeks cumulative toxicity study joined by a two-week recovery period with "GALBEN TH" by oral application on rats – A histopathological examination revealed changes in the thyroid, which was not re-examined at the end of the recovery period.

Effects observed at the dose level of 100 mg/kg/day were limited to a slightly significantly increased liver weight in both sexes (+17% in males, and +13% in females). There were no other changes observed at this dose level, which was therefore considered the NOAEL of the study.

In the 5-week rat study, the NOAEL is 100 mg/kg bw per day on the basis of the significant increase in liver weight. Histopathology revealed treatment-related changes in the liver and thyroid.

Other findings in few control and test animals, consisting of slight lymphocytic-histiocytic infiltrations in the liver, lung, heart, kidney, urinary bladder, intestine, epididymis, and prostate which, together with lymphoid hyperplasia in mesenteric lymph nodes, were considered most likely due to latent infections.

Other findings occurring in both control and test group included infiltration of the uterine stroma by eosinophils, and congestion of the spleen, which was considered by the authors to be related to the sacrifice of the animals.

No histopathology was conducted at the end of the recovery period.

Summary of histopathology (No. of affected animal/No. of animals examined)

Organ & Lesion	Sex and dose level (mg/kg/day)			
	Male		Female	
	0	800 / 4000	0	800 / 4000
Liver - Fatty infiltration				
Single hepatocytes	6/10	6/10	8/10	5/10
Diffuse small droplet - slight	0/10	3/10	2/10	4/10

Organ & Lesion	Sex and dose level (mg/kg/day)			
	Male		Female	
	0	800 / 4000	0	800 / 4000
Thyroid - Hyperplasic elevation of the follicular epithelium				
Slight	1/10	3/10	0/10	2/10
Moderate	0/10	3/10	0/10	0/10
Thyroid				
tendency to formation of microfollicular structure^	0/10	2/10	0/10	0/10

^: lesion only mentioned in the pathology report text, and not included in the Individual findings table

Carcinogenic NOAEL

A systemic NOAEL for benalaxyl at dose level of 100 ppm (corresponding to 4.42/5.64 mg/kg bw per day for males and females, respectively), based on heart weight changes together with changes in LDH and K; the carcinogenic NOAEL is 100 ppm (corresponding to 4.42/5.64mg/kg bw per day for males and females respectively), based on the occurrence of astrocytomas in brain (a rare tumour) was set. Clinical chemistry showed dose-related increases in lactic dehydrogenase (LDH) in male rats at 18 months, attaining statistical significance only at 1000 ppm in comparison to controls. Males at 1000 ppm also showed increased serum potassium levels in comparison to controls at 18 months only.

Liver: A total of 16 primary hepatocellular tumours were observed, representing a population frequency of 3%, which is compatible with the frequency of spontaneous hepatocellular neoplasms. Although the incidence of hepatocellular neoplasm was found to be greater in females at 1000 ppm, the difference was not

statistically significant; there was no dose-response observed in the treatment groups and no primary hepatocellular tumours were found in males at 1000 ppm.

Thyroid: The morphological distinction between benign and malignant parafollicular cell neoplasms was considered inadequate and therefore, these neoplasms were simply described as parafollicular cell tumours. (see B.6.5.1.1 Lifetime oral dosing studies in rats: combined oncogenicity and chronic toxicity of GALBEN technical (M 9834))

The administration of Benalaxyl Technical at doses up to and including a dose of 275 mg/kg bw per day was concluded to be negative in the Micronucleus assay. Based upon the results, the high dose for the definitive assay was set at 275 mg/kg bw per day, which was estimated to be the maximum tolerated dose (MTD) in each animal sex. (see B.6.4.2.1 In Vivo Micronucleus Assay in Rats using Benalaxyl Technical). All criteria for a valid study were met.

Also, the fact that the increase was observed in one sex does not reduce the concern because it is considered likely that there a difference in susceptibility between the sexes for these types of tumors due to differences in function of this tissue between males and females.

Additional factors which may increase the carcinogenic potential of benalaxyl are the following, also mentioned in the short summary of studies including other species, targeted toxicity and systemic toxicity:

There are several reasons why a tumour observed in animals may be judged to be not relevant for humans or may be judged to be of lower concern. In most of these cases the tumour arises via a mode of action which does not occur in humans (see this Section Part K of CLP Criteria Guidance).

In some cases the tumour may arise in a tissue known to be overly susceptible in the species tested to development of certain tumours and consequently may be judged to be less relevant for humans. In a few cases a tumour may occur in a tissue with no equivalent in humans.

Tumours occurring in tissues with no human equivalent

Some of the commonly used animal species have some tissues with no equivalent in humans. Tumours occurring in these tissues include the following types:

Selected, statistically significantly different, non-neoplastic findings at terminal sacrifice (No. affected animals)

Organ & lesion	Sex and dose level (ppm)							
	Male				Female			
	0	4	100	1000	0	4	100	1000
Oesophagus – No. examined	54	52	55	54	54	54	54	55
Hyperkeratosis	1	5	8*	2	6	6	6	6
Harderian glands – No. examined	53	52	55	54	54	55	54	54
Chronic/lymphocytic inflammation/fibrosis	8	10	8	19*	12	14	11	11
Large intestine – No. examined	52	50	54	54	54	55	52	55
Submucosal, lymphoid hyperplasia	2	3	9*	3	3	5	4	4
Liver – No. examined	54	52	55	54	54	55	54	55
Dilated, fluid-filled sinusoids	4	5	13*	9	3	1	1	2
Cervical lymph nodes – No. examined	46	51	50	52	44	53	52	47
Increased macrophages (sometimes containing haemosiderin)	7	1*	0*	2	1	0	1	1
Mesenteric lymph nodes – No. examined	51	49	47	51	52	52	53	53
Congestion	6	2	3	1	0	7*	1	2
Mammary glands – No. examined	46	47	31	51	54	54	54	55
Glandular/lobular hyperplasia	12	4*	1*	6	4	2	3	4
Prostate – No. examined	54	51	54	53				
Chronic prostatitis	11	11	2*	12				
Spleen – No. examined	54	52	55	54	54	54	54	54
Haemosiderosis	18	9	4*	9	33	21*	33	35
Reticuloendotelial cell hyperplasia	2	9*	7	7	3	7	1	3
Erythroid hypoplasia	1	2	1	1	6	0*	2	4

*: $p \leq 0.05$

Non – neoplastic: Amyloidosis was observed in the liver's mice at ≥ 250 ppm in males. This dose exceed the MTD. HCD for amyloidosis in the liver were provided and all the data were within those HCD. Amyloidosis was also reported in the kidneys, spleen and adrenal glands, salivary glands, stomach and intestine, and Zymbal glands (Anonymous, 1985 and 1988b). (see B.6.5.1.1 Lifetime oral dosing studies in rats: combined oncogenicity and chronic toxicity of GALBEN technical (M 9834)).

The macroscopic observations, specific for set the classification criteria based on CLP regulation were observed also in rats. The most frequent finding at interim kill was milk cysts (galactoceles) in mammary glands, which were observed in females of all groups, including the control. An increase in relative heart weight (10%) was noted in male rats at 1000 ppm at 24 months and the absolute ovary weights of female rats at 4 and 100 ppm were significantly decreased at 24 months.

In a few cases a tumour may occur in a tissue with no equivalent in humans:

Neoplastic: Urinary bladder tumours (transitional bladder tumour) were observed in males at 3000 ppm (3 tumours out of 60 animals). Mammary glands: Fibroadenomas were extremely frequent among all female groups (range: 38–52 %); adenocarcinomas ranged 4 to 9% in all groups. Reticulum cell sarcoma was the most frequent malignant neoplasm showing multiple organ involvement in ten rats (2.3%). Adrenal's medullary tumours -3 tumours in males at 100 ppm, which showed distant metastases to lungs and/or liver. Brain: Intracerebral astrocytomas and ependymoma. Liver: A total of 16 primary hepatocellular tumours were observed, representing a population frequency of 3%, which is compatible with the frequency of spontaneous hepatocellular neoplasms. Although the incidence of hepatocellular neoplasm was found to be greater in females at 1000 ppm, the difference was not statistically significant. Pancreas: Islet cell adenomas were more common in males and noted with similar frequency among all male groups (range 7 to 11%). Parathyroid: Solitary distinct nodules within the parathyroids were all classified as adenomas, as no distinction was possible

between “nodular” hyperplasia and adenoma; however taking in consideration the incidence, the effect was not attributed to administration. Adenomas of the pituitary were extremely frequent in all female groups (range 78 to 89%) and not uncommon among males (range 36 – 46%). Thyroid: parafollicular cell hyperplasia and tumours in males and females (see CA_B.6.5.1.1).

About the mechanistic activity of benalaxyl could be mentioned the study CA 5.3.1/02, the dietary study of 28 days, in rat, at concentrations of 1000, 5000 and 10000 ppm which it caused an increase in liver weight and microsomal protein and cytochrome P450 content associated with a marked induction of hepatic CYP2B and UDP-glucuronyltransferase (UDP-GT). As results was the increases of excretion of T4 and a lower plasma T4 levels feedback to the pituitary gland and cause increased plasma thyroid stimulating hormone (TSH) levels (Anonimous, 2008). The sustained increase in plasma TSH level may result in thyroid follicular cell hypertrophy, followed by thyroid hyperplasia and/or neoplasia with longer term exposure.

Comparing with human thyroid reactivity, it need to be made a correction, considering as essential to be mentioned the lack of T4-binding globulin (TBG) and shorter half-life of T4 in rats compared to humans. These are some of the reason for that it is not allow to extrapolate the thyroid hypertrophy, hyperplasia, and/or neoplasia with microsomal enzyme induction in rats versus humans. Follicular cell hypertrophy on the thyroid was observed in the 5-week and 13-week studies in the rat, mainly at high dose. In the dogs, effects on prostate weight, atrophy of seminiferous tubules and increase in relative testes weights were observed. The experts’ meeting at EFSA agreed that the evidence available is weak (EAMS modality without an in-depth testing procesure according with ED GD EFSA/ECHA and, taking into consideration lack of data on mode of action, an endocrine disrupting potential of the test substance cannot be excluded and conclusions cannot be reached (data gap) in the view of avoid its contribution on benalaxyl carcinogen potential.

An in-depth assessment of genotoxicity, without in vitro positive results in the study In Vivo Micronucleus Assay in Rats using Benalaxyl Technical (see CA 5.4.2/01), some findings from 2nd RF study, could be relevant in proposed classification, as mortality (1/3 m and 1/3 f at 300 mg/kg bw/d 2/3 m and 1/3 f at 400 mg/kg bw/d) and clinical sign (lethargy and piloerection at 400 mg/kg (m + f) at 300 mg/kg bw/d (m);lethargy, piloerection and prostration at 300 mg/kg bw/d (f)). This study stated the MTD = 275 mg/kg bw/d (lethargy, piloerection and prostration). The MTD is the highest dose of the test agent during the bioassay that can be predicted not to alter the animal’s normal longevity from effects other than carcinogenicity. Data obtained from a sub-chronic or other repeated dose toxicity study are used as the basis for determining the MTD. Excessive toxicity, for instance toxicity at doses exceeding the MTD, can affect the carcinogenic responses in bioassays. Such toxicity can cause effects such as cell death (necrosis) with associated regenerative hyperplasia, which can lead to tumour development as a secondary consequence unrelated to the intrinsic potential of the substance itself to cause tumours at lower less toxic doses.

The scientific knowledge leading to a comprehensive MoA may influence a carcinogenic classification under CLP. MoA constitutes key data according to the CLP Regulation (CLP; Annex I, 1.1.1.5 and 3.7.2.1.1, 3.7.2.3.2.) and it is crucial for an efficient and transparent decision-making process under, for example CLH. About the mechanistic activity of benalaxyl could be mentioned the study CA 5.3.1/02, the dietary study of 28 days, in rat, at concentrations of 1000, 5000 and 10000 ppm which it caused an increase in liver weight and microsomal protein and cytochrome P450 content associated with a marked induction of hepatic CYP2B and UDP-glucuronyltransferase (UDP-GT). As results was the increases of excretion of T4 and a lower plasma T4 levels feedback to the pituitary gland and cause increased plasma thyroid stimulating hormone (TSH) levels (Anoninous, 2008). The sustained increase in plasma TSH level may result in thyroid follicular cell hypertrophy, followed by thyroid hyperplasia and/or neoplasia with longer term exposure.

Comparing with human thyroid reactivity, it need to be made a correction, considering as essential to be mentioned the lack of T4-binding globulin (TBG) and shorter half-life of T4 in rats compared to humans. These are some of the reason for that it is not allow to extrapolate the thyroid hypertrophy, hyperplasia, and/or neoplasia with microsomal enzyme induction in rats versus humans. Follicular cell hypertrophy on the thyroid was observed in the 5-week and 13-week studies in the rat, mainly at high dose. In the dogs, effects on prostate weight, atrophy of seminiferous tubules and increase in relative testes weights were observed. The experts’ meeting at EFSA agreed that the evidence available is weak (EAMS modality without an in-depth testing procesure according with ED GD EFSA/ECHA and, taking into consideration lack of data on mode of

action, an endocrine disrupting potential of the test substance cannot be excluded and conclusions cannot be reached (data gap) in the view of avoid its contribution on benalaxyl carcinogen potential.

Also the incidence of malignance was considered the most critical effect. No HCD were available for the performing laboratory. Incidences of astrocytoma were available, but information on severity was missing.. In such cases the CLP guidance suggests a comparison with the historical control data As discussed above the observed incidence in malignancy is just a fact without a presence of performing laboratory historical data. Also, it is unclear whether the stated HC data for the Dossier studies include the results of the different periods of time. Therefore, comparison with the historical control is not considered conclusive. The data set from the Applicant Dossier, of those older historical controls (from 1977 to 1979) which were not reliable on, due to the lack of information about the protocol/techniques of preservation/microscopic examination as well as time of sacrifice of surviving animals. The large frequence and distribution in all mice groups from the studies, higher incidence in males than in female and the high mortality concluded a treatment related.(see B.6.5.1.1).

Endocrine disrupting potential: At 200 ppm (corresponding to 6.5 mg/kg bw per day) significant testicular findings (atrophy of seminiferous tubules were noted in the histopathological summary of organs and tissues microscopic examination) (see B.6.3.2.5 Study of the toxicity of repeated oral administration of product M 9834 (GALBEN) to Beagle dogs at the dosage levels of 10, 200 and 800 ppm for 52 weeks).

A summarised results of endocrine disruptor potential of benalaxyl are presented in the table below:

Study, Species, Doses (ppm), mg/kg bw/d	**NOAEL (ppm) mg/kg bw/d**	**LOAEL (ppm) mg/kg bw/d**	**Effect classification**	**Critical effects**	**Reference**
5w, oral, gavage/intubation Rat (Wistar), M+F 0, 10, 100, 800/4000	100 M+F	800/4000 M+F	Target organ toxicity Systemic toxicity EATS-mediated	Organ target weight: relative increasing Liver weight ↑51% (M) and↑80 % (F) Reversible after the 2-week recovery period In life observation: - Clinical chemistry and haematology: Change: Liver toxicity; Reversible after the 2-week recovery period Organ histopathology: Effect indicative of thyroid 2/10 vs 0/10 in controls; Not indicated as a base for NOAEL setting; not re-examined at the end of the recovery period Changes: Hyperplasic elevation of the follicular epithelium No histopathology at the end of the recovery period; 800/4000 mg/kg bw/day indicates that dosage of the high dose level (800 mg/kg bw/day) was continuously increased up to 4000mg/kg bw/day	RAR-08_Vol-3CA_B.6.3.1.1 CA 5.3.1/01 Anonymous (1982) A Range finding study; NOAEL verified in subchronic study; Subacute oral in rodents In – house method No GLP
4w, oral, feed Rat (CD) only M	<120 m	120 M	In vivo mechanistic	Clinical chemistry: Phase I enzyme induction	RAR-08_Vol-3 CA_B.6.3.1.2

Study, Species, Doses (ppm), mg/kg bw/d	**NOAEL (ppm) mg/kg bw/d**	**LOAEL (ppm) mg/kg bw/d**	**Effect classification**	**Critical effects**	**Reference**
0, 120, 600, 1200				(in vivo) ↑Cytochrome P450 and 7-Pentoxyresorufin O-dependentylase Phase II enzyme induction (in vivo) ↑p-Nitrophenol UDP-glucuronosyl transferase Effect indicative of EATS	CA 5.3.1/01 Anonymous (2001) A Mechanistic study to investigate liver enzyme induction relevant to demonstrate increased clearance of thyroid hormones in rat In – house method No GLP
13w, oral, feed Rat, Sprague-Dawley (Crl:CD (BR)) 0, 0.56, 5.80, 58.87, 637.73, 1051.84 M 0, 0.66, 6.69, 71.51, 783.56, 1277.24 F	58.9 M 71.5 F	637.7 M 783.6 F	Target organ toxicity Systemic toxicity EATS - mediated	Organ target weight: relative increasing Liver weight: ↑48 % M and ↑61% F Lobulation; Darker liver M + F In life observation: – Clinical chemistry and haematology Change: Liver toxicity M+F Organ histopathology: Thyroid histopathology – no effect M+F Testis histopathology - no effects were observed up to 1051.84 mg/kg bw. Effect indicative of EAS Thyroid weight – no effect Effect indicative of thyroid	RAR-08_Vol-3 CA_B.6.3.2.2 CA 5.3.2/02 Anonymous (1982) Repeated dose 90-day oral toxicity study Assimilated OECD 408 GLP
90d, oral, feed Rat Sprague-Dawley (Crl:CD) 0, 0.6, 62, 677 M 0, 0.7, 74, 745 F	62 M 74 F	677 M 745 F	Target organ toxicity Systemic toxicity EATS - mediated	Organ target weight: relative increasing Liver weight: ↑41% M and ↑55% F Hepatocellular hypertrophy M + F In life observation: – Clinical chemistry and haematology: ↓ Body weight M + F Change: Liver toxicity M + F Organ histopathology: Testis histopathology - no effects up to 677 mg/kg bw Effect indicative of EAS Thyroid histopathology: M - Follicular cell hypertrophy (mild: 7/10 vs 2/10 in control group, moderate: 2/10 vs 0/10 in control group) Not reported as basis for NOAEL F – no effect Effect indicative of thyroid	RAR-08_Vol-3 CA_B.6.3.2.3 CA 5.3.2/03 Anonymous (2014b) CombinedToxicity/Neurotoxicity study; reproductive endpoints (sperm parameters and estrous cyclicity) were evaluated according to OECD 416 to address a data gap in the two-generation study Repeated dose 90-day oral toxicity study in rodents OECD 408 GLP

Study, Species, Doses (ppm), mg/kg bw/d	**NOAEL (ppm) mg/kg bw/d**	**LOAEL (ppm) mg/kg bw/d**	**Effect classification**	**Critical effects**	**Reference**
				Reproductive effect: Estrus cyclicity – no effect Effect indicative of EAS Sperm morphology + motility - no effect on spermatogenesis endpoints (mean testicular and cauda epididymal sperm numbers and sperm production rate, motility, progressive motility and morphology) Effect indicative of EAS No relevant effect observed	
90d, oral, capsule, Dog, Beagle 0, 7.5, 25, 75/200 M + F	25 M + F	75/200 M + F	Target organ toxicity Systemic toxicity EATS - mediated	Organ target weight: relative increasing Liver weight:↑26% M + F In life observation: – Clinical chemistry and haematology: ↑ALP (M + F) Organ histopathology: Testis histopathology - no effect up to the highest dose of 75/200 mg/kg bw Thyroid histopathology – no effect Effect indicative of thyroid Effect indicative of EAS Thyroid weight – no effect Effect indicative of thyroid	RAR-08_Vol-3 CA_B.6.3.2.4 CA 5.3.2/04 Anonymous (2014b) Dosage level was increased from 75 to 200 mg/kg bw/day during week 4 because there were no clinical signs of toxicity Repeated dose 90-day oral toxicity study in non-rodents OECD 408 and OECD 424 GLP
52wd weeks by oral, feed, Dog, Beagle, 0, 0.32,6.5,25.2 M	6.5 M	25.2 M	Target organ toxicity Systemic toxicity EATS - mediated	Macroscopic examination and organ weight determinations did not reveal any abnormalities. In life observation: - Clinical chemistry and haematology – no effect up to the highest dose of 25.2 mkd; - Clinical signs - no effect up to the highest dose of 25.2 mkd; - Food consumption - no effect up to the highest dose of 25.2 mkd; - Mortality - no effect up to the highest dose of 25.2 mkd; Organ histopathology: - Testis histopathology – Changes in Atrophy of the seminiferous tubules Effect indicative of EAS	RAR-08_Vol-3 CA_B.6.3.2.5 CA 5.3.2/05 Anonymous (1982) A chronic study Guidelines: Test method was not specified in the report (In house method), but detailed test procedure was included in report (along with RBM Standard Operating Procedures) and complied to a great extent with OECD Guideline 409 GLP

Study, Species, Doses (ppm), mg/kg bw/d	NOAEL (ppm) mg/kg bw/d	LOAEL (ppm) mg/kg bw/d	Effect classification	Critical effects	Reference
				- Thyroid histopathology - no effect up to the highest dose of 25.2 mkd; Effect indicative of T	
2y oral, feed, Rat, Crl:CD(SD), 0,0.18,4.42,44.3 M 0,0.23,5.64,56.3 F	0.18 M 0.23 F	44.3 M 56.3 F	Target organ toxicity Systemic toxicity EATS - mediated	Organ weight: ↑Heart weight (not accompanied NOAEL systemic) In life observation: -Clinical chemistry and haematology - ↑K and LDH No relevant effect observed, no carcinogenic effects Organ histopathology: -Thyroid histopathology – no effect; NOAEL carcinogenicity; based on males; thyroid weight was not measured Effect indicative of T	RAR-08_Vol-3 CA_B.6.5.1.1 CA 5.5/01 Anonymous (1983) A combined chronic toxicity/carcinogenicity GLP
78w, feed, Mouse, Swiss, 0,44.93,180.87,558.87 M 0.42.93,174.26,521.73 F	44.93 M 42.93 F	558.87 M 521.73 F	Target organ toxicity Systemic toxicity EATS - mediated	Organ weight: Thyroid weight – no effect M + F Effect indicative of T Liver histopathology: Amyloidosis in liver and multiple tissues (kidneys, spleen, adrenal glands at higher dose levels); effect indicative for MTD<44.93 Liver histopathology: Amyloidosis in liver and multiple tissues (kidneys, spleen, adrenal glands at higher dose levels); effect indicative for MTD<42.9 Mortality: in M at 180.87 was 68.3 % and 75 % in the high dose level No effect indicative for EATS but offer the values for NOAEL systemic toxicity Urinary bladder histopathology – Tumours Effect dose above MTD	RAR-08_Vol-3 CA_B.6.5.2.1 CA 5.5/02 Anonymous (1985) A combined chronic toxicity /carcinogenicity GLP
29w, oral, feed, Rat, Sprague-Dawley 0,6.97,66.57,333.29 M Adult (F0) 0,7.90,79.64,397.73 F Adult (F0)	6.97 M 7.90 F	333.29 M 397.73 F	Target organ toxicity EATS - mediated	Organ weight: Liver weight: Relative ↑50 % M NOAEL parental based on females = 79.64 Relative and absolute ↑ 17.7 % NOAEL parental based on females Reproductive: Sensitive to, but not diagnostic of, EATS, no effect, generate NOAEL	RAR-08_Vol-3 CA_ B.6.6.1.1 CA 5.6.1/01 Anonymous (1983) Two-generation reproduction study CA 5.6.1/01 GLP

Study, Species, Doses (ppm), mg/kg bw/d	NOAEL (ppm) mg/kg bw/d	LOAEL (ppm) mg/kg bw/d	Effect classification	Critical effects	Reference
				reproductive = 333.29 M and 397.73 F Developmental: Litter/pup weight: ↓ pup weight during lactation, generate NOAEL offspring = 79.64 F Sensitive to, but not diagnostic of, EATS Pup development: ↓Organ weight changed (organs not specified) generate NOAEL offspring = 79.64 F Sensitive to, but not diagnostic of, EATS Adult (F0) = Organ histopathology: Female Cervix histopathology = No effect up to the highest dose of 397.73 mkd but not relevant for EAS – modality because of important deviations (age of balanoprenputial separation and vaginal opening, anogenital distance, coagulating gland weight and histopathology, epidymis weight and histopathology, seminal vesicles weight not measured Adult (F0) = Organ histopathology: Male Epididymis histopathology = No effect up to the highest dose of 333.29 mkd Organ weight: Epididymis weight = No effect up to the highest dose of 333.29 mkd M + F Abnormalities: Genital abnormalities = No effect up to the highest dose of 397.73 mkd Ovary weight + Vagina histopathology + Ovary histopathology = No effect up to the highest dose of 397.73 mkd Prostate weight + Prostate	

Study, Species, Doses (ppm), mg/kg bw/d	NOAEL (ppm) mg/kg bw/d	LOAEL (ppm) mg/kg bw/d	Effect classification	Critical effects	Reference
				histopathology (with seminal vesicles and coagulating glands) + Testis histopathology = No effect up to the highest dose of 333.29 mkd Uterus weight (with EATS-mediated) + Uterus histopathology = No effect up to the highest dose of 397.73 mkd	
10d, oral, Gavage/Intubation, Rat CD(SD)BR 0,12.5,50,200 F	12.5 F	200 F	EATS-mediated	NOAEL maternal = 200 No relevant effect observed Adult (F0) Fetus M + F at 200 mkd but Abnormalities and presence of anomalies – incomplete ossification of the cranial bones (parietal, intraperitoneal, supraoccipital) generate NOAEL developmental Sensitive to, but not diagnostic of, EATS	RAR-08_Vol-3 CA_ B.6.6.2.1 CA 5.6.2/01 Anonymous (1982) Prenatal developmental toxicity study In house method in compliance with OECD 414 GLP
14d, oral, Gavage/Intubation, Rat Crl: CD(SD) 0,15,50,150,300 F Adults (F0) 0, 15, 50, 150 Fetus M + F	15 F 15 F	300 F 150 F	Systemic toxicity	Maternal toxicity: In life observation: ↓Body weight and generate the maternal NOAEL Due to lack of toxicity at all doses an additional cohorts Developmental toxicity: No relevant effect observed at 150mkd Sensitive to, but not diagnostic of, EATS	RAR-08_Vol-3 CA_ B.6.6.2.2 CA 5.6.2/02 Anonymous (2015) Prenatal developmental toxicity study OECD 414 GLP
22d, oral, Gavage/Intubation, Purity: NOT INDICATED Rabbit, New Zealand White 0,5,50, 250 F Adult (FO) F 50 Fetus M + F 50			Systemic toxicity	In life observation: ↓Body weight - % adjusted b.w.change (day 0 to 28) Fetal development: Retarded skeletal development; Decreased crown, rump length	RAR-08_Vol-3 CA_ B.6.6.2.3 CA 5.6.2/03 Anonymous (1983) OECD 414 GLP

10.9.2 Comparison with the CLP criteria

As it is stated in CLP Regulation a Classification of a substance as a carcinogen is based on consideration of the strength of the evidence of available data for classification with considerations of all other relevant information (weight of evidence) being taken into account as appropriate.

Carcinogen Category 2 based on tumour data from a structural analogue together with substantial support from consideration of other important factors such as formation of common significant metabolites.

The evidence of mutagenic activity in vivo may indicate that a substance has a potential for carcinogenic effects.
Benalaxyl was evaluated for its carcinogenicity in long-term studies, conducted in rats, mice and dogs. This effect was supported by results from other mechanistic or mutagenicity studies.

Benalaxyl is a chemical substance which induce tumours, increase tumour incidence and/or malignancy or shorten the time to tumour occurrence. Prevalence of benign tumours are considered to have the potential to progress to malignant tumours and are generally included in a malignance risk. It is necessary a delimitation between the structure of benalaxy which could induce cancer by any route of exposure, from the carcinogenic potential and its potency which are related of route, level, pattern and duration of exposure.
The induction of only benign tumours usually provides a lower strength of evidence for carcinogenicity than the induction of malignant tumours and will usually support Category 2 (CLP Annex I, 3.6.2.2.3).

There are marked differences among the general tumour incidence in the various experiments. These differences must be partly due to natural fluctuations and partly to the fact that the number of apparently normal tissues and organs (where, however, microscopic tumours are often found) submitted to systematic histopathological investigation, varied to some extent from experiment to experiment, in agreement with specific protocol.
Relating to bladder tumours, it was noted that transitional cell carcinomas (1 in males and 2 in females) were observed only in controls of the more recent experiments, started in 1981. These tumours were observed only at microscopic examination.

Some benign tumours, for example brain tumours, may be of concern in themselves. However, unusual tumour types or tumours occurring with reduced latency may add to the weight of evidence for the carcinogenic potential of a substance, even if the tumours are not statistically significant
Carc. 2 (H351 – Suspecting of causing cancer) may be warranted according to CLP Regulation criteria. This proposal for classification was reported in the RAR and in the EFSA conclusion (EFSA Journal 2020;18(1):5985).

As it is stated in CLP Regulation a Classification of a substance as a carcinogen is based on consideration of the strength of the evidence of available data for classification with considerations of all other relevant information (weight of evidence) being taken into account as appropriate.

Benalaxyl was evaluated for its carcinogenicity in long-term studies, conducted in rats, mice and dogs. This effect was supported by results from other mechanistic or mutagenicity studies.

According to Regulation EC No 1272/2008 (CLP), Table 3.6.1, classification for carcinogens is based on:
CATEGORY 1: Known or presumed human carcinogens A substance is classified in Category 1 for carcinogenicity on the basis of epidemiological and/or animal data. A substance may be further distinguished as:
- Category 1A, known to have carcinogenic potential for humans, classification is largely based on human evidence, or

- Category 1B, presumed to have carcinogenic potential for humans, classification is largely based on animal evidence.

The classification in Category 1A and 1B is based on strength of evidence together with additional considerations (see section 3.6.2.2). Such evidence may be derived from:

- human studies that establish a causal relationship between human exposure to a substance and the development of cancer (known human carcinogen); or

- animal experiments for which there is sufficient (1) evidence to demonstrate animal carcinogenicity (presumed human carcinogen).

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.

***CATEGORY 2**: Suspected human carcinogens 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 (see section 3.6.2.2). Such evidence may be derived either from limited(1) evidence of carcinogenicity in human studies or from limited evidence of carcinogenicity in animal studies.*

As there is no human data available for benalaxyl that may be relevant for carcinogenicity, criteria for category 1A are not fulfilled.

For classification in category 1B evidence may be derived from *"[..] animal experiments for which there is sufficient (1) evidence to demonstrate animal carcinogenicity (presumed human carcinogen) [..] 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."*

Sufficient evidence from animal studies is explained as "*a causal relationship has been established between the agent and an increased incidence of malignant neoplasms or of an appropriate combination of benign and malignant neoplasms in (a) two or more species of animals or (b) two or more independent studies in one species carried out at different times or in different laboratories or under different protocols. [..]"*

A classification of substance in **Category 2** (suspected human carcinogens) "*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 (see section 3.6.2.2). Such evidence may be derived either from limited(1) evidence of carcinogenicity in human studies or from limited evidence of carcinogenicity in animal studies*."

Limited evidence from animal studies is explained as "*data suggest a carcinogenic effect but are limited for making a definitive evaluation because, e.g. (a) the evidence of carcinogenicity is restricted to a single experiment; (b) there are unresolved questions regarding the adequacy of the design, conduct or interpretation of the studies; (c) the agent increases the incidence only of benign neoplasms or lesions of uncertain neoplastic potential; or (d) the evidence of carcinogenicity is restricted to studies that demonstrate only promoting activity in a narrow range of tissues or organs*."

The significance of tumours observed in the chronic and carcinogenicity studies, *i.e are* discussed below based on considerations included in the CLP guidance:

(a)tumour type and background incidence;

Rats:
Events: 19 neoplasms from 65 rats in a lifetime oral dosing studies in rats: combined oncogenicity and chronic toxicity
Mortality during the study period: 47 - 61% rats which died, killed when moribund or killed at termination were necropsied.
Dose level of 100 ppm (4.42/5.64 mg/kg bw per day for male and female, respectively) is general available for the tumours occurrence in both sexes, with an increased incidence at 1000 ppm in males more than in females and the absence of dose response at lower doses in both sexes.
Adrenal's medullary tumours -3 tumours in males at 100 ppm, which showed distant metastases to lungs and/or liver
Brain: Intracerebral astrocytomas and ependymoma

Liver: A total of 16 primary hepatocellular tumours were observed, representing a population frequency of 3%, which is compatible with the frequency of spontaneous hepatocellular neoplasms. Although the incidence of hepatocellular neoplasm was found to be greater in females at 1000 ppm, the difference was not statistically significant
Mammary glands: Fibroadenomas were extremely frequent among all female groups (range: 38–52 %); adenocarcinomas ranged 4 to 9% in all groups
Reticulum cell sarcoma was the most frequent malignant neoplasm showing multiple organ involvement in ten rats (2.3%)
Pancreas: Islet cell adenomas were more common in males and noted with similar frequency among all male groups (range 7 to 11%).
Parathyroid: Solitary distinct nodules within the parathyroids were all classified as adenomas, as no distinction was possible between "nodular" hyperplasia and adenoma; however taking in consideration the incidence, the effect was not attributed to administration.
Adenomas of the pituitary were extremely frequent in all female groups (range 78 to 89%) and not uncommon among males (range 36 – 46%).
Thyroid: parafollicular cell hyperplasia and tumours in males and females
No HCD were available for the performing laboratory. Incidences of astrocytoma were available, but information on severity was missing.
The incidence range observed among the studies varied from 0/100 to 3/60 in male (5%) and 0/100 to 2/50 in female rats, and the incidences observed were within the ranges. The incidences for males were 1.9% (low dose), 1.8% (mid dose), 3.7% (high dose), and zero for females of all groups. For ependymoma, the article did not show a range, but we only have one case in the high dose females, 1.8%, so it is less concerned. A female euthanized in extremis (D80), gross observations included a firm lobulated mass on thymus, enlarged spleen and mediastinal and renal lymph nodes, and depressed areas in the kidneys; the cause of death of this animal was malignant lymphoma.
Astrocytoma was considered the most critical effect. No HCD were available for the performing laboratory. Incidences of astrocytoma were available, but information on severity was missing. According to the literature, astrocytoma is a rare tumour in Sprague Dawley rats. All the experts of PRAS 182 meetings, September 2018, agreed to lower the carcinogenic NOAEL at 100 ppm (4.42/5.64 mg/kg bw per day for male and female, respectively) considering the increased incidence at 1000 ppm in males and the absence of dose response at lower doses.
The mechanistic evidence on the human relevance of the bladder tumours in rats is inconclusive.

Mice:
Urinary bladder tumours (transitional bladder tumour) were observed in males at 3000 ppm (3 tumours out of 60 animals); this dose exceed the MTD. Such tumours were not observed in females or with the lower dose levels. Two of these tumours were detected only at microscopic examination and were at a very early stage, and one was observed at gross examination at necropsy.
Respective latency time were 45, 54, and for the tumour observed at necropsy, 68 weeks from the start of the treatment.
(b) potential progression of lesions to malignancy; multi-site response
Benalaxyl presented a large occurrence of tumours and a multi-site response as it is seen in Table Selected neoplastic findings at terminal sacrifice (No. affected animals) in section 10.9.1 of this report.

Rats:
- Lifetime oral dosing studies in rats: combined oncogenicity and chronic toxicity of GALBEN technical (M 9834)(1983)
This is the study with specific results for carcinogenicity. Multiple tumours (or lesion) on organs were observed, such us: liver, brain, mammary glands, pancreas, and pituitary gland.

- An Oral (Gavage) Prenatal Developmental Toxicity Study of Benalaxyl in Rats (2015)
Enlarged liver, correlated with hepatocellular hypertrophy, in a single female at 450/300 mg/kg/day also a higher liver weights (17.8% and 17.2%, absolute and relative to net body weight, respectively) compared to

the control group; Centrilobular to midzonal hepatocellular hypertrophy, minimal in degree, was noted in 12 of 25 females compared to none in the control, but no degenerative changes were noted.

Follicular cell hypertrophy on the thyroid was observed in the 5-week and 13-week studies in the rat, mainly at high dose.

Dog:
Study of the toxicity of repeated oral administration of product M 9834 (GALBEN) to Beagle dogs at the dosage levels of 10, 200 and 800 ppm for 52 weeks. (1982)
A rather spontaneous incidence of seminiferous tubules atrophy in Beagle dogs is reported in the literature, and the findings of the one-year study are considered likely secondary to general toxicity, more evident in 2/6 dogs.

Mice:
3 lesions in male mice treated at the highest dose level of 3000 ppm were originally diagnosed as “transitional cell tumours of the urinary bladder”, a type of tumours considered by the study pathologist rather uncommon. However, pathology peer reviews of the original slides determined these to be “submucosal mesenchymal tumour” of the mouse urinary bladder, a lesion non-epithelial in origin, unique to the mouse urinary bladder, and with no counterpart in any other species including humans and therefore of no relevance for risk assessment in humans.

Rats:
At the scheduled necropsy on GD20, enlarged liver, correlated with hepatocellular hypertrophy, was observed in a single female at 450/300 mg/kg/day also a higher liver weights (17.8% and 17.2%, absolute and relative to net body weight, respectively) were recorded in the 450/300 mg/kg/day group females compared to the control group; (the differences were significant by correlation with microscopically observations). Centrilobular to midzonal hepatocellular hypertrophy, minimal in degree, was noted in 12 of 25 females compared to none in the control, but no degenerative changes were noted.
Follicular cell hypertrophy on the thyroid was observed in the 5-week and 13-week studies in the rat, mainly at high dose.
In the 5-week rat study, the NOAEL is 100 mg/kg bw per day on the basis of the significant increase in liver weight; at 800/4000 mg/kg/day were seen reversible changes in haematological and clinical chemistry parameters, and a significant increase in liver weight (+51% in males, and +80% in females, also reversible).

A general toxicity phase animals primarily involved the liver and thyroid glands and included higher mean gamma glutamyltransferase values, gross observation of a swollen liver (1 female), and histologic evidence of hepatocellular hypertrophy and thyroid follicular cell hypertrophy at 10000 ppm; hepatocellular hypertrophy in females and higher mean liver weights were also recorded at ≥1000 ppm. Additional test substance-related findings included serum chemistry alterations of higher mean cholesterol in the females at 10000 ppm; and higher mean total protein, albumin, and cholesterol values in the males at ≥1000 ppm.

Summary of treatment-related microscopic findings at 0, 100, 1000 and 10000 ppm in rats revealed presence of hepatocellular hypertrophy which was characterized by expansion of the hepatocellular cytoplasm associated with a distribution of the changes, predominantly centrilobular and extended to a more generalised pattern with increased severity of the change. Follicular cell hypertrophy in the thyroid glands was characterized by expansion of the cytoplasm of the thyroid follicular cells and a decrease in the amount of follicular colloid.
The same dose level 1000 ppm is considered the dose-response for the adverse-effects of benalaxyl precluding carcinogenicity.

Dog:
In the dogs, effects on prostate weight, atrophy of seminiferous tubules and increase in relative testes weights. Atrophy of the seminiferous tubules was observed in the 12-month dog study at the highest dietary concentration of 800 ppm (equivalent to 25 mg/kg bw/d). Findings were seen with a low incidence (in two of

six dogs), are known to occur spontaneously in untreated animals, and are shown to be associated with body weight effects in the more severely affected of the two dogs.
At 1000 ppm, a relative liver weights were increased by less than 15% in both males and females, and liver histopathology at this dose level only showed minimal hepatocellular hypertrophy in 3 females only. Thyroid follicular hypertrophy was also observed at the same incidence and with the same degree as controls in males, and at the same degree than controls but in only one more female at 1000 ppm, as was observed at the 10-fold lower dose level. Albumin and total protein levels, were significant statistic higher than control, in males only. However, in the 90-d toxicity study, there was no dose-relationship on prostate weights, and no histologic correlate and therefore the findings were considered non-treatment-related. A rather spontaneous incidence of seminiferous tubules atrophy in Beagle dogs is reported in the literature, and the findings of the one-year study are considered likely secondary to general toxicity, more evident in 2/6 dogs.

Mice:
The 13-week mouse study was considered as a range-finding study, considering that an increase liver weight at 1000 ppm was equivocal and not associated with histopathological changes.
In the mouse study, 3 lesions in male mice treated at the highest dose level of 3000 ppm were originally diagnosed as “transitional cell tumour of the urinary bladder”, a type of tumours considered by the study pathologist rather uncommon. However, pathology peer reviews of the original slides determined these to be “submucosal mesenchymal tumour” of the mouse urinary bladder, a lesion non-epithelial in origin, unique to the mouse urinary bladder, and with no counterpart in any other species including humans and therefore of no relevance for risk assessment in humans.

Support from Conclusion of ED assessment

Thyroid-modality.
The adversity is related only with the target organ, liver, respectively.
Without adversity it is not fulfilled criteria of ED Guidance.
The pituitary effect appears as a great intracranial pressing and haemoragies produced by astrocytomas; unconclusive data.
There are missing a properly study *in vitro* performed on three species (for a clear adsorption, distribution, metabolism, excretion properties - ADME of benalaxyl).

EATS-modalities

There are no available studies investigating EATS-mediated endocrine activity (*in vivo* and *in vitro* mechanistic data and ToxCAST data were considered inconclusive).
.
A definition of MoA as a most biological plausible link between adverse effects and/or endocrine effects could not be identified for ED properties of benalaxyl.

HCD (historical control data)
The incidence of malignance was considered the most critical effect. No HCD were available for the performing laboratory. Incidences of astrocytoma were available, but information on severity was missing. In such cases the CLP guidance suggests a comparison with the historical control data As discussed above the observed incidence in malignancy is just a fact without a presence of performing laboratory historical data. Also, it is unclear whether the stated HC data for the Dossier studies include the results of the different periods of time. Therefore, comparison with the historical control is not considered conclusive.
The data set provided from the Applicant Dossier, are some older historical controls (from 1977 to 1979) which are not reliable on, due to the lack of information about the protocol/techniques of preservation/microscopic examination as well as time of sacrifice of surviving animals. The large frequence and distribution in all mice groups from the studies, higher incidence in males than in female and the high mortality concluded a treatment related.

HCD were collected from 1977 - 1981 with not relevant information (from 1977 to 1979), due to the lack of information about the protocol/techniques of preservation/microscopic examination as well as time of sacrifice of surviving animals.

Therefore, classification in Carc. Cat. 2 is proposed based on a treatment related increase in mammary a heart weight changes together with changes in LDH and K; () is 100 ppm (), based on the occurrence of astrocytomas (a rare tumour) and the dissemination of amyloidosis in various organs and tissues, noted three lesions as a submucosal mesenchymal tumours of the mouse urinary bladder. Tumours occurring only at excessive doses associated with severe toxicity generally have a more doubtful potential for carcinogenicity in humans. If a test compound is only found to be carcinogenic at the highest dose(s) used in a lifetime bioassay, and the characteristics associated with doses exceeding the MTD as outlined above are present, this could be an indication of a confounding effect of excessive toxicity. This may support a classification of the test compound in Category 2.
Life-time exposure to benalaxyl resulted in an increased frequency of thyroid adenomas in rats and hepatocellular adenomas in mice. Human relevance cannot be excluded; however, the tumour types are mainly benign. MTD seems to have been met in the rat study but not in the mouse study. Overall, data is considered as "limited" evidence of carcinogenicity and classification in Carc. 2 H351 is proposed for benalaxyl.

This proposed classification for benalaxyl is reported in an updated RAR and in the EFSA Conclusion (EFSA Journal 2020;18(1):5985), including the results of the experts'meeting on ED potential of benalaxyl.

10.9.3 Conclusion on classification and labelling for carcinogenicity

Based on the available data (long term studies) discussed above, increased incidence of enlarged of rare tumors (maligns and benigns) was observed in rats, mice, dogs and rabitts. Life-time exposure to benalaxyl resulted in an increased frequency of thyroid adenomas in rats and hepatocellular adenomas in mice also human relevance cannot be excluded. However, the multi - targeted organs and various tumour types are considered sufficient evidence to classify benalaxyl as Category 2

Benalaxyl should be classified Carc. 2; H351 - "Suspected of causing cancer".

RAC evaluation of carcinogenicity

Summary of the Dossier Submitter's proposal

The carcinogenic potential of benalaxyl has been investigated in a 2-year study in rats and an 18-month study in mice. The DS proposed classification in Category 2. The basis for this proposal is not very clear from the CLH-report, but astrocytomas in the rat study appear to have played a role in the reasoning.

Comments received during consultation

Comments were received from 1 MSCA and a manufacturer. The commenting MSCA supported Carc. 2 based on a low incidence of astrocytoma in the 2-year rat study, noting that this proposal is in line with the EFSA conclusion (EFSA, 2020).

The manufacturer proposed no classification, arguing that astrocytoma is a spontaneous brain tumour with a high incidence in Sprague-Dawley (SD) rats. They provided historical

control data (HCD) from publicly available sources (summarised under 'additional key elements') since reliable HCD from the performing laboratory is not available.

Additional key elements

Astrocytoma in male SD rats: published historical control data

Reliable HCD from the laboratory where the 2-year rat study was conducted cannot be obtained as the laboratory no longer exists. Therefore, industry collected HCD for SD rats from publicly available sources. The in-life phase of the 2-year rat study with benalaxyl started in December 1980. Crl:CD(SD) rats for the study were obtained from Charles River Breeding Laboratories, Wilmington, US.

Published historical control data for astrocytoma in male Crl:CD(SD) rats

Reference	**Strain; supplier** **Testing facility** **No. of groups; date** **Study duration; no. of male animals per group**	**HCD for malignant astrocytoma in the brain (males)**
Giknis and Clifford (2004)	Crl:CD(SD); Charles River Laboratories 8 facilities 30 studies; 1992-2002 2 years; 50-180/group	Mean: 1.2% Range: 0-4.3% Found in 13/30 groups
Nagatani *et al.* (2013)	Crl:CD(SD), Charles River Laboratories Japan BOZO Research Center, Japan 28 groups; 1996-2009 Duration not specified; 50-75/group	Mean: 2.0% Range: 0-6.7% (max. incidence 4/60) Found in 18/28 groups
Bertrand *et al.* (2014)	Crl:CD(SD); Charles River UK Charles River Edinburgh, UK 4 studies; 2002-2013 2 years; at least 50/group	Mean: 1.5%
Baldrick (2005)	Crl:CDBR; Charles River UK Covance Laboratories, UK 13 studies, 2 control groups per study; 1991-2002 2 years; 50-65/group	Mean: 1.6% Range: 0-5.0%
Gopinath (1986)	Crl:CD(SD); Charles River Huntingdon Research Centre, UK 42 studies; 'over the past 5 years' Duration not specified	Mean: 1.1% Range: 0-5.0% (max. incidence 3/60)

None of this historical control information is directly relevant because the data come from other laboratories and were conducted beyond the 5-year time window, except for

Gopinath (1986). On the other hand, the supplier was Charles River in all cases (although from different locations) and the data are quite consistent. Thus, they can be taken into account to a limited extent in the assessment of carcinogenicity.

Assessment and comparison with the classification criteria

As the presentation of the data in the CLH-report is not very clear, the RAC analysis is primarily based on the full study reports to the two carcinogenicity studies and on the RAR (draft Renewal Assessment Report, 2018).

2-year dietary study in rats (1985; 5.5/01)

The rats (Crl:CD(SD), 65/sex/group) were administered benalaxyl at dietary levels of 0, 4, 100 and 1000 ppm (top dose equivalent to 44/56 mg/kg bw/d m/f). 10 animals per sex and group were sacrificed after 1 year.

The treatment had no effect on survival, body weight, clinical signs, haematology, clinical chemistry parameters or organ weights. As to histopathology, the study authors concluded that there was no evidence of a treatment-related non-neoplastic or neoplastic effects. The brain tumour incidence is shown in the table below.

Brain tumours in the 2-year rat study				
Dose (ppm)	**0**	**4**	**100**	**1000**
Dose (mg/kg bw/d) m/f	0	0.18/0.23	4.4/5.6	44/56
No. examined, males	54	52	55	54
Astrocytoma, males; (day of death)	0	1 (647)	1 (630)	2 (654, 582)
Ependymoma, males	0	0	0	0
No. examined, females	54	55	54	55
Astrocytoma, females	0	0	0	0
Ependymoma, females (day of death)	0	0	0	1 (500)

Malignant astrocytoma was observed in 0, 1, 1, and 2 males in the control, low, mid and high dose group respectively. Astrocytoma was considered to be the cause of death in all 4 affected males, the same applies to the single top dose female with ependymoma (sacrificed moribund on day 500). The time to death or unscheduled sacrifice due to moribund condition in top dose males with astrocytomas does not appear to be shortened compared to the lower dose groups.

HCD from the performing laboratory is not available. No astrocytoma was observed in control males. A single astrocytoma occurred in males at the low dose, a dose so low (0.2 mg/kg bw/d) that it could almost be considered a "second control". In this regard, RAC notes that the dose spacing in this study is too wide (10 to 25-fold) and does not follow OECD TG 453.

The published HCD for male Crl:CD(SD) rats provided by industry, although of limited

relevance, indicate a mean background incidence of 1-2% and a maximum incidence of about 5% (see 'additional key elements'). Thus, 1 astrocytoma per group is well within the spontaneous background incidence. The incidence of 2 at the top dose may slightly exceed the normal background, but the increase is not statistically significant on a pairwise comparison, the dose-response relationship is not particularly strong, and the incidence lies within the broader (although less relevant) HCD range. RAC concludes that there is insufficient evidence of treatment-related increase in brain tumours in rats.

The rationale for top dose selection is not provided in the study report. RAC notes that the top dose of 1000 ppm did not cause general toxicity. The main effects at a 10-fold higher dose of 10000 ppm in 90-day rat studies (5.3.2/02, /03) were slightly reduced body weight (<10%), increased cholesterol (by about 50%), increased liver weight (by ca. 40-55%), hepatocellular hypertrophy and steatosis, and thyroid follicular cell hypertrophy. This information indicates that a dose higher than 1000 ppm could have been tested without inducing excessive toxicity in a long-term rat study. Consequently, the available information on carcinogenic potential in rats is considered inconclusive due to low dosing.

18-month dietary study in mice (1985; 5.5/02)

Swiss mice (60/sex/group) were administered benalaxyl at dietary levels of 0, 250, 1000 and 3000 ppm (top dose equivalent to 559/522 mg/kg bw/d m/f).

The survival of males was reduced at the mid- and high-dose (statistically significant in a trend test). Female survival was not affected. There was no remarkable effect on body weight, clinical signs, haematology or clinical chemistry parameters. An increase in liver weight was detected in top dose females (relative by 27%). The treated males showed a higher incidence of amyloidosis (spleen, kidneys) and nephritis in the histopathological examination. A correlation between mortality rate and frequency of amyloidosis appears to exist in males. The top dose selection was based on a 90-day range-finding study where 5000 ppm (803/908 mg/kg bw/d m/f) caused a liver weight increase in both sexes (relative by 33%/53% m/f).

The only potentially treatment-related tumour in this study was urinary bladder proliferative lesion in 3 top dose males. These lesions were initially identified as transitional cell carcinomas by the study pathologist, but later, on a review by an external pathology working group (2001; 5.5/05), they were re-classified as submucosal mesenchymal tumours. This revised diagnosis was accepted in the EFSA assessment (EFSA, 2020). All three lesions were found in animals found dead during the study; two were detected on microscopic examination only (time of death weeks 46 and 55) and one also on gross pathology (week 68; a 4-mm nodule). Relevant HCD is not available. The background incidence of this lesion is generally difficult to determine due to a wide variety of diagnostic terms used in the past (including 'vegetative change' or 'decidual-like reaction') and differences in trimming procedure (often found in the trigone on microscopic examination, may be missed on cross-sectioning).

The nature and neoplastic potential of these mesenchymal proliferative lesions are controversial (Frazier *et al.*, 2012). They may be considered benign tumours and human relevance cannot be excluded (cf. the analysis in the RAC opinion on bifenthrin, 2011). Nevertheless, the incidence of these tumours in the mouse study with benalaxyl was low and occurred in presence of general toxicity. Therefore, this finding is not considered to

warrant classification.

Histopathological findings and survival in the 18-month mouse study				
Dose (ppm)	**0**	**250**	**1000**	**3000**
Dose (mg/kg bw/d) m/f	0	45/43	181/174	559/522
Survival (%), males	62	58	32	25
Survival (%), females	72	83	85	70
No. examined, males	60	60	60	60
Kidney amyloidosis, males	10	12	28**	21**
Spleen amyloidosis, males	9	22**	29**	26**
Amyloidosis in any organ, males	14	24	37**	37**
Kidney abscesses and nephritis, males	13	15	14	24*
Urinary bladder submucosal mesenchymal tumour, males	0	0	0	3

Statistically significant difference from control: *, $p \leq 0.05$; **, $p \leq 0.01$

Conclusion

The available studies did not show any neoplastic effects warranting classification. However, the carcinogenicity potential of benalaxyl has not been fully investigated due to low dosing in the rat carcinogenicity study. Consequently, RAC concludes that **no classification** for carcinogenicity is warranted based on **inconclusive data**.

10.10 Reproductive toxicity

Not evaluated in this report.

10.11 Specific target organ toxicity-single exposure

The most relevant information for a proposed classification of benalaxyl as STOT SE is covered by acute toxicity studies in form terms of a clinical observations, also macro- and microscopic pathological examinations that can reveal the hazards that may not be life-threatening but could indicate a functional impairement.

Acute toxicity studies are included in Section 10.1.In this section, they are mentioned those specific results for nervous system from two acute neurotoxicity studies which were carried out during the period of renewal of benalaxyl as active substance.

10.11.1 Short summary and overall relevance of the provided information on specific target organ toxicity – single exposure

The intention for a proposal of classification of benalaxyl as specific target organ toxicity –single exposure was concluded in the Report of Risk Assessmen sent to EFSA based on the evidences derived from additional studies on acute neurotoxicity performed. The study which promoted this proposal was an old acute oral toxicity study on mice (1980), having as results a possible neurological signs that states the following observation: *"*death occurred in the first 24 hours following treatment by gavage and was preceded by loss of the sense of equilibrium, uncoordinated movements, and asthenia*"*.

The proposal considered available based on the weight of evidence revealed in acute oral toxicity studies and acute neurotoxicity studies.

Based on the weight of evidence, benalaxyl has not a significant neurotoxic potential and all observed acute effects (i.e. tremor, clonic convulsions) were considered as a results of the high single dose from acute oral toxic study. This highest dose was used as lethal doses also in the acute neurotoxicity study. Therefore, no classification for specific target organ toxicity, by a single exposure for benalaxyl was proposed.
In this report, the proposal of classification as STOT SE; H371 is justified by the results from those two acute neurotoxicity studies and taking into account specific reaction for a nervous system effects available.

Table 21: Summary table of animal studies on STOT SE

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Relevant information about the study (as applicable)**	**Results**	**Reference**
An Oral (Gavage) Dose Range-Finding Acute Neurotoxicity Study of Benalaxyl in Rats Oral (gavage) Rats Crl:CD(SD) 3/sex/group Remarks: All criteria for a valid study were met Guidelines: N/A: Dose range-finding study GLP/QA: Yes/Yes Acceptable	Benalaxyl tech. Lot/batch: PL 13-0055 Purity: 98.4%. Vehicle: 0.5% (w/v) methylcellulose in deionized water A single dose to 4 G (G2-G5) Range-finding study previous a neurobehavioral further study. <u>Initial dose levels</u>: 200, 600, and 2000 mg/kg bw (Groups 2, 3, and 4, respectively). Group 5 - 400 mg/kg bw (additional dose level) Group 1 – control group, received the vehicle in the same condition of study. Group 6 -	Body weight: no statistically significant differences were noted. Clinical signs: 1 male at 600 mg/kg bw per day/ Group 3 - 2 and/or 4 hours following dose administration: - Slightly soiled fur appearance - drooping eyelids - ↓respiratory rate - rales - slightly to moderately impaired mobility - dragging body - low arousal were noted 1 male - 400 and 600 mg/kg bw (G3 and G4) and 1 to 2 females in the 200 and 400 (G2 and G3) at approx... 2, 4, or 8 hrs following dose administration: - red deposits around the eyes, nose, and/or mouth Group 6: additional animals at 2000 mg/kg bw: 2 m + 2 f Similar findings to those noted at 2000 mg/kg bw in the main phase were confirmed in an additional phase, where both females administered the test substance at 2000 mg/kg bw had clonic convulsions at approximately 2 hours following dose administration. The time of peak effect was considered 2 hours following dose administration based on the severity and nature of the findings at this time (lightly soiled fur appearance, drooping	Group 3: 400 mg/kg bw/day is the dose level without death and specific sign for nervous system as a target.	RAR-08_Vol-3 CA_B.6.7.1.1 Reference number: CA 5.7.1/01 Anonymous (2014c)

	additional animals at 2000 mg/kg bw : 2 M + 2 F (to confirm the previous incidence of convulsions at 2000 mg/kg bw)	eyelids, decreased respiratory rate, impaired mobility, dragging body, clonic convulsions, low arousal, and circling). Benalaxyl could be classified with H371 STOT SE Category 2, according with criteria of the CLP Regulation based on the effects at 400 mg/kg bw/day.		
An Oral (Gavage) Acute Neurotoxicity Study of Benalaxyl in Rats Oral (gavage) Rats Sprague-Dawley (Crl:CD (SD)) OECD TG 424 and OPPTS 870.6200 Criteria are met Statistics was performed GLP/QA: Yes/Yes Acceptable	Benalaxyl tech. PL13-0055 Purity: 98.4% Vehicle: 0.5% (w/v) carboxymethyl cellulose aqueous solution Phase 1 3G (G2 - G4) of 10 m/10f at 200, 400, and 1000 mg/kg bw Phase 2 3G (G1-as a single dose to G3) of 10f at 0, 50, and 100 mg/kg bw.	Acute neurotoxicity screening battery (FOB): functional observational battery, locomotor activity, and neuropathological assessments. Mortality and clinical signs: Phase 1 At 200 mg/kg bw and above – a single dose -1000 mg/kg bw (m) and 200, 400, and 1000 mg/kg bw (f), ≤ 4.5 hrs following dose administration; The majority of animals that were found dead or euthanized in extremis were noted with clonic convulsions the same with FOB findings at the time of peak effect on study day 0, only. 1000 mg/kg bw one male was found dead approx. 2 hrs following dose administration (clonic convulsions during the continuous 2-hour post-dosing observations) and one male more – dead after approx. 4.5 hrs following dose administration (no clinical observations) One female in 400 mg/kg bw group was euthanized *in extremis* approximately 3 hrs following dose administration after being noted with increased respiration; this female was also noted with: - Splayed hind limbs and immobility by the clinical veterinarian. Two of the aforementioned females (that in the 400 mg/kg bw group and one in the 1000 mg/kg bw group) were euthanized or found dead prior to completion of the motor activity testing. In addition to the clonic convulsions noted above, one female in the 1000 mg/kg bw group was also noted with clonic convulsions approx... 3 hrs following dose administration on day 0; however, this female survived to the scheduled euthanasia. Control group was survived Phase 2 females in the 100 mg/kg bw G3: repetitive movement of the mouth and jaws which correlated with similar home cage FOB findings at the time of peak effect on study day 0 FOB findings were recorded for all animals prior to the initiation of dose administration (pre-test), at the time of peak effect (approximately 2 hours post-dosing) on day	400 and 1000 mg/kg bw/day (m): Motor activity assessments: ↑ mean total motor activity values 200, 400, and 1000 mg/kg bw/day (f) at the time of peak effect: Changes in the pattern of habituation in these groups. similar to control group (D0) ↑mean total motor activity counts ↑in non-ambulatory movements, possibly due to additional episodes of clonic activity during the motor activity session. No effects were noted during Phase 2. 400 mg/kg bw/day is the dose level which reveals the signs specific for a classification as STOT SE 2	RAR-08_Vol-3 CA_ B.6.7.1.2 Reference number: CA 5.7.1/01 Anonymous (2014a)

		0, and on days 7 and 14. In the 100 mg/kg bw group, 3 females were noted with repetitive movement of the mouth and jaws and 2 females were noted with salivation on the day of dose administration during the 2-hour continuous post-dosing observations; these findings were considered test substance-related. Control group was survived FOB and motor activity assessment: females in the 200, 400, and 1000 mg/kg bw ↓ rearing counts groups at peak time, compared to the control group. Phase 2, 100 mg/kg bw (f): repetitive movement of mouth and jaws Motor activity assessments ↑motor activity values 400 and 1000 mg/kg bw/day (m) 200, 400, and 1000 mg/kg bw/day (f) at peak time: Changes in the pattern of habituation in these groups.		

10.11.1 Short summary and overall relevance of the provided information on specific target organ toxicity – single exposure

In the second acute neurotoxicity study, *An Oral (Gavage) Acute Neurotoxicity Study of Benalaxyl in Rats* (Anonimous, 2016a based on dose-respons observed at the dose levels administrated on rats in the first dose-range finding - DRF study a maximum dose level of 1000 mg/kg bw/d was selected in view of avoiding the rate of mortality of rats and allowing a properly FOB and motor activity assessments.
Benalaxyl as active substance was administered as a single dose to 3 groups (Groups 2 - 4) of 10 male and 10 female rats at dose levels of 200, 400, and 1000 mg/kg bw (Phase 1).
Due to mortality noted at the lowest dose level of 200 mg/kg and above in the females during the initial dosing phase, an additional phase (Phase 2) was added to determine a no-observed-adverse-effect level (NOAEL) for female neurotoxicity.
In Phase 2, benalaxyl or the vehicle was administered orally by gavage as a single dose to 3 groups (Groups 1-3) of 10 female rats at dose levels of 0, 50, and 100 mg/kg.

Unfortunately, from Phase 1 of the acute neurotoxicity study, on a single dose of benalaxyl resulted in mortality and/or moribundity for males at 1000 mg/kg bw/d and for females at 200, 400, and 1000 mg/kg bw/d within approximately 4.5 hours following dose administration; the majority of animals that were found dead or euthanized in extremis were noted with clonic convulsions.
In addition, test substance-related FOB findings (lower mean rearing counts) for females at 200, 400, and 1000 mg/kg bw/d and/or higher mean total motor activity values for males at 400 and 1000 mg/kg bw /d at the time of peak effect on day 0 and dose level of 200 mg/kg bw was considered to be the no-observed-adverse-effect level (NOAEL) for acute neurotoxicity in male rats.

For Phase 2, females in the 100 mg/kg bw /group were noted with clinical findings of repetitive movement of the mouth and jaws which correlated with similar home cage FOB findings at the time of peak effect on study day 0 only. No test substance-related effects were noted for females in the 50 mg/kg bw/ group. Therefore, the NOAEL for acute neurotoxicity in female rats was considered to be 50 mg/kg bw/group.

Concentration of 400 mg/kg bw is relevant for nervous system effects because of females which were alive before 4 hours as at 200 mg/kg bw giving the possibility to mention an increased respiration, (+ splayed hindlimbs and immobility) which were noted after 2 h post dose observation. Time of death and observation time(s) has contradictory, as seen in the table below:

Motor activity assessments revealed higher mean total motor activity values for males at 400 and 1000 mg/kg bw and for females at 200, 400, and 1000 mg/kg bw at the time of peak effect on the day of dosing, indicating a change in the pattern of habituation in these groups.
Significantly lower mean rearing counts were noted for females in the 200, 400, and 1000 mg/kg bw/ groups compared to the control group at the time of peak effect on the day of dosing. At 400 mg/kg bw in mail rats was observed a higher mobility during testing period of 14 days than at 200 mg/kg bw.

Results of necropsy were essential to consider mortality as a reason for an acute toxicity proposed classification but for stated a STOT SE as a classification the key is proving the reasonable effects of benalaxyl without mortality at the dose levels of interest and histopathological observations
Brain weights and measurements were unaffected by administration of benalaxyl at any dose level. No test substance-related microscopic lesions were observed in any of the central or peripheral nervous system tissues examined from 5 animals/sex in the control and 1000 mg/kg bw groups.

Minimal axonal degeneration was observed sporadically in both control and 1000 mg/kg group animals in the sciatic nerve, peroneal nerve, tibial nerve, lumbar dorsal root fibres, lumbar ventral root fibres, and lumbar spinal nerve.

10.11.2 Comparison with the CLP criteria

Specific target organ toxicity (single exposure) is defined as specific, non-lethal target organ toxicity arising from a single exposure to a substance or mixture. Relevant information for STOT SE is covered by acute toxicity studies in form of clinical observations, and macroscopic and microscopic pathological examination that can reveal hazards that may not be life-threatening but could indicate functional impairment. Acute toxicity studies are included in section 10.1.

STOT-SE should be considered where there is clear evidence of toxicity to a specific organ, when it is observed in the absence of a classification for lethality (see Section 3.8 of the CLP Guidance).

STOT SE 1 and 2

STOT-SE Category 1 and 2 are assigned on the basis of findings of ‘significant’ or ‘severe’ toxicity.In this context, ‘significant’ means changes which clearly indicate functional disturbance or morphological changes which are toxicologically relevant. ‘Severe’ effects are generally more profound or seriouis than ‘significant’ effects and are of considerably adverse nature with significantimpact on health.

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).

According to the guidance value ranges for single-dose exposures laid down in the CLP criteria (Annex I 3.8.2.1.9.3), benalaxyl could be classified as category 1, if::

a. reliable and good quality evidence from human cases or epidemiological studies; or

b. observations from appropriate studies in experimental animals in which significant and/or severe toxic effects of relevance to human health were produced at generally low exposure concentrations. Guidance dose/concentration values are provided below (see 3.8.2.1.9) to be used as part of weight-of-evidence evaluation.’

Benalaxyl is letal for rats at DL_{50} = 2000 mg/kg bw/day in acute oral study, a single dose. There are not enough dose levels and a sufficient number of species of animals to conduct acute studies on benalaxyl to those sever relevant effects on animals, which could be appropriate for be extrapolated to human.

Criteria for Cat 1 are not fulfilled for classification of benalaxyl as STOT SE 1.

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 2 are defined (Section 3.8.2.1.9.3 of Annex I of the CLP Regulation) as 2000 ≥ C > 300 mg/kg bw (oral route, rat); 2000 ≥ C > 1000mg/kg bw (dermal route, rat) and 5.0 ≥ C > 1.0 mg/L (inhalation route, rat, dust/mist/fume).

Regulation EC No 1272/2008 (CLP), section 3.8.1 states that:

"Acute toxicity refers to lethality and STOT-SE to non-lethal effects. However, care should be taken not to assign both classes for the same toxic effect, essentially giving a "double classification", even where the criteria for both classes are fulfilled. In such case the most appropriate class should be assigned."

It should be noted that it is already proposed to classify benalaxyl for acute toxicity Cat. 4 on the basis of the LD50 studies with cut-off values of 300-2000 mg/kg bw.

STOT SE 2 would thus be a more sensitive endpoint since neurotoxicity findings was observed at dose levels below 1000 mg/kg bw/day, with a NOAEL of 200 mg/kg bw/d (m) and 50 (f) mg/kg bw/d. Although clinical effects observed after short term exposure were without histopathological correlations and a high mortality in the acute toxicity study, presumably caused by the neurotoxic effects. According to the CLP criteria mortalities observed within 72 hours after the first treatment can be considered an acute effect.

Based on the available data it was demonstrated that the neurotoxic effects can lead to mortality at dose levels that are below the classification criteria for acute toxicity Cat. 4. (300-2000 mg/kg bw). Thus, these effects are considered relevant for classification.

As neurotoxic effects consistently occur directly after dosing at dose levels below the limit values and also below the cut-off value for acute tox 4, it is proposed to classify benalaxyl for STOT SE (nervous system) Cat 2, H371.

STOT SE 3

STOT SE3 includes narcotic effects and respiratory tract irritation. These are target organ effects for which a substance does not meet the criteria to be classified in Categories 1 or 2.

Narcotic effects were not observed in acute toxicity studies related benalaxyl. An inhalatory study was performed.

10.11.3 Conclusion on classification and labelling for STOT SE

Benalaxyl should be classified. STOT SE 2; H371 - "nervous system"

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

Summary of the Dossier Submitter's proposal

The DS proposed classification with STOT SE 2; H371 (nervous system) based on clinical sings of neurotoxicity observed in the main acute neurotoxicity study in rats (5.7.1/02), occurring not only below the proposed ATE for acute oral toxicity (2000 mg/kg bw), but also below the cut-off for classification as Acute Tox. 4 (300 mg/kg bw).

Comments received during consultation

Comments were received from 1 MSCA and a manufacturer. The manufacturer was of the view that the mortality in the acute neurotoxicity study may not have been due to neurotoxicity. Further, they mentioned inconsistency with other studies and lack of neurotoxicity in the subchronic neurotoxicity study. Still, they supported the DS's proposal.

The MSCA, who also commented on acute toxicity (see above), questioned whether the mortality in the acute neurotoxicity studies was a consequence of clonic convulsions or simply concomitant with the convulsions. They pointed out that the convulsions should not be used for classification in two hazard classes (acute toxicity and STOT SE).

Assessment and comparison with the classification criteria

The following table provides a detailed overview of findings related to neurotoxicity in the acute neurotoxicity studies (5.7.1/01, 02) below the proposed oral ATE of 1000 mg/kg bw. Description of effects at ≥ 1000 mg/kg bw can be found in the acute toxicity section.

Neurotoxicity-related findings below 1000 mg/kg bw in the acute neurotoxicity studies			
Dose (mg/kg bw); study (dose range-finding or main)	**No. of animals per group**	**Mortality; clinical sings in animals found dead or sacrificed *in extremis***	**Findings potentially related to neurotoxicity in survivors**
600 (drf)	3 m, 3 f	–	1 m clonic convulsions, decreased respiratory rate, gasping, drooping eyelids, slightly impaired mobility, dragging body, low arousal (2-4 h post-dosing)
400 (drf)	3 m, 3 f	–	–
400 (main)	10 m, 10 f	1 f: increased respiration, splayed hindlimbs and immobility at 2 h, euthanized at 3 h	Reduced no. of rearing counts (f 3.2 vs 9.3), increased motor activity (m, f) (day 0)
200 (drf)	3 m, 3 f	–	–
200 (main)	10 m, 10 f	1 f: clonic convulsions and vocalization at 2 h, found dead at 4 h	Reduced no. of rearing counts (f 3.9 vs 9.3), increased motor activity (f) (day 0)
100 (main)	10 f	–	3 f repetitive movement of the mouth and jaws, 2 f salivation (2 h post-dosing)
50 (main)	10 f	–	–

m = male, f = female

One male (out of 3) showed clonic convulsions and other clinical signs of toxicity at 600 mg/kg bw in the range-finding study. This animal survived to the scheduled sacrifice. However, 600 mg/kg is relatively close to the proposed acute oral ATE of 1000 mg/kg bw (causing 20% mortality in males), so the clinical signs at 600 mg/kg bw are considered to be of limited relevance for a STOT SE classification.

One female (out of 13) at 400 mg/kg bw was killed *in extremis* due to increased respiration, splayed hindlimbs and immobility. Death, preceded by convulsions, also occurred in 1 female (out of 13) at 200 mg/kg bw. As both animals died, clinical signs in these two animals are considered to be covered by the acute toxicity classification.

In the rest of the animals no notable clinical signs of toxicity were observed at 200, 400 or 600 mg/kg bw.

Repetitive movement of the mouth and jaws, possibly representing small-scale convulsions, was reported in 3 out of 10 females at a non-lethal dose of 100 mg/kg bw. Repetitive movement of the mouth and jaws was also noted in one female with convulsions at 2000 mg/kg bw (range-finding study, additional phase) but not at other doses (200-1000 mg/kg bw). Although this finding might indicate neurotoxicity, the absence of this finding at 200 and 400 mg/kg bw makes the interpretation difficult.

Further, an increase in total motor activity in the latter part of the testing session was observed from 400 mg/kg bw in males and from 200 mg/kg bw in females. The increase was attributed to a change in the pattern of habituation. In addition, the number of rearing counts was considerably reduced in females from 200 mg/kg bw. The alterations in locomotor activity and rearing, although suggestive of neurotoxicity, are not considered sufficiently severe to trigger classification.

No evidence of neurotoxicity was observed in a 90-day dietary neurotoxicity study in rats (5.3.2/03) conducted by the same laboratory as the acute toxicity studies. The top dose in this study was 10,000 ppm (677/745 mg/kg bw/d m/f).

A gavage prenatal developmental toxicity (PNDT) study, in rats (2015; 5.6.2/02) of the same strain from the same laboratory and breeder (Charles River) but in a different location of the breeder, reported no clinical signs of toxicity at a dose of 300 mg/kg bw, just below that causing mortality after a single dose (450 mg/kg bw) in this repeated dose study.

Overall, the acute neurotoxicity studies provide some indications of neurotoxicity at doses below those causing mortality. However, these are not considered sufficiently consistent or adverse to warrant a STOT SE classification in addition to the proposed acute oral toxicity classification (Acute Tox. 4, ATE = 1000 mg/kg bw). Further, the available studies do not provide evidence of other specific target organ effects relevant for a STOT SE classification in categories 1, 2 or 3. Therefore, RAC considers that for STOT SE **no classification** is warranted.

10.12 Specific target organ toxicity-repeated exposure

Not evaluated in this report.

10.13 Aspiration hazard

Not evaluated in this report.

11 EVALUATION OF ENVIRONMENTAL HAZARDS

Benalaxyl is a fungicide active substance considered under Directive 91/414/EEC (subsequently Regulation 1107/2009) for representative use as a foliar spray. Available environmental fate and ecotoxicology studies have been considered and summarised in the Renewal Assessment Report, 2018 (RAR, Volume 3, Annex B8 and Annex B9) and the renewal of approval dossier.

11.1 Rapid degradability of organic substances

Benalaxyl is considered not readily biodegradable. Hydrolytic degradation of the active substance and metabolites > 10 %. Benalaxyl is stable to hydrolysis at pH 4 and 9; pH 9 (50ºC): DT_{50} = 55 days; pH 9 (70ºC): DT_{50} = 19 h.

Table 22: Summary of relevant information on rapid degradability

Method	Results	Remarks	Reference
Hydrolysis OECD guideline 111	Benalaxyl is stable to hydrolysis at pH 4 and 7. pH 9 (50ºC): DT50 = 55 days pH 9 (70ºC): DT50 = 19 h A DT_{50} of 157 days at 20°C was extrapolated from the results at the higher temperatures	The study is considered acceptable	RAR B.8.2.1.1 CA 7.2.1.1/01 Anonymous (1982)
Water-sediment degradation OECD guideline 309 ^{14}C-benalaxyl	0.3% ÷ 0.4% AR Benalaxyl does not undergo any significant biodegradation/ mineralisation in natural waters within the study period of 62 days.	GLP, Study acceptable	RAR B.8.2.2.2 CA 7.2.2.2/01 Anonymous (2015b)
Ready biodegradability OECD guideline 301 F	The degradation rate of benalaxyl based on the $ThOD_{NH4}$, was –2.1 and based on $ThOD_{NO3}$ the percentage of biodegradation of benalaxyl reached –2.0%. Results indicate Benalaxyl is "not readily biodegradable"	GLP, Study acceptable	RAR B.8.2.2.1 CA 7.2.2.1/01 Anonymous (1999)
Aerobic Transformation/ Degradation in Soil OECD guideline 307	^{14}C-benalaxyl degraded to 1.1% AR to 45.4% under aerobic conditions for 117 days NER: 22% - 50% after 117 d.	GLP, Study acceptable	RAR B.8.1.1.1.1 CA 7.1.1.1/03 Anonymous (2015a)

Aerobic mineralisation in surface water

Benalaxyl is stable to hydrolysis at pH 4 and 7. At pH 9 and 50ºC a DT50 of 55 days was calculated. Using the Arrhenius equation DT_{50} values at 20 and 25ºC at pH 9 were calculated as 157 days and 86 days, respectively. The main hydrolysis product was identified as benalaxyl acid (M9, DL-alanine, N-2,6-xylyl-N-phenylacetyl).

Benalaxyl is not easily photolysed under natural sunlight conditions during June – August at 45º 28' N, 3º 10' W coordinates since 60%AR is still present as benalaxyl after 64 days of exposure. At least 15 unidentified compounds were detected but none of them individually represented more than 5% of the initially applied radioactivity.

Anonymous, (2015b), Aerobic Mineralisation of ^{14}C-benalaxyl in Surface Water - Simulation Biodegradation Test, FMC Corporation Agricultural Solution, Harlan, GLP, OECD guideline 309, Commission Regulation (EU) No 283/2013

The degradation, transformation and mineralisation of ^{14}C-benalaxyl at two concentrations was studied in natural pond water according to OECD 309. Natural water (300ml), pH 8.18, was treated with ^{14}C-benalaxyl at 10.5 µg/l (nominal 10 µg/L) and 106.7µg/l (nominal 100 µg/L) and incubated in the dark at 23.4 ± 0.9°C for 62 days. Sterile controls (100µg/L nominal) and bio-controls (with benzoic acid at 10µg/L nominal) were also set up. Test vessels were connected to a flow-through system and continuously agitated and aerated with humidified air in the dark. Volatile compounds were trapped in ethylene glycol (only for ^{14}C-benalaxyl test vessels) and sodium hydroxide. Duplicate test samples were taken at day 0, 7, 14, 21, 28, 42 and 62. Radioactivity in water samples was determined by LSC. Samples were then partitioned in hexane and the organic phase analysed by HPLC and TLC. Mineralisation of benalaxyl was negligible accounting for only 0.3% to 0.4% AR at the low and high test concentration, respectively. Insignificant transformation/degradation of benalaxyl was observed throughout the study period and therefore no DT_{50}s/DT_{90}s could be derived. It can be concluded that benalaxyl does not undergo any significant biodegradation/mineralisation in natural waters within the study period of 62 days.

11.1.1 Ready biodegradability

Adequate data to assess the ready biodegradability of Benalaxyl were evaluated during the first approval of the active substance. A summary of the data evaluated is presented below.

Anonymous (1999), Ready Biodegradability of Benalaxyl techic in a manometric respiratory test. GLP, OECD guideline 301 F

The ready biodegradability of benalaxyl (96.68% radiochemical purity) was investigated in a Manometric Respiratory Test (Anonymous, 1999) over a period of 28 days at 22ºC in the dark with the a.s applied to activated sludge at a concentration of 30 mg suspended solids per litre. The biodegradation was followed by the oxygen uptake of the micro-organisms during the exposure period.

After 28 days of exposure the degradation rate of benalaxyl based on the ThODNH4, was –2.1. Based on ThODNO3 the percentage of biodegradation of benalaxyl reached –2.0% after 28 days. This values indicates that benalaxyl was not degraded by the activated sludge and can therefore be considered as “not readily biodegradable”.

11.1.2 BOD_5/COD

No data available.

11.1.3 Hydrolysis

Benalaxyl is stable to hydrolysis at pH 4 and 7 (Anonymous, 1982). At pH 9 and 50ºC a DT_{50} of 55 days was calculated. Using the Arrhenius equation DT_{50} values at 20 and 25ºC at pH 9 were calculated as 157 days and 86 days, respectively. The main hydrolysis product was identified as benalaxyl acid (M9, DL-alanine, N-2,6-xylyl-N-phenylacetyl).

Anonymus, 1982, GALBEN Hydrolysis, OECD guideline 111.The buffered aqueous solutions (0.05 M) of 1.5 to 15 mg/l benalaxyl (99.2% purity) were incubated in the dark at pH 4, 7 and 9 for a period of 5 days at 50ºC and 70º Celsius.

Analysis was carried out by GLC (a.s.) and HPLC (benalaxyl acid).

Benalaxyl was found to be stable to hydrolysis at pH 4 and 7. At pH 9 and 50ºC 53% benalaxyl remained after 5 days (DT_{50} of 55d). At 70ºC and pH 9 the DT_{50} of benalaxyl was of 19 hours. At this temperature and pH values of 4 or 7 the hydrolysis of benalaxyl was not tested.

Based on the findings, hydrolytic constants (Kh) at lower temperatures were extrapolated using the Arrhenius equation. Thus, at pH 9 and 20ºC the Kh is 0.0044d-1 with a corresponding DT50 of 157 days; at 25ºC the Kh value is of 0.0081d-1 and the DT_{50} is of 86 days. The main hydrolysis product at pH 9 is benalaxyl acid. This compound was referred to be more stable than the active substance.

Benalaxyl is stable to hydrolysis at pH 4 and 7. At pH 9 it has a DT_{50} of 55 days at 50°C and 19 hours at 70°C. A DT_{50} of 157 days at 20°C was extrapolated from the results at the higher temperatures

11.1.4 Other convincing scientific evidence

11.1.4.1 Field investigations and monitoring data (if relevant for C&L)

No data available.

11.1.4.2 Inherent and enhanced ready biodegradability tests

No data available.

11.1.4.3 Water, water-sediment and soil degradation data (including simulation studies)

Water-Sediment

Adequate data to assess the degradation of benalaxyl in sediment/water systems were evaluated during the first EU approval. The rates of degradation in the sediment/water study of Anonymus (1997) have been re-evaluated according to the recommendations of the FOCUS Kinetics Group (FOCUS 2006, 2011).

The degradation of benalaxyl in two sediment/water systems was modelled according to the recommendations of the FOCUS Kinetics Guidance document. Benalaxyl degraded with SFO DT_{50}s in the total system of 141.9 to 199.4 days at 20°C (χ^2 error 2.596 to 4.261%).

Summary of route and rate of degradation in aquatic systems

Benalaxyl is categorised as “not readily biodegradable”.

Since the original review was performed, a new data requirement to address the aquatic mineralisation of active substances has been stipulated under Regulation 1107/2009. A study has been performed to the OECD guideline 309 and benalaxyl was stable under the conditions of the test.

In two natural water/sediment systems from Italy (20ºC, high organic loamy sand pond aquatic system and a low organic sandy river aquatic system) benalaxyl was observed to dissipate from the water compartment according to a biphasic process. Levels of radioactivity in the surface water of the Pond system decreased from 97%AR (0 h) to 16%AR at the end of the study. Benalaxyl declined from 94% AR to 1.4% AR after 100 days. In the River system, a similar but slower decline was observed. Levels of radioactivity in the surface water decreased from 95% AR (0 h) to 41% AR at 100 days. Benalaxyl decreased from 92% AR to 26% AR. In the original evaluation benalaxyl was concluded to dissipate from the water phase following a biphasic pattern with 1st order DT_{50} values of 5 and 10 days, respectively for the Pond and River systems for the first phase. The second phase was slower and the corresponding 1st order DT_{50} values were 32 and 61 days for the Pond and River systems. Extractable radioactivity from the Pond sediment increased from 5.7% of AR at 0 h to 68% AR after 100 days with a corresponding increase of benalaxyl levels from 5.7% AR to 53% AR (Maximum of 71% AR in sediment at 60 days). In the River sediment levels of extractable radioactivity increased from 5.6% AR at 0 h to 52% AR after 100 days of incubation with benalaxyl levels increasing from 5.6% AR to 43% AR. Unextractable radioactivity reached a maximum of 8.1%AR after 100 days (River aquatic sediment). No CO_2 was detected in the River system. In the Pond system CO_2 was only detected sporadically and reached a maximum of 0.4%AR at the end of the study.

The main degradation products found in both systems were identified M1 (max. of 7.6% AR and 7.7% AR after 100 days, respectively in the Pond and River total systems) and benalaxyl acid (M9, max. of 7.79% AR and 6.11% AR after 100 and 60 days, respectively in the Pond and River total systems).

DT_{50}s in the whole sediment/water systems have been recalculated according to FOCUS (2006, 2011) guidance. Benalaxyl degraded with SFO DT_{50}s in the total system of 141.9 to 199.4 days at 20°C (χ^2 error 2.596 to 4.261%).

Soil

Data to assess the aerobic rate of degradation of benalaxyl in soil were evaluated during the first EU review and no further data were considered necessary. For further details, please refer to the DAR and addenda for benalaxyl (Portugal, 2000, 2003).

Rate of degradation in soil

Aerobic degradation of the active substance

Detailed summaries of the studies in support of the original approval (DAR, 2000, 2003) are presented below. Due to concerns regarding possible shortcomings in the existing soil metabolism studies (e.g. high rates used, enantiomeric ratios not determined) a soil metabolism study in four soils has been conducted.

DT_{50}s were available from five soils investigated in the studies of Anonymous (1982) and Anonymous (1981), as summarised below. Benalaxyl was concluded to degrade with DT_{50}s of 36 to 100 days at 22°C.

The rates of degradation in the aerobic soil degradation studies of Anonymous (1982, 1981) have been re-evaluated according to the recommendations of the FOCUS Kinetics Group (FOCUS 2006, 2011) in the study of Anonymous (2009).

The study investigated the aerobic degradation of ^{14}C-benalaxyl (M 9834, DL-alanine, N-(2,6-dimethylphenyl)-N(phenylacetyl)-methyl ester) in a silt loam soil in the dark over 133 days. ^{14}C-benalaxyl was applied to 100 g of soil at a concentration of 50 mg a.s./kg and maintained at 22 ± 2°C and 40% water holding capacity. Samples were taken at 7, 14, 28, 56, 77, 98 and 133 DAT for extraction and analysis.

^{14}C-benalaxyl degraded throughout the study to 11.7% AR after 133 DAT. The major ^{14}C metabolites Compound A ((methyl-N-(2,6-xylyl)-N-malonyl alaninate; M1), Compound B (N-(2,6-xylyl)-N-malonyl alanine; M2) and Compound C (benalaxyl-acid; M9) were identified. Throughout the study compounds A, B, and C reached a maximum of 31.0% AR (133DAT), 34.1%AR (98 DAT) and 4.9% AR (28 DAT), respectively. A total of 18.8% of the applied radioactivity (AR) was present as bound residues after 133 days. No loss of total ^{14}C and no evolution of $^{14}CO_2$ was ascertained to occur.

The aerobic degradation scheme of benalaxyl in soil (aerobic condition) was defined.

The rate of degradation of benalaxyl (methyl-N-(phenylacetyl)-N-(2,6-xylyl)-alaninate;) was investigated by Anonymous (1981) in four recently collected Italian farmland soils: Triulzi (a loam tending to sandy loam); Linate (loam); Cantonazzo (clay loam); and Badia Polesine (sandy loam). Unlabelled benalaxyl was applied to 100 g (dry weight [dw] of soil) with 40% moisture content at a nominal rate of 0.5 or 5 mg a.s./100g dw soil. The rate of degradation of benalaxyl in Triulzi soil was also determined when the water content was raised to 69% (with sterile and fresh soil). Samples were maintained in the dark at 22 ± 2°C and water lost via evaporation was replaced. Samples were taken at 0, 7, 14, 28, 56, 70, 105 and 143 DAT or for soils with 69% maximum water holding capacity (MWHC) 0, 7, 18 29, 56 and 68 days post application or day 0, 84, 112, 212 post application for sterile soils. Soils were extracted using methanol/water (90:10 v/v) and analysed via HPLC and TLC.

Procedural recoveries at different levels of benalaxyl both in soil and water ranged from between 90 and 106%. Total recoveries of benalaxyl during the study from samples taken were between 94.5 to 98.5%.

This decline in benalaxyl was characterised by an initial lag phase where degradation was slow, followed by a period of rapid degradation. For a 50 mg/kg dose of benalaxyl the concentration of benalaxyl declined from 48.0-50.0 mg/kg (0 DAT) to 4.25-9.52 mg/kg at the end of the study (143-158 DAT). When the dose was 10

times lower (5 mg/kg soil), the concentration of benalaxyl declined from 4.86-5.52 mg/kg (0 DAT) to 0.25-0.72 (between 98 DAT and 143 DAT).

An increase in moisture content (from 40% MWHC to 69% MWHC) increased the rate of degradation of benalaxyl in the Triulzi soil. With an application of 5 mg/kg to the soil, benalaxyl declined from 4.86 mg/kg benalaxyl (0 DAT) to 0.72 mg/kg at 143 DAT when the soil was at 40% MWHC compared to a decline of 5.21–5.62 mg/kg (0 DAT) to 0.44–1.00 mg/kg at 68 DAT when at 60% MWHC. Under sterile conditions at 69% MWHC, degradation was much reduced and benalaxyl declined from 5.26 mg/kg (0 DAT) to 4.40 mg/kg at 212 DAT. This indicates that degradation is through microbial action and that benalaxyl is stable under sterile conditions.

The rate of degradation increased when benalaxyl was applied to a previously treated soil (Cantonazzo soil) indicating that the lag phase (due to microbial activity) was eliminated. When a dose of 5 mg/kg was applied to fresh Cantonazzo soil, levels reduced from 5.39 mg/kg (0 DAT) to 0.69 mg/kg at 73 DAT, following re-application at 108 days after the initial treatment levels of benalaxyl then declined from 5.70 (at DAT 0 of the second application) to 0.8 mg/kg at day 54 (after the second application).

Determination of modelling endpoints for benalaxyl and soil metabolites from laboratory degradation studies, using Modelmaker 4.0, according to FOCUS kinetics was drawn by Anonymous (2009).

The decline of benalaxyl in two laboratory studies was modelled according to the recommendations of the FOCUS Kinetics Guidance document. A lag-phase was noted in all soils and the HS-SFO or SFO model satisfactorily describes the decline of benalaxyl in all soils. DT_{50}s of 18.1 to 57.8 days for were obtained for the decline phase under the conditions of the studies and modelling endpoints of DT_{50}s 19.7 to 39.3 days normalised to 20°C and pF2 were obtained.

11.1.4.4 Photochemical degradation

Benalaxyl is not easily photolysed under natural sunlight conditions during June – August at 45º 28’ N, 3º 10’ W coordinates since 60%AR is still present as benalaxyl after 64 days of exposure. At least 15 unidentified compounds were detected but none of them individually represented more than 5% of the initially applied radioactivity. Benalaxyl is not prone to photolysis.

11.2 Environmental transformation of metals or inorganic metals compounds

Not relevant for this proposal.

11.3 Environmental fate and other relevant information

Adsorption and desorption in soil

Data to assess the adsorption/desorption of benalaxyl were evaluated during the first EU review. For further details, please refer to the DAR and addenda for benalaxyl (Portugal, 2000, 2003).

The adsorption and desorption behaviour of benalaxyl was investigated (Anonymous, 1993) at 20 ± 1 ºC in three different German soils (soil I: sandy loam, $pH_{(H2O)}$ 4.6 and 1.8 % OC; soil II: sandy loam, $pH_{(H2O)}$ 5.8 and 1.5 % OC; and soil III: sandy loam, $pH_{(H2O)}$ 7.8 and 1.1 % OC)) using a batch equilibrium method.

The tests were carried out with the three soils using the following concentration and adsorption time:

Test series 1	Concentration: 200 µg/l; 1 mg/l; 5 mg/l Time: 2h; 4h; 6h; 16h
Test series 2	Concentration: 40 µg/l; 200 µg/l Time: 1h; 3h; 5h; 8h (only with 200 µg/l)
Test series 3	Concentration: 200 µg/l; 500 µg/l; 1 mg/l; 5 mg/l Time: 10 min; 30 min; 60 min; 90 min
Desorption test	Concentration: 200 µg/l Time (adsorption): 16h; Time (desorption): 2 x 16h

In all cases, 5 g dry soil was pre-equilibrated for 48h in 10 ml of 0.01 M $CaCl_2$ in water. For the blank test a soil: solution ratio of 1:5 was used (no test substance) for the three soils. For the control test, the 0.01 M $CaCl_2$ solution with the test substance was used.

A soil: solution ratio of 1:5 (w/w) was used for the three soils. The steady phase (equilibration time) was reached in less than 10 minutes and so the Freundlich isotherm parameters (adsorption constant and freundlich exponent) were calculated from a linear plot for the three soils using the results at 90 minutes (test series 3).

The average mass balance was 105 ± 20 % for all three soils. The results presented in the report were calculated incorrectly. The corrected K_f, K_{foc} and 1/n values are summarised in the following table.

Corrected Kocs for benalaxyl from Anonymous (1993)

Soil	%oc	pH (H_2O)	K_f	1/n	K_{oc}	K_{om}
Sandy loam I	1.8	4.6	4.77	0.6624	265	154*
Sandy loam II	1.5	5.8	8.33	0.6295	555	322
Sandy loam III	1.1	7.8	7.77	0.7141	707	410
Worst case (only 2 soils)				0.6295	555	322

*considered unreliable as it is only based on 3 concentrations, one of which only has a single replicate.

Anonymous, 2018, [^{14}C]Benalaxyl Determination of Adsorption/Desorption Behavior in Two Soils, GLP, OECD Guideline 106 (2000): Adsorption/Desorption using a Batch Equilibrium Method. OPPTS 835.1230: Adsorption/Desorption (Batch Equilibrium)

The adsorption and desorption behaviour of ^{14}C-benalaxyl was investigated (Anonymous, 2018) in two different soils (Lufa 2.2: sandy loam, pH 5.4 and 1.8 % OC; and RefeSol 06-A: silty clay, pH 7.2 and 3.0 % OC) using a batch equilibrium method. Following preliminary tests (Tier 1 and 2), the adsorption isotherms (Tier 3) for both soils were calculated at a soil/solution ratio of 1/10 (4 g soil and 40 mL of 0.01 M $CaCl_2$), and at five test item concentrations (0.5, 0.25, 0.05, 0.025 and 0.005 mg/L). An adsorption equilibration time of 24 hours was used. All the tiers were carried out at 20 - 25°C and in the dark.

The Freundlich adsorption coefficients ($K_{F, abs}$) were 8.216 and 17.951 L/kg for the Lufa 2.2 and RefeSol 06-A soils, respectively. Freundlich adsorption coefficients related to organic carbon content ($K_{Foc, abs}$) for the two soils were 453.9 and 598.4 L/kg and the adsorption 1/n values were 0.9151 and 0.9377, an arithmetic mean of the results of the 2 soils can be used in exposure modelling (K_{Foc} = 526.15; 1/n = 0.93 for benalaxyl).

For the desorption experiments, a 24 hours desorption time was used to determine the desorption parameters.

The Freundlich desorption coefficient ($K_{F, des}$) were 9.566 and 24.795 L/kg for the Lufa 2.2 and RefeSol 06-A soils, respectively. Freundlich desorption coefficients related to organic carbon content ($K_{Foc, des}$) for the two soils were 528.5 and 826.5 L/kg and the adsorption 1/n values were 0.9117 to 0.9941.

At the 48-hour sampling (Tier 1 and 2), the mass balance was between 100.1% AR and 104.2% AR for both soils and all soil/solution ratios after extraction and combustion. The parental mass balance was >95% for all samples.

At the desorption equilibrium at 48-hour sampling (Tier 3), the mean mass balance was between 97.1% AR and 100.8% AR for both soils using the highest and lowest concentrations (0.5 and 0.005 mg/L) and the soil/solution ratio of 1/10 after extraction and combustion.

In the Tier 3 experiments at a soil to solution ration of 1/10, the mass balances (radioactivity mass balance) after 24 hours of desoprtion was ≥ 95%. The average parental mass balance after 24 hours of desoprtion ranged from 89.2 % to 97.6 % AR; therefore, the test item was considered to be stable for at least 24 hours.

No adsorption to the surface of the test vessel over the test period was detected in control samples from Tier1/Tier 2.

It was noted that in one (Anonymous, 1993) of these studies, the reliability of the results cannot be confirmed taking into consideration the low values for the 1/n (deviating from the linear adsorption) and the quite poor fitting. The new study, on the contrary, is well conducted and results are appropriately presented even though only 2 soils were investigated and only 2 reliable endpoints would be available.

11.4 Bioaccumulation

Table 23: Summary of relevant information on bioaccumulation

Method	Results	Reference
Partition coefficient n-octanol/water Method EEC A8	Log P_{ow} for Benalaxyl: 3.54 at 20°C and at pH=6.1	dRAR CA B.2.7 Anonymous (1995) N. 94/1087.B
Flow-Through Bluegill Bioconcentration/Depuration Study with ^{14}C-Galben In house method complying with US updated requirements No GLP	BCF = 57	dRAR B.9.2.2.3 CA 8.2.2.3/01 Anonymous (1985)
Flow-Through Bluegill Bioconcentration/Depuration Study with ^{14}C-Galben – Metabolite Identification Phase	Galben represented 50-60% of the total radioactivity (TR) found in the edible fraction	RAR B.9.2.2.3 CA 8.2.2.3/02 Anonymous (1985)

11.4.1 Estimated bioaccumulation

No data available.

11.4.2 Measured partition coefficient and bioaccumulation test data

Benalaxyl has a log P_{OW} of 3.54 at 20°C and at pH=6.1, which indicates a low bioaccumulation potential.

The bioconcentration potential of benalaxyl in fish was investigated on Bluegill sunfish exposed to the a.s. at a concentration of 0.054 mg as/l under flow through conditions for up to 28 days, followed by a 14 days depuration period (Anonymous, 1985).

Benalaxyl concentration in fish reached a plateau level within 3 days of exposure corresponding to a BCF value of 57. Depuration was also rapid with a half life of less than 6 hours and more than 98% residues were eliminated from fish within 14 days indicating that benalaxyl can be considered as a non bioaccumulable substance.

Benalaxyl appears to be oxidised in fish giving rise to polar compounds and carboxy derivatives, that latter can be further metabolised to glucuronic acid and sulphate conjugated compounds.

One GLP Study of Anonymous (1995) regarding Octanol/water partition coefficient of benalaxyl, according to Method EEC A8 performed.

This study aimed to determine the partition coefficient n-octanol/water of Benalaxyl at 20ºC.

The following result was obtained for Benalaxyl: Log Pow for Benalaxyl: 3.54 at 20°C and at pH=6.1

With regard to bioaccumulation of Benalaxyl, two 28 days dynamic studie on bioconcentration by bluegill sunfish were carried out.

Study 1: The Flow-Through Bluegill Bioconcentration/Depuration Study with 14C-Galben, Anonymus 1985, no GLP, included in the DAR (2000) for benalaxyl and the endpoint is listed in the Review Report (2004). The

EU agreed BCF value in fish is 57 indicating that benalaxyl can be considered as a non bioaccumulable substance

The bioconcentration potential of Galben in bluegill sunfish was evaluated using 14C-Galben. Bluegill sunfish were exposed to 14C-Galben using a flow-through system, at a concentrations of 0.0524 mg a.s./L, in addition to a control. Fish were exposed to the test concentrations for 28 days. For the elimination of 14C residues (depuration), the test fish were placed in clean water for 14 days.

Temperature and dissolved oxygen concentration were measured daily in each test aquarium. Water alkalinity, hardness and pH were measured weekly during the uptake and depuration phases.

14C residues in the water were measured on Days 1, 3, 7, 10, 14, 21 and 28 during the exposure phase and Days 1, 3, 7 and 14 during depuration, from both treated and control. 14C residues in fish were measured on Days 1, 3, 7, 10, 14, 21, and 38 from the treated tank and Days 1, 14 and 28 in the control, during the exposure phase. During depuration samples were taken on Days 1, 3, 7 and 14 in the treated tank and Day 14 from the control. Samples were analysed for [14C] residues using liquid scintillation counts (LSC).

Throughout the exposure phase, 14C-Galben values in water averaged 0.046 mg a.s./L. Comparison of the values obtained during the exposure period showed that the residue levels reached a plateau within 3 days of initiation of exposure. On a whole fish basis, the residues plateaued at an average value of approximately 2.6 mg/kg, thus the bioconcentration ratio was 2.6:0.046 or 57:1. During the depuration period, the residues were eliminated from the fish tissue with a half life of less than 6 hours. After 14 days of depuration, the residue level in the whole fish had declined to approximately 2% of the plateau phase.

As the study was conducted according to an in-house method, the occurrence of deviations cannot be assessed against these methods Fish used in the study were treated for ISH ten days prior to test initiation. Current guidelines (OECD 305) state there must be at least a 14 day period following any treatment. Mortalities and other adverse effects were not recorded. Fish were not weighed and lipid content was not measured throughout the study. Fish were not fed for the duration of the study. TOC was not measured. The steady state BCF and kinetic BCF were not reported.

According to current OECD 305 guidelines, as the dissolved oxygen did not remain > 60% throughout the duration of the test and mortality was not recorded, the validity of the study according to current guidelines cannot be confirmed.

Study 2: The Flow-Through Bluegill Bioconcentration/Depuration Study with 14C-Galben – Metabolite Identification Phase, Author: Anonymous, 1985, no GLP. The study is included in the DAR (2000) for benalaxyl. Benalaxyl appears to be oxidised in fish giving rise to polar compounds and carboxy derivatives, that latter can be further metabolised to glucuronic acid and sulphate conjugated compounds. Edible and non-edible fractions were taken for metabolite identification using methods described in the previous report. Extracts were analysed by thin-layer chromatography (TLC). Galben represented 50-60% of the total radioactivity (TR) found in the edible fraction.

The results show that Galben comprised greater than 50% of the total 14C-resdiue in the edible fraction of bluegill sunfish exposed to 14C-Galben in a dynamic flow-through system. G7A, G8 and G14 were also present, but at levels less than 10%. With the exception of a polar species in one of the edible fractions, no metabolite comprised more than 10% of the total 14C-residue. The very low 14C-residue levels and limited amount of sample precluded further identification of polar species.

For the visceral samples, the results show Galben to be present only ion unconjugated form, G6 and G14 in conjugated form and G8 in both forms. Based on the nature of the hydrolytic enzyme preparation used, the conjugates are glucuronides and/or sulfates. After hydrolysis, no unidentified species comprised more than 10% of the total 14C-residue.

According CLP Regulation for organic substances the potential for bioaccumulation shall normally be determined by using the octanol/water partition coefficient, usually reported as a log Kow. The relationship between the log Kow of an organic substance and its bioconcentration as measured by the bioconcentration factor (BCF) in fish has considerable scientific literature support. Using a cut-off value of log Kow ≥ 4 is intended to identify only those substances with a real potential to bioconcentrate. While this represents a potential to bioaccumulate, an experimentally determined BCF provides a better measure and shall be used in

preference if available. A BCF in fish of ≥ 500 is indicative of the potential to bioconcentrate for classification purposes. Some relationships can be observed between chronic toxicity and bioaccumulation potential, as toxicity is related to the body burden.

11.5 Acute aquatic hazard

Benalaxyl has a log P_{OW} of 3.54 at 20°C and at pH=6.1, less than 4, and a BCF in fish of 57 which indicates a low bioaccumulation potential.Evaluation of acute aquatic hazard for benalaxyl is based on studies which are considered fully validated test. presented in the table below and relevant studies for the classification purposes are also briefly summarised below.

The available acute toxicity data for relevant metabolites of benalaxyl (M1 - Compound A; M2 - Compound A; F4 acetyl; F7/F8; M9 - Benalaxyl acid) revealed toxicity values > 100 mg/L. Therefore, the studies with these metabolites are not described here, no relevance for classification.

Table 24: Summary of relevant information on acute aquatic toxicity

Method	Species	Test material	Results[1]	Reference
Fish				
96-hour (semi- static study) OECD 203 and EC method C.1 GLP	Rainbow Trout (*Oncorhynchus mykiss)*	Benalaxyl technical (purity: 98.4 % Benalaxyl (analysed))	LC_{50} = 4.8 mg/L (based on geom. mean measured concentrations)	RAR B.9.2.1. CA 8.2.1/06 Anonymous (2014a)
96-hour (semi- static study) OECD 203 and EC method C.1. GLP	Rainbow trout (*Oncorhynchus mykiss*)	Benalaxyl Isomer R (purity: 98.6 %)	LC_{50} = 4.9 mg/L (based on mean measured concentrations)	RAR B.9.2.1. CA 8.2.1/07 Anonymous (2014b)
96-hour (semi- static study) OECD 203 and EC method C.1. GLP	Rainbow trout (*Oncorhynchus mykiss*)	Benalaxyl Isomer S (purity: 98.3 %)	LC_{50} = 5.0 mg/L (based on mean measured concentrations)	RAR B.9.2.1. CA 8.2.1/08 Anonymous (2014c)
Aquatic invertebrates				
48 hours (static) OECD 202 and EC method C.2 GLP	*Daphnia magna*	Benalaxyl technical (purity: 98.4 % Benalaxyl (analysed))	EC_{50} = 15 mg/L (based on measured concentrations)	DRAR B.9.2.4.1 CA 8.2.4.1/02 Anonymous (Harris, S., 2014a)
48 hours (static) OECD 202 and EC method C.2 GLP	*Daphnia magna*	Benalaxyl Isomer R (purity: 98.6 % (98.8 total isomers, R/S ratio: 99.8/0.2))	EC_{50} = 13 mg/L (based on measured concentrations)	RAR B.9.2.4.1 CA 8.2.4.1/03 Anonymous (2014b)
48 hours (static) OECD 202 and EC method C.2 GLP	*Daphnia magna*	Benalaxyl Isomer S (purity: 98.3 %; (R/S ratio: 0/100))	EC_{50} = 17 mg/L (based on measured concentrations)	RAR B.9.2.4.1 CA 8.2.4.1/04 Anonymous (2014c)
48 hours	*Daphnia magna*	Benalaxyl	**EC_{50} = 0.59 mg/L**	RAR

Method	Species	Test material	Results[1]	Reference
(static) OECD 202, Part I, Guideline (1984) GLP		(purity: 96.6 % Benalaxyl (analysed))	(based on measured concentrations)	B.9.2.4.1 CA 8.2.4.1/01 Anonymus (1993)
Algae				
72 hours (static system) OECD 201 and EC method C.3 GLP	Pseudokirchneriella subcapitata	Benalaxyl technical (purity: 98.4 %)	E_rC_{50} = 3.5 mg/L E_yC_{50} = 0.56 mg/L (based on geometric mean measured concentrations)	RAR B.9.2.6.1 CA 8.2.6.1/02 Anonymous (2014a)
72 hours (static system) OECD 201 and EC method C.3 GLP	Pseudokirchneriella subcapitata	Benalaxyl Isomer R (purity: 98.6 %)	E_rC_{50}= 3.4 mg/L E_yC_{50} = 0.85 mg/L (based on geometric mean measured concentrations)	RAR B.9.2.6.1 CA 8.2.6.1/03 Anonymous (2014b)
72 hours (static system) OECD 201 and EC method C.3 GLP	Pseudokirchneriella subcapitata	Benalaxyl Isomer S (purity: 98.3 %)	E_rC_{50}= 3.4 mg/L E_yC_{50}= 0.086 mg/L (based on geometric mean measured concentrations)	RAR B.9.2.6.1 CA 8.2.6.1/04 Anonymous (2014c)

[1] Indicate if the results are based on the measured or on the nominal concentration

11.5.1 Acute (short-term) toxicity to fish

With regard to acute (short-term) toxicity to fish of benalaxyl, eight studies were carried out. Five of these studies (Anonymous, 1979a, 1979b; 1979c; 1980; 1984) were evaluated during Annex I inclusion of benalaxyl and they were accepted as supportive information only. Three studies (Anonymous, 2014a; 2014b; 2014c) was submitted for the purpose of EU renewal. These studies are not considered relevant for the classification purposes.

Acute toxicity data with benalaxyl technical and its isomers (Isomer R and Isomers S) was available on one fish specie - rainbow trout (see Table above). All tests were conducted according to the OECD test guideline 203 and GLP and no significant deviations from the test guideline were identified. The 96 h LC_{50} is 4.8 mg/L (based on geom. mean measured concentrations) for rainbow trout as no mortality or sublethal effects were observed in the study. The acute toxicity data for fish demonstrate both isomers to be of equal toxicity and the endpoints are comparable with that for benalaxyl technical (Isomer R: 96 h LC_{50} = 4.9 mg/l; Isomer S: 96 h LC_{50} = 5.0 mg/l).

Study 1: Anonymous, 2014a, Benalaxyl technical: Acute Toxicity to Rainbow Trout *(Oncorhynchus mykiss*), GLP, OECD 203 and EC method C.1

The 96-hour acute toxicity of benalaxyl technical to rainbow trout (*Oncorhynchus mykiss*) was determined under semi-static conditions, in a dose response test. The nominal test concentrations were 10, 18, 32, 56 and 100% v/v saturated solution, plus a control tested in parallel. Analysis of freshly prepared media for the 18, 32, 56 and 100% v/v saturated solutions at 0 and 72 hours showed mean measured concentrations of 3.4, 6.8, 11 and 22 mg benalaxyl technical/L, respectively. Seven fish in a single replicate were tested per treatment group.

The study was conducted in accordance with the referred guidelines. The test was considered to be valid given that none of the control fish died or showed signs of stress during the test and that the oxygen concentration at the end of the test was >60% of ASV (6.2 mg CVL) in the control and test vessels.
Based on mean measured concentrations, the 96-hour LC_{50} for benalaxyl technical and rainbow trout (*Oncorhynchus mykiss*) is 4.8 mg/L from geomean of the highest concentration causing no mortalities and the lowest concentration causing 100% mortality. The NOEC and LOEC were determined to be 3.4 and 6.8 mg a.s./L, respectively, based on mean measured concentrations.

Study 2: Anonymous, 2014b, Benalaxyl isomer R: Acute Toxicity to Rainbow Trout (*Oncorhynchus mykiss*), GLP, OECD 203 and EC method C.1

The 96-hour acute toxicity of benalaxyl isomer R to rainbow trout (*Oncorhynchus mykiss*) was determined under semi-static conditions, in a dose response test. A preliminary media preparation trial indicated that a dissolved test item concentration of approximately 28 mg/L was obtained from a saturated solution method of preparation indicating this to be the limit of water solubility of this item under test conditions. Following a preliminary range-finding test, fish were exposed, in groups of seven, to an aqueous solution of the test item over a range of concentrations of 10, 18, 32, 56 and 100% v/v saturated solution for a period of 96 hours at a temperature of approximately 14 °C. Analysis of freshly prepared media for the 18, 32, 56 and 100% v/v saturated solutions at 0 and 72 hours showed mean measured concentrations of 3.6, 6.7, 12 and 21 mg benalaxyl isomer R/L, respectively.

The study was conducted in accordance with the referred guidelines. The test was considered to be valid. Based on mean measured concentrations, the 96-hour LC_{50} for benalaxyl isomer R and rainbow trout (Oncorhynchus mykiss) equalled 4.9 mg/L (95% CI: 3.6 – 6.7 mg/L). The NOEC and LOEC were determined to be 3.6 and 6.7 mg/L, respectively.

Study 3: Anonymous, 2014c, Benalaxyl isomer S: Acute Toxicity to Rainbow Trout (Oncorhynchus mykiss), GLP, OECD 203 and EC method C.1

The 96-hour acute toxicity of benalaxyl isomer S to rainbow trout (*Oncorhynchus mykiss*) was determined under semi-static conditions, in a dose response test. A preliminary media preparation trial indicated that a dissolved test item concentration of approximately 28 mg/L was obtained from a saturated solution method of preparation indicating this to be the limit of water solubility of this item under test conditions. Following a preliminary range-finding test, fish were exposed, in groups of seven, to an aqueous solution of the test item over a range of concentrations of 10, 18, 32, 56 and 100% v/v saturated solution for a period of 96 hours at a temperature of approximately 14°C. Analysis of freshly prepared media for the 10, 18, 32, 56 and 100% v/v saturated solutions at 0 and 72 hours showed mean measured concentrations of 2.2, 4.1, 7.1, 11 and 21 mg Benalaxyl isomer S/L, respectively.

The study was conducted in accordance with the referred guidelines. The test was considered to be valid given that none of the control fish died or showed signs of stress during the test and that the oxygen concentration at the end of the test was >60% of ASV (6.2 mg CVL) in the control and test vessels.
Based on mean measured concentrations, the 96-hour LC_{50} for benalaxyl isomer S and rainbow trout (Oncorhynchus mykiss) equalled 5.0 mg/L (95% CI: 4.2 – 5.8 mg/L). The NOEC and LOEC were determined to be 2.2 and 4.1 mg/L, respectively.

11.5.2 Acute (short-term) toxicity to aquatic invertebrates

With regard to acute (short-term) toxicity to aquatic invertebrates of benalaxyl, four studies were carried out. One of these studies (Anonymous, 1993) was evaluated during Annex I inclusion of benalaxyl and it was fully accepted. Three studies (Anonymous, 2014a, 2014b, 2014c) was submitted for the purpose of EU renewal.

Acute toxicity data with benalaxyl technical and its isomers (Isomer R and Isomers S) was available on Daphnia magna (see Table above). All tests were conducted according to the OECD test guideline 202 and GLP and no significant deviations from the test guideline were identified.

The 48-hour acute toxicity of benalaxyl to *Daphnia magna* was determined in a static system, with groups of 20 daphnids per treatment (Anonymous, 1993). According to the current OECD 202 Guideline, the test is considered valid since no mortality occurred in the controls and the dissolved oxygen concentration at the end of the test was >3 mg/L (actual 96% which corresponds to approx. 8mg/L) in the control and test vessels.

The 48 hour EC_{50} value for benalaxyl and *Daphnia magna* was calculated to be 0.59 mg/L. The No Observed

Effect Concentration after both 24 and 48 hours exposure was 0.18 mg/L respectively.

The studies are relevant for the classification purposes.

Study 1: Anonymous, 1993, Acute Immobilisation Test on *Daphnia magna*, GLP, OECD 202 and EC method C.2

The 48-hour acute toxicity of benalaxyl to *Daphnia magna* was determined in a static system, with groups of 20 daphnids per treatment. Nominal concentrations were 0.18, 0.32, 0.58, 3.2 and 5.8 mg/L, with four replicates per treatment. A solvent control was also tested. The number of immobilised daphnids in each replicate test vessel was recorded at 24 and 48 hours of exposure.

Temperature, dissolved oxygen content and pH were measured during the test but the frequency was not reported. Adherence to the OECD 202 Guideline can therefore not be confirmed. The report did not confirm the conduct of a reference test. The sampling of test solutions for analysis of test concentrations was not reported, therefore concentrations cannot be confirmed. There was no confirmation if the daphnids were fed during the test.

According to the current OECD 202 Guideline, the test is considered valid since no mortality occurred in the controls and the dissolved oxygen concentration at the end of the test was >3 mg/L (actual 96% which corresponds to approx. 8mg/L) in the control and test vessels. As no analysis of test concentration was measured, it must be noted that this study was originally included in the DAR (2000) for supportive information only.

The 48 hour EC50 value for benalaxyl and Daphnia magna was calculated to be 0.59 mg/L. The No Observed Effect Concentration after both 24 and 48 hours exposure was 0.18 mg/L respectively.

Study 2: Anonymous, 2014a, Benalaxyl technical: Daphnia sp., 48-Hour Acute Immobilization Test GLP, OECD 202 and EC method C.2

The acute toxicity of benalaxyl technical to *Daphnia magna* was determined in a 48 hour immobilisation test under static conditions. A preliminary media preparation trial indicated that a dissolved test item concentration of approximately 25 mg/L was obtained from a saturated solution method of preparation indicating this to be the limit of water solubility of this item under test conditions. Daphnids were exposed to an aqueous solution of the test item at concentrations of 10, 18, 32, 56 and 100% v/v saturated solution, plus a control tested in parallel. Analysis of the 18, 32, 56 and 100 % v/v saturated solution test preparations showed measured test concentrations to range from 4.18 to 24.4 mg/L at 0 hours, and from 4.44 to 24.4 mg/L at 48 hours. There was no significant change < 80% (98.3 to 106.2%) in the measured concentrations at 48 hours and so the results are based on 0-Hour measured test concentrations only.

A total of 100 organisms (5 per replicate, 4 replicates per concentration) were exposed to the five concentrations of the test substance, a control and a reference substance (Potassium dichromate), for 48 hours under static conditions.

The study was conducted in accordance with the referred guidelines. The test was considered to be valid given that no more than 10% of the control daphnids showed signs of disease or stress (e.g. discoloration or unusual behavior) during the test and that the dissolved oxygen concentration at the end of the test was >3 mg/L in the control and test vessels.The 48 hour EC_{50} value for benalaxyl technical and *Daphnia magna* was estimated to be 15 mg/L (measured) with 95% confidence limits of 13 - 17 mg/L. The No Observed Effect Concentrations after 24 and 48 hours exposure were 8.4 and 4.2 mg/L respectively. The Lowest Observed Effect Concentrations after 24 and 48 hours exposure were 14 and 8.4 mg/L respectively.

Study 3: Anonymous, 2014b, Benalaxyl isomer R: Daphnia sp., 48-Hour Acute Immobilization Test, GLP, OECD 202 and EC method C.2

The acute toxicity of benalaxyl isomer R to *Daphnia magna* was determined in a 48 hour immobilisation test under static conditions. A preliminary media preparation trial indicated that a dissolved test item concentration of approximately 28 mg/L was obtained from a saturated solution method of preparation indicating this to be

the limit of water solubility of this item under test conditions. Daphnids were exposed to an aqueous solution of the test item at concentrations of 10, 18, 32, 56 and 100% v/v saturated solution, plus a control tested in parallel. Analysis of the 32, 56 and 100% v/v saturated solution test preparations showed measured test concentrations to range from 9.15 to 27.3 mg/L at 0 hours, and from 9.53 to 27.2 mg/L at 48 hours. There was no significant change < 80% (99.4 to 104.2%) in the measured concentrations at 48 hours and so the results are based on 0-Hour measured test concentrations only.

A total of 100 organisms (5 per replicate, 4 replicates per concentration) were exposed to the five concentrations of the test substance, a control and a reference substance (Potassium dichromate), for 48 hours under static conditions.

The study was conducted in accordance with the referred guidelines. The test was considered to be valid given that none of the control daphnids showed immobilization or other signs of disease or stress and that the dissolved oxygen concentration at the end of the test was >3 mg/L in the control and test vessels.

The 48 hour EC_{50} value for benalaxyl isomer R and *Daphnia magna* was estimated to be 13 mg/L (measured) with 95% confidence limits of 12 - 15 mg/L. The No Observed Effect Concentrations after both 24 and 48 hour exposures was 9.2 mg/L. The Lowest Observed Effect Concentrations after both 24 and 48 hour exposures was 15 mg/L respectively.

Study 4: Anonymous, 2014c, Benalaxyl isomer S: Daphnia sp., 48-Hour Acute Immobilization Test, GLP, OECD 202 and EC method C.2

The acute toxicity of benalaxyl isomer S to *Daphnia magna* was determined in a 48 hour immobilisation test under static conditions. A preliminary media preparation trial indicated that a dissolved test item concentration of approximately 28 mg/L was obtained from a saturated solution method of preparation indicating this to be the limit of water solubility of this item under test conditions. Daphnids were exposed to an aqueous solution of the test item at concentrations of 10, 18, 32, 56 and 100% v/v saturated solution, plus a control tested in parallel. Analysis of the 18, 32, 56 and 100 % v/v saturated solution test preparations showed measured test concentrations to range from 6.66 to 25.0 mg/L at 0 hours, and from 6.31 to 24.4 mg/L at 48 hours. There was no significan t change < 80% (96.4 to 104.4%) in the measured concentrations at 48 hours and so the results are based on 0-Hour measured test concentrations only.

A total of 100 organisms (5 per replicate, 4 replicates per concentration) were exposed to the five concentrations of the test substance, a control and a reference substance (Potassium dichromate), for 48 hours under static conditions.

The study was conducted in accordance with the referred guidelines. The test was considered to be valid given that none of the control daphnids showed immobilization or other signs of disease or stress and that the dissolved oxygen concentration at the end of the test was >3 mg/L in the control and test vessels.

The 48 hour EC_{50} value for benalaxyl isomer S and *Daphnia magna* was estimated to be 17 mg/L (measured) with 95% confidence limits of 15 - 20 mg/L. The No Observed Effect Concentrations after both 24 and 48 hours exposures was 6.7 mg/L. The Lowest Observed Effect Concentrations after both 24 and 48 hours exposures was 8.8 mg/L respectively.

11.5.3 Acute (short-term) toxicity to algae or other aquatic plants

With regard to acute (short-term) toxicity to algae of benalaxyl, four studies were carried out. One of these studies (Anonymous, 1982) was evaluated during Annex I inclusion of benalaxyl and it was accepted as supportive information only. Three studies (Anonymous, 2014a; 2014b; 2014c) was submitted for the purpose of EU renewal.

Acute toxicity data with benalaxyl technical and its isomers (Isomer R and Isomers S) was available on *Pseudokirchneriella subcapitata*. All tests were conducted according to the OECD test guideline 201 and GLP standards and no significant deviations from the test guideline were identified. The study for benalaxyl technical is not considered relevant for the classification purposes. The 72-hour E_rC_{50} based on growth

inhibition is 3.5 mg/L and E_yC_{50} based on yield is 0.086 mg/L. Results that were based on nominal concentrations were analytically verified and the measured concentrations were in the range of 40% to 183% of the initial nominal concentrations. The acute toxicity data on algae also demonstrates the R-isomer (72-hour E_rC_{50} = 3.4 mg/L; 72-hour E_yC_{50} = 0.85 mg/L;) to be of equivalent toxicity to technical benalaxyl. Although the algae endpoint for the S-isomer (72-hour E_rC_{50} = 3.4 mg/L; 72-hour E_yC_{50} = 0.086 mg/L;) is lower than that for the active substance, it is within an order of magnitude of the active substance endpoint.

Study 1: Anonymous, 1982, Toxicity Study with Technical Galben on Selenastrum capricornutum, GLP, OECD 201

A static toxicity test was conducted to determine the effects of the test substance Technical Galben on the growth of the unicellular green alga, Selenastrum capricornutum. Three replicates, of five test concentrations of 0.01, 0.1, 1.0, 10 and 100 mg a.s./L and a solvent control were prepared. Selenastrum capricornutum was kept under test conditions for 96 hours. Cell density in each test vessel was monitored at 24, 48, 72 and 96 hours after the start of the test**.**

No reference was made to the conduct of a reference test to assess laboratory conditions, thus it cannot be confirmed when the last reference test was performed. As the 1981 guideline does state a reference substance may occasionally be tested, this may be considered to not have negatively impacted on the validity of the study.

According to the current OECD 201 (2011) guideline, deviations were noted. No reporting of a reference test was observed within the study which, according to the guidelines is desirable at least twice a year. No reference was made to observations of health at the end of the test, although microscopic examination was carried out to complete the cell count. Test concentrations were arranged in a series of > 3.2 spacing factor. The composition of the algal medium differs to that in current guidelines but conforms to the 1981 guideline. No analysis of test concentrations was reported for the duration of the study. The guideline states concentrations should be analysed if an analytical procedure is available. As the study was conducted in 1982, such a procedure may not have been available. No coefficient of variation was reported throughout the study. The effect of Galben on yield was not evaluated in conjunction with growth rate, as specified in the 2011 guideline.

According to the 1981 version of OECD 201, under which the test was conducted, the validity would be questionable as no analysis of test concentration was conducted. However, the guideline does state this is to be determined where practicable. Also, the other two conditions for validity were met. These were the control exhibiting log phase growth within 48 hours and a standing crop at 96 hours of 105 cells, in addition to one test concentration demonstrating no significant decrease in growth compared to the control and one concentration showing growth inhibition of more than 50% at 96 hours were met. As a consequence of the date of the study, it was not conducted according to GLP. It must be noted that the study was originally included in the DAR (2000) for supportive information only.

According to the current OECD 201 guideline (2011), coefficient of variation is not reported, but biomass increase in control increased by more than a factor of 16 within the 72-hour test period (actual:600). The validity of the study cannot therefore be concluded, based on current guidelines.
Based on nominal concentrations, the 96-hour E_rC_{50} was determined to be 2.4 mg a.s./L.

Study 2: Anonymous, 2014a, Benalaxyl technical: Algal Growth Inhibition Test , GLP, OECD 201 and EC method C.3

The 72-hour toxicity of benalaxyl technical to the single cell green alga *Pseudokirchneriella subcapitata* was determined in a static system. A preliminary media preparation trial indicated that a dissolved test item concentration of approximately 25 mg/L was obtained from a saturated solution method of preparation indicating this to be the limit of water solubility of this item under test conditions. Algae were exposed to solutions of the test item at nominal concentrations of 0.16, 0.50, 1.6, 5.0, 16 and 50% v/v saturated solution

(three replicate flasks per test item concentration) for 72 hours, under constant illumination and shaking at a temperature of 24 ± 1°C. Control cultures were run in parallel (six replicate flasks). A positive control study (Harlan Study Number 41303826) using potassium dichromate as the reference item was also conducted. Chemical analysis of the test preparations at 0 hours showed measured test concentrations ranging from 0.031 to 12 mg/L whilst concentrations in the range of 0.031 to 9.3 mg/L were observed at 72 hours (40% to 183% of the 0-Hour measured test concentrations). Given the variability in the results obtained at 0 and 72 hours it was considered appropriate to calculate the results based on the geometric mean measured test concentrations only, which were determined to be 0.031, 0.066, 0.31, 0.96, 2.7 and 10 mg/L in the 0.16, 0.50, 1.6, 5.0, 16 and 50% v/v saturated solutions, respectively.

The study was conducted in accordance with the referred guidelines. The test was considered to be valid given that none deficiencies. The following data show that the cell concentration of the control cultures increased by a factor of 176 after 72 hours. This increase was in line with the OECD Guideline that states the enhancement must be at least by a factor of 16 after 72 hours.

Mean cell density of control at 0 hours: 6.14×10^3 cells per mL

Mean cell density of control at 72 hours: 1.08×10^6 cells per mL

The mean coefficient of variation for section by section specific growth rate for the control cultures was 6% and hence satisfied the validation criterion given in the OECD Guideline which states the mean must not exceed 35%.

The coefficient of variation for average specific growth rate for the control cultures over the test period (0 - 72 h) was 3% and hence satisfied the validation criterion given in the OECD Guideline which states that this must not exceed 7%.

The 72-hour E_rC_{50} was determined to be 3.5 mg/L, the 72-hour E_yC_{50} was 0.56 mg/L. The No-Observed-effect concentration (NOEC) for both growth rate and yield was determined to be 0.066 mg/L (all endpoints based on mean measured concentrations).

Study 3: Anonymous, 2014b, Benalaxyl isomer R: Algal Growth Inhibition Test, GLP, OECD 201 and EC method C.3

The 72-hour toxicity of benalaxyl isomer R to the single cell green alga *Pseudokirchneriella subcapitata* was determined in a static system. A preliminary media preparation trial indicated that a dissolved test item concentration of approximately 28 mg/L was obtained from a saturated solution method of preparation indicating this to be the limit of water solubility of this item under test conditions. Algae were exposed to solutions of the test item at nominal concentrations of 0.16, 0.50, 1.6, 5.0, 16 and 50% v/v saturated solution (three replicate flasks per test item concentration) for 72 hours, under constant illumination and shaking at a temperature of 24 ± 1°C. Control cultures were run in parallel (six replicate flasks). A positive control study (Harlan Study Number 41303826) using potassium dichromate as the reference item was also conducted. Chemical analysis of the test preparations at 0 hours showed measured test concentrations ranging from 0.059 to 11.3 mg/L whilst concentrations in the range of 0.071 to 11.3 mg/L were observed at 72 hours (88% to 121% of the 0-Hour measured test concentrations). Given the variability in the results obtained at 0 and 72 hours it was considered appropriate to calculate the results based on the geometric mean measured test concentrations only, which were determined to be 0.065, 0.13, 0.35, 1.3, 3.6 and 11.3 mg/L in the 0.16, 0.50, 1.6, 5.0, 16 and 50% v/v saturated solutions, respectively.

The study was conducted in accordance with the referred guidelines. The test was considered to be valid given that none deficiencies. The following data show that the cell concentration of the control cultures increased by a factor of 176 after 72 hours. This increase was in line with the OECD Guideline that states the enhancement must be at least by a factor of 16 after 72 hours.

Mean cell density of control at 0 hours: 6.14×10^3 cells per mL

Mean cell density of control at 72 hours: 1.08×10^6 cells per mL

The mean coefficient of variation for section by section specific growth rate for the control cultures was 6% and hence satisfied the validation criterion given in the OECD Guideline which states the mean must not exceed 35%.

The coefficient of variation for average specific growth rate for the control cultures over the test period (0 - 72 h) was 3% and hence satisfied the validation criterion given in the OECD Guideline which states that this must not exceed 7%.

The results of the laboratory study on the effects of benalaxyl isomer R on *Pseudokirchneriella subcapitata*, strain CCAP 278/4 demonstrate the 72-hour E_rC_{50} to be 3.4 mg/L, the 72-hour E_yC_{50} to be 0.85 mg/L (all endpoints based on geometric mean measured concentrations). The No-Observed-effect concentration (NOEC) was determined to be 0.35 and the Low-Observed Effect Concentration (LOEC) equalled 1.3 mg/L.

Study 4: Anonymous, 2014c, Benalaxyl isomer S: Algal Growth Inhibition Test, GLP, OECD 201 and EC method C.3

The 72-hour toxicity of benalaxyl isomer S to the single cell green alga *Pseudokirchneriella subcapitata* was determined in a static system. A preliminary media preparation trial indicated that a dissolved test item concentration of approximately 28 mg/L was obtained from a saturated solution method of preparation indicating this to be the limit of water solubility of this item under test conditions. Algae were exposed to solutions of the test item at nominal concentrations of 0.16, 0.50, 1.6, 5.0, 16 and 50% v/v saturated solution (three replicate flasks per test item concentration) for 72 hours, under constant illumination and shaking at a temperature of 24 ± 1°C. Control cultures were run in parallel (six replicate flasks). A positive control study (Harlan Study Number 41303826) using potassium dichromate as the reference item was also conducted. Chemical analysis of the test preparations at 0 hours showed measured test concentrations ranging from 0.042 to 12.4 mg/L whilst concentrations in the range of 0.042 to 12.6 mg/L were observed at 72 hours (93% to 102% of the 0-Hour measured test concentrations). Given that no significant decline in measured concentration occurred between 0 and 72 hours it was considered appropriate to calculate the results based on the 0-Hour measured test concentrations only, which were determined to be 0.042, 0.13, 0.41, 1.3, 3.8 and 12.4 mg/L in the 0.16, 0.50, 1.6, 5.0, 16 and 50% v/v saturated solutions, respectively.

The study was conducted in accordance with the referred guidelines. The test was considered to be valid given that none deficiencies. The following data show that the cell concentration of the control cultures increased by a factor of 176 after 72 hours. This increase was in line with the OECD Guideline that states the enhancement must be at least by a factor of 16 after 72 hours.

Mean cell density of control at 0 hours: 6.14×10^3 cells per mL

Mean cell density of control at 72 hours: 1.08×10^6 cells per mL

The mean coefficient of variation for section by section specific growth rate for the control cultures was 6% and hence satisfied the validation criterion given in the OECD Guideline which states the mean must not exceed 35%.

The coefficient of variation for average specific growth rate for the control cultures over the test period (0 - 72 h) was 3% and hence satisfied the validation criterion given in the OECD Guideline which states that this must not exceed 7%.

The results of the laboratory study on the effects of benalaxyl isomer S on *Pseudokirchneriella subcapitata*, strain CCAP 278/4 demonstrate the 72-hour E_rC_{50} to be 3.4 mg/L, the 72-hour E_yC_{50} to be 0.086 mg/L (all endpoints based 0-Hour measured concentrations). The No-Observed-effect concentration (NOEC) was determined to be < 0.042 and the Low-Observed Effect Concentration (LOEC) equalled 0.042 mg/L.

11.5.4 Acute (short-term) toxicity to other aquatic organisms

No data are available.

11.6 Long-term aquatic hazard

Evaluation of chronic aquatic hazard for benalaxyl is based on studies which are considered valid in the Renewal Assessment Report of benalaxyl. All valid studies are presented in the table below and relevant studies for the classification purposes are also briefly summarised below.

Table 25: **Summary of relevant information on chronic aquatic toxicity**

Method	Species	Test material	Results[1]	Reference
Fish				
30 days (ELS; flow-through) OECD 210 (2013) GLP	Zebrafish (*Danio rerio*)	Benalaxyl Technical (purity: 98.4%)	NOEC = 0.079 mg/L (body weight) (based on measured concentrations)	RAR B.9.2.2.1 CA 8.2.2.1/02 Anonymous (2014)
Invertebrates				
21 days (flow though) OECD 202, Part II, Guideline (1984) GLP	*Daphnia magna*	Benalaxyl Technical (purity: 96.6%)	**NOEC = 0.03 mg/L** (based on measured average concentrations)	RAR B.9.2.5.1 CA 8.2.5.1/01 Anonymous (1992b)
28 days (spiked water) OECD 207 and BBA-Guideline proposal (1995) GLP	*Chironomus riparius*	Benalaxyl Technical (purity: 96.68% ± 0.95%)	NOEC = 3.13 mg/L (based on nominal concentrations)	RAR B.9.2.5.3 CA 8.2.5.3/01 Anonymous (1998)
Algae				
72 hours (static system) OECD 201 and EC method C.3 GLP	*Pseudokirchneriella subcapitata*	Benalaxyl technical (purity: 98.4%)	NOEC = 0.066 mg/L (based on geometric mean measured concentrations)	RAR B.9.2.6.1 CA 8.2.6.1/02 Anonymous (2014a)
72 hours (static system) OECD 201 and EC method C.3 GLP	*Pseudokirchneriella subcapitata*	Benalaxyl Isomer R (purity: 98.6 %)	NOEC = 0.35 mg/L (based on geometric mean measured concentrations)	RAR B.9.2.6.1 CA 8.2.6.1/03 Anonymous (2014b)
72 hours (static system) OECD 201 and EC method C.3 GLP	*Pseudokirchneriella subcapitata*	Benalaxyl Isomer S (purity: 98.3 %)	NOEC < 0.042 mg/L (based on geometric mean measured concentrations)	RAR B.9.2.6.1 CA 8.2.6.1/04 Anonymous (2014c)

[1] Indicate if the results are based on the measured or on the nominal concentration

11.6.1 Chronic toxicity to fish

Two studies testing the chronic (long-term) toxicity of benalaxyl, towards two different fish species are available. One of these studies (Anonymous, 1992a) was evaluated during Annex I inclusion of benalaxyl and the second study (Anonymous, 2014) was submitted for the purpose of EU renewal.

A prolonged toxicity test on Rainbow Trout (*Oncorhynchus mykiss*) was performed with benalaxyl (Jonas, W., 1992a). The study was conducted according to the OECD guideline 204 and GLP. Based on survival and growth of fish during this study, the NOEC (21 d) is 0.49 mg a.s./L (based on measured concentrations). This study is not relevant for the classification purposes.

The long-term toxicity of benalaxyl to fish was investigated with Zebrafish (*Danio rerio*) eggs and larvae in a 30 d post hatch toxicity test under flow-through conditions according to the OECD guideline 210 (GLP) (Anonymous, 2014). A NOEC of hatching success was determined to be 8.33 mg/L. The NOEC for survival, length, body weight (wet and dry weight) are 2.68, 0.85, 0.079 mg/L, respectively. This study is not relevant for the classification purposes.

Study 1: Anonymous, 1992a, Benalaxyl Prolonged Toxicity Test on the Rainbow Trout (*Oncorhynchus mykiss*), GLP, OECD 204 (1984)

The 21-day post hatch toxicity of benalaxyl to rainbow trout (*Oncorhynchus mykiss*) was determined in flow-through test, with groups of 10 fish per treatment exposed to 0.034, 0.060, 0.109, 0.196, 0.352, 0.634, 1.142, 2.056 and 3.70 mg a.s./L. A control and solvent control were also tested.

Fish were observed on days 1, 4, 7, 9, 11, 14, 16, 18 and 21 for mortality and sublethal effects of exposure. Temperature, dissolved oxygen content and pH were measured on the observation days. On days 1, 11 and 21, 200 ml of the test concentrations were sampled from the middle of the containers, filled into glass bottles and stored at ≤ 4°C until analysis by GC.

The study was conducted in accordance with the referred guidelines.

Under OECD 204 guidelines deviations were noted in the maintenance of the fish and observations. Aquaria were aerated in the flow-through test, although this is considered acceptable for semi-static procedures only. Fish were not fed during the test, contrary to the recommendation of a minimum of daily feeding in the guideline. Although fish were observed on Days 1, 4, 7, 9, 11, 14, 16, 18 and 21 and dead fish were removed during these observations, the guideline states that fish should be inspected daily and dead fish removed when observed. As there are no current guidelines in place for this test, any deviations cannot be determined.

The test was considered to be valid. None of the control fish died or showed signs of stress during the test and the oxygen concentration was > 60% of the air saturation value throughout the study.

As there are no current guidelines in place for this test, the validity according to current criteria cannot be determined.

The NOEC for rainbow trout was determined to be 0.49 mg a.s./L, based on measured concentrations.

Study 2: Anonymous, 2014, Toxicity of Benalaxyl Technical to Zebrafish (*Danio rerio*) in an Early-Life Stage Test, GLP, OECD 210 (2013)

The 30-day post hatch toxicity of benalaxyl technical to zebrafish (Danio rerio) was determined in an early-life stage test. Fertilised zebrafish eggs were exposed to the test item in a flow-through system at test item concentrations of 0.079, 0.26, 0.85, 2.68 and 8.33 mg/L and a control. The eggs and larvae were observed daily for any sublethal effects and mortality.

The study was conducted in accordance with the referred guidelines. The test was considered to be valid given that none deficiencies. All endpoints were based on mean measured concentrations. The NOEC for hatching success of zebrafish eggs was determined to be 8.33 mg/L. The NOEC for survival, length and wet- and dry-body weight are 2.68, 0.85, 0.079 mg/L respectively.

11.6.2 Chronic toxicity to aquatic invertebrates

One study is available testing the chronic toxicity of benalaxyl towards the water flea, Daphnia magna (Anonymous, 1992b). The study was conducted in accordance with the referred guidelines**.** According to the OECD 202 (1984) guidelines the study can be considered valid. There was a minor deviation in light intensity noted under current OECD 211 guidelines, with lux not being within the recommended range of 1000-1500. Taking into consideration the OECD 211 (2012) guidelines, mortality of the parent animals in the control was not more than 20% and the mean number of living offspring produced per parent animal surviving at the end of the test is 60, the study remains valid and acceptable for risk assessment.

The 21 d EC_{50} (immobilisation) was calculated to be 0.12 mg/l, the EC_0 (immobilisation, 21d) was 0.03 mg/l, and the EC_0 (reproduction, 21d) was 0.03 mg/l. An overall NOEC of 0.03 mg/**L** was established. All values are based on measured average concentrations. This study is relevant for the classification purposes**.**

Study: Anonymous, Benalaxyl Reproduction Test on *Daphnia magna*, GLP, OECD 202, Part II, Guideline (1984)

The toxicity of Benalaxyl to *Daphnia magna* was determined over 21 days under flow-through conditions, with groups of 40 daphnids (2 replicates of 20) per treatment exposed to nominal concentrations of 0.041, 0.122, 0.34, 1.1 and 3.3 mg/L. A control and solvent control were also prepared. The number of immobilised daphnids, observations of abnormal behaviour and the number of young daphnia (F1) were recorded on Days 1, 4, 7, 9, 11, 14, 16, 18 and 21. Temperature, pH and dissolved oxygen were measured in each test item and control solution on observations days. Samples were taken on Days 1, 11 and 21 for analysis of Benalaxyl by gas chromatography (GC).

The mean measured concentrations represented 78% ± 25% of nominal values for the test duration. Based on measured average concentrations, the 21-day EC_{50} (immobilisation, 21d) of 0.12 mg/l, EC_0 (immobilisation, 21d) of 0.03 mg/l, and EC_0 (reproduction, 21d) of 0.03 mg/l and an overall NOEC of 0.03 mg/l were established.

The study was conducted in accordance with the referred guidelines. According to the OECD 202 (1984) guidelines the study can be considered valid. There was a minor deviation in light intensity noted under current OECD 211 guidelines, with lux not being within the recommended range of 1000-1500.

Taking into consideration the OECD 211 (2012) guidelines, mortality of the parent animals in the control was not more than 20% and the mean number of living offspring produced per parent animal surviving at the end of the test is 60, the study remains valid and acceptable for risk assessment.

The 21 d EC_0 (immobilisation and reproduction) was calculated to be 0.03 mg/l. The NOEC value is 0.03 mg/L (based on measured average concentrations).

11.6.3 Chronic toxicity to algae or other aquatic plants

With regard to chronic (long-term) toxicity to algae of benalaxyl, four studies were carried out. One of these studies (Anonymous, 1982) was evaluated during Annex I inclusion of benalaxyl and it was accepted as supportive information only. Three studies (Anonymous, 2014a; 2014b; 2014c) was submitted for the purpose of EU renewal.

Chronic toxicity data with benalaxyl technical and its isomers (Isomer R and Isomers S) was available on Pseudokirchneriella subcapitata (see Table above). All tests were conducted according to the OECD test guideline 201 and GLP standards and no significant deviations from the test guideline were identified. The

study for benalaxyl technical is not considered relevant for the classification purposes. For benalaxyl the No-Observed-effect concentration (NOEC) for both growth rate and yield was determined to be 0.066 mg/L and the Low-Observed Effect Concentration (LOEC) equalled 0.031 mg/L (all endpoints based on mean measured concentrations). These studies are not relevant for the classification purposes.

11.6.4 Chronic toxicity to other aquatic organisms

One study is available testing the chronic toxicity of benalaxyl towards the *Chironomus riparius* (Anonymous, 1998). The test was conducted according to the OECD 207 and BBA-Guideline proposal (1995) and GLP standards. The value for the LC_{50} was calculated to be 17.7 mg a.s./L. The NOEC was 3.13 mg a.s./L and the LOEC was 6.25 mg a.s./L caused by the delay in development of larvae at and above concentrations of 6.25 mg a.s./L. Results were based on nominal concentrations. This study is not relevant for the classification purposes.

Study: Anonymous, Assessment of Side Effects of Benalaxyl Technical on the Larvae of Midge, *Chironomus riparius* with the Laboratory Test Method, GLP, OECD 207 and BBA-Guideline proposal (1995)

The effects of Benalaxyl on the life cycle of the midge (*Chironomus riparius*) via the water column were determined under static conditions with artificial sediment for a period of 28 days. The test substance was applied to the water column at nominal concentrations of 0.78, 1.56, 3.13, 6.25, 12.5, 25, 50 and 100 mg a.s./L. There were four replicates per test concentration, each containing twenty five larvae. Test vessels were observed three times per week to make a visual assessment of any behavioural difference compared with the control. During the period of expected emergence (normally starting at Day 10 and lasting until Day 24) a daily check of emerged midges was performed. The sex and number of emerging adults were recorded daily.

Temperature, oxygen concentration and pH were recorded at test start and end, and twice a week in all vessels for the duration of the test.

Samples of the overlying water, pore water and sediment were taken and separated 1 hour, 7 days and 28 days after application from the 1.56 mg a.s./L, 25 mg a.s./L and the blank. Analysis was reported in a separate study by Isagro Ricerca, number 2232.

The study was conducted in accordance with the referred guidelines. According to the OECD 207 and BBA-Guideline proposal (1995) guidelines the study can be considered valid.

Under the BBA guideline (1995) deviations were noted in timing of egg mass sampling prior to insertion of larvae, pH exceeding the limit as a consequence of algal growth, and observations not being conducted on Day 24. These were not considered to have a negative impact on the study. According to the current OECD 219 guidelines deviations were noted with the egg masses being sampled between 3 and 4 days before insertion into the test system (4 to 5 recommended), a higher number of larvae being introduced into each vessel than recommended (20) and the light intensity being slightly higher than the 500-1000 recommendation. None of these are thought to negatively impact the study.

According to the guidelines under which the study was conducted (OECD 207 and BBA-Guideline proposal (1995) as emergence in the control was higher than 70% (actual: 92%, 93% and 97%) and the mean development time for larvae was not more than 20 days after application (i.e. a development rate of 0.05) and not shorter than 10 days (i.e. a development rate of 0.1), the study was considered valid. Under OECD 219 the study would be considered valid as the controls had at least a 70% emergence at the end of the test, emergence to adults from control vessels occurred between 12 and 23 days and at the end of the test the oxygen concentration was at least 60% air saturation and pH was between 6 and 9.

The value for the LC_{50} was calculated to be 17.7 mg a.s./L. The NOEC was 3.13 mg a.s./L and the LOEC was 6.25 mg a.s./L caused by the delay in development of larvae at and above concentrations of 6.25 mg a.s./L. The maximum acceptable toxicant concentration (MATC) was 4.42 mg a.s./L. Results were based on nominal concentrations.

11.7 Comparison with the CLP criteria

11.7.1 Acute aquatic hazard

Acute aquatic toxicity data for benalaxyl are available for fish, aquatic invertebrates and algae, covering the three trophic levels that need to be assessed for CLP classification.

The criteria for Category Acute 1 in line with Table 4.1.0 from the Guidance on the Application of the CLP Criteria are:

96 hr LC_{50} (for fish)	≤ 1 mg/l and/or
48 hr EC_{50} (for crustacea)	≤ 1 mg/l and/or
72 or 96 hr E_rC_{50} (for algae or other aquatic plants)	≤ 1 mg/l.

The 96 h LC_{50} in fish (*Rainbow trout*) was 5.1 mg/L – the criteria does not fulfilled for aquatic acute toxicity category 1.

For aquatic invertebrates (*Daphnia magna*) the 48 h EC_{50} immobilisation was. 0.59 mg a.s./L lower than 1 mg/l; the classification criteria fulfilled for aquatic acute toxicity category 1.

The 72 h E_rC_{50} in Pseudokirchneriella subcapitata was 3.5 mg a.s./L - the criteria does not fulfilled for aquatic acute toxicity category 1.

Based on the available data it is concluded that benalaxyl fulfilled the criteria for classification as Aquatic Acute Category 1 (≤ 1 mg/l) according to the CLP based on effect at daphnia magna. As the lowest acute toxicity endpoint ranging < 0.1 to ≤ 1 mg/L, the corresponding Acute M-factor should be 1, based on the criteria set in Table 4.1.3 of the CLP Regulation.

11.7.2 Long-term aquatic hazard (including bioaccumulation potential and degradation)

The ready biodegradability of benalaxyl (96.68% radiochemical purity) investigated in a Manometric Respiratory Test (OECD 301 F) over a period of 28 days at 22ºC in the dark with benalaxyl tech. at a concentration of 24.9 mg/l applied to activated sludge value, based on ThODNO3 the percentage of biodegradation of benalaxyl reached –2.0% after 28 days. This values indicates that benalaxyl was not degraded by the activated sludge and can therefore be considered as “not readily biodegradable”.
Benalaxyl does not meet the criterion of rapid degradation, > 70 % within a 28 day period the aquatic environment.

An experimental bioconcentration study in fish is available. In the experimental study, whole fish BCF values for Benalaxyl (BCF = 57) were less than the CLP trigger value of 500 indicating a low potential for bioaccumulation.
Benalaxyl does not meet the CLP criterion (BCF ≥ 500) as a bioaccumulative substance.
In addition, the log P_{OW} of benalaxyl is 3.54 (at 20°C, pH = 6.1 and purity = 99.4%) which is below the CLP criterion of log Pow > 4.

Long-term aquatic toxicity data for benalaxyl are available for fish, aquatic invertebrates and algae, covering the three trophic levels that need to be assessed for CLP classification.
The criteria for Aquatic Chronic Toxicity Category 1 (for non rapidly degradable substances) in line with Table 4.1.0 from CLP Regulation:

NOEC or ECx(for fish)	≤ 0.1 mg/l and/or
NOEC or ECx (for crustacea)	≤ 0.1 mg/l and/or

NOEC or ECx (for algae or other aquatic plants) ≤ 0.1 mg/l.

The lowest NOEC value is the measured 21d-NOEC of 0.03 mg a.s./L for Daphnia magna. This value is ≤ 0.1 mg/L, and since benalaxyl is considered to be 'not rapidly degradable', it should be classified according to Regulation (EC) No. 1272/2008 as:

Aquatic Chronic Toxicity Category1 with a chronic M-factor = 1. based on 0.01 < NOEC ≤ 0.1 mg/L, according to CLP criteria stipulated in Annex I, p.4, Table 4..1.3.

11.8 CONCLUSION ON CLASSIFICATION AND LABELLING FOR ENVIRONMENTAL HAZARDS

Based on the CLP Regulation (1278/2008) criteria and available data, the proposal for classification for benalaxyl is:

Hazard Class and Category code(s)	M factor	Hazard Statement
Aquatic Acute Category 1, H400	1	Very toxic to aquatic life
Aquatic Chronic Category 1, H410	1	Very toxic to aquatic life with long lasting effects

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier Submitter's proposal

Benalaxyl is a fungicide which is currently classified as Aquatic Acute 1, H400 and Aquatic Chronic 1, H410 in Annex VI of the CLP Regulation. No M-factors have been set. The DS proposed to add M-factor of 1 to both acute and chronic classification based on the 48-hour EC_{50} for *Daphnia magna* of 0.59 mg/L (0.1 < EC_{50} ≤ 1 mg/L) and on the 21-day NOEC of 0.03 mg/L (0.01 < NOEC ≤ 0.1 mg/L, not rapidly degradable) for *Daphnia magna*.

Degradation

In an OECD TG 111 study, the buffered aqueous solutions of 1.5 to 15 mg/L benalaxyl (99.2% purity) were incubated in the dark at pH 4, 7 and 9 for a period of 5 days at 50ºC and 70º C. Benalaxyl was stable to hydrolysis at pH 4 and 7. At pH 9, DT_{50} values were 55 days at 50°C and 19 hours at 70°C. DT_{50} values of 157 days and 86 days at 20°C and 25ºC, respectively, were extrapolated from the results at the higher temperatures. The main hydrolysis product was identified as benalaxyl acid (M9= DL-alanine, N-2,6-xylyl-N-phenylacetyl).

Benalaxyl was not easily photolysed under natural sunlight conditions during June – August at 45º 28' N, 3º 10' W coordinates since 60% Applied Radioactivity (AR) was still present as benalaxyl after 64 days of exposure. At least 15 unidentified compounds were detected but none of them individually represented more than 5% of the initially applied

radioactivity.

In an aerobic surface water simulation test (GLP, OECD TG 309), degradation, transformation and mineralisation of ^{14}C-benalaxyl at two concentrations was studied in natural pond water. Natural water, pH 8.2, was treated with ^{14}C-benalaxyl at 10.5 µg/L and 106.7 µg/L and incubated in the dark at 23.4 ± 0.9°C for 62 days. Sterile controls and bio-controls were also set up. Duplicate test samples were taken at day 0, 7, 14, 21, 28, 42 and 62. Mineralisation of benalaxyl was negligible accounting for only 0.3% to 0.4% AR at the low- and high-test concentration, respectively. Insignificant transformation/degradation of benalaxyl was observed throughout the study period and therefore no DT_{50}/DT_{90} values could be derived. It was concluded that benalaxyl does not undergo any significant biodegradation/mineralisation in natural waters within the study period of 62 days.

In a GLP OECD TG 301 F Manometric respiratory test, ready biodegradability of benalaxyl (96.68% radiochemical purity) was investigated over a period of 28 days at 22ºC in the dark at a concentration of 30 mg suspended solids per litre. After 28 days of exposure the degradation rate of benalaxyl was –2.1. The DS considered that benalaxyl was not degraded by the activated sludge and can therefore be considered as "not readily biodegradable".

In a water/sediment study evaluated during the first EU review benalaxyl was observed to dissipate from the water compartment in two natural water/sediments according to a biphasic process. In the original evaluation benalaxyl was concluded to dissipate from the water phase with 1st order DT_{50} values of 5 and 10 days, respectively for the Pond and River systems, for the first phase. The second phase was slower and the corresponding 1st order DT_{50} values were 32 and 61 days for the Pond and River systems. No CO_2 was detected in the River system. In the Pond system CO_2 was only detected sporadically and reached a maximum of 0.4% AR at the end of the study. The main degradation products found in both systems were identified M1 (methyl-N-(2,6-xylyl)-N-malonyl alaninate) (max. < 10% AR) and M9 (benalaxyl acid) (< max. 10% AR). DT_{50} values in the whole sediment/water systems have been recalculated according to FOCUS (2006, 2011) guidance. Benalaxyl degraded with SFO DT_{50} values in the total system of 141.9 to 199.4 days at 20°C.

The DS presented summaries of the soil degradation studies in support of the original approval (Draft Assessment Report (DAR), 2000, 2003) in the CLH-Report. Due to concerns regarding possible shortcomings in the existing soil metabolism studies the route of degradation of benalaxyl was examined in four soils (loamy sand, clay, loam and silt loam) under aerobic conditions for 117 days according to OECD TG 307 (GLP). ^{14}C-benalaxyl degraded to 1.1% AR to 45.4% at the end of the study. Total non-extractable radioactivity reached a maximum of 22% to 50%. The RAR 2018 (Vol.3-Annex B.8) specified that $^{14}CO_2$ ranged from 3.5% AR to 17.1% AR in all four soils and organic volatiles were less than 0.1% AR. Two degradation products occurred at >5% at any sampling interval and were identified as M9 (10.1% AR) and M1 (45.2% AR).

Bioaccumulation

Benalaxyl has a log P_{OW} of 3.54 at 20°C and at pH=6.1 (Method EEC A 8, shake-flask method) which indicates a low bioaccumulation potential.

Bioconcentration potential of benalaxyl in fish was investigated in the non-GLP study

using in house method complying with US updated requirements. Bluegill sunfish were exposed at a concentration of 0.0524 mg as/L under flow-through conditions for up to 28 days. Benalaxyl concentration in fish reached a plateau level within 3 days of exposure corresponding to a whole fish BCF value of 57. As the study was conducted according to an in-house method the validity of the study could not be assessed. Due to lack of information and deficiencies in complying with the OECD TG 305 conditions DS was of the opinion that the validity of the study according to current guidelines cannot be confirmed.

Aquatic toxicity

Acute Aquatic toxicity

***Table**: Summary of reliable information on acute aquatic toxicity*

Method	Species	Test material (purity)	Results	Reference
Fish				
96-hour (semi-static) OECD TG 203, EC C.1 GLP	*Oncorhynchus mykiss*	Benalaxyl technical (98.4%)	LC_{50} = 4.8 mg/L (1 mm no solvent used, saturated solutions	RAR B.9.2.1. CA 8.2.1/06 Anonymous (2014a)
96-hour (semi-static) OECD TG 203, EC C.1 GLP	*Oncorhynchus mykiss*	Benalaxyl Isomer R (98.6%)	LC_{50} = 4.9 mg/L mm no solvent used, saturated solutions	RAR B.9.2.1. CA 8.2.1/07 Anonymous (2014b)
96-hour (semi-static) OECD TG 203, EC C.1 GLP	*Oncorhynchus mykiss*	Benalaxyl Isomer S (98.3%)	LC_{50} = 5.0 mg/L mm no solvent used, saturated solutions	RAR B.9.2.1. CA 8.2.1/08 Anonymous (2014c)
Aquatic invertebrates				
48 hours (static) OECD TG 202, EC C.2 GLP	*Daphnia magna*	Benalaxyl technical (98.4%)	EC_{50} = 15 mg/L measured (2 no solvent used, saturated solutions	DRAR B.9.2.4.1 CA 8.2.4.1/02 Anonymous (2014a)
48 hours (static) OECD TG 202, EC C.2 GLP	*Daphnia magna*	Benalaxyl Isomer R (98.6%) R/S ratio: 99.8/0.2	EC_{50} = 13 mg/L measured (3 no solvent used, saturated solutions	RAR B.9.2.4.1 CA 8.2.4.1/03 Anonymous (2014b)
48 hours (static) OECD TG 202, EC C.2 GLP	*Daphnia magna*	Benalaxyl Isomer S (98.3%) (R/S ratio: 0/100)	EC_{50} = 17 mg/L measured (4 no solvent used, saturated solutions	RAR B.9.2.4.1 CA 8.2.4.1/04 Anonymous (2014c)
48 hours (static) OECD TG 202, Part I (1984) GLP	*Daphnia magna*	Benalaxyl (96.6 %)	**EC_{50} = 0.59 mg/L** nominal, measured conc. not available acetone used as solvent (*	RAR B.9.2.4.1 CA 8.2.4.1/01 Anonymous (1993)

Algae				
72 hours (static) OECD TG 201, EC C.3 GLP	*Pseudokirchneriella subcapitata*	Benalaxyl technical (98.4%)	E_rC_{50} = 3.5 mg/L gmm (13-20% of nominal) no solvent used, saturated solutions	RAR B.9.2.6.1 CA 8.2.6.1/02 Anonymous (2014a)
72 hours (static) OECD TG 201, EC method C.3 GLP	*Pseudokirchneriella subcapitata*	Benalaxyl Isomer R (98.6%)	E_rC_{50}= 3.4 mg/L gmm (21-40% of nominal) no solvent used, saturated solutions	RAR B.9.2.6.1 CA 8.2.6.1/03 Anonymous (2014b)
72 hours (static) OECD TG 201, EC method C.3 GLP	*Pseudokirchneriella subcapitata*	Benalaxyl Isomer S (98.3%)	E_rC_{50}= 3.4 mg/L (5 measured no solvent used, saturated solutions	RAR B.9.2.6.1 CA 8.2.6.1/04 Anonymous (2014c)

(* the details concerning the dose preparation technique were taken from the DAR 2018 Volume 3CA B-9
gmm = based on geometric mean measured concentrations
mm = based on mean measured concentrations
(1 geomean of the highest concentration causing no mortalities and the lowest concentration causing 100% mortality
(2 There was no significant change < 80% (98.3 to 106.2%) in the measured concentrations at 48 hours and so the results are based on 0-Hour measured test concentrations only.
(3 There was no significant change < 80% (99.4 to 104.2%) in the measured concentrations at 48 hours and so the results are based on 0-Hour measured test concentrations only.
(4 There was no significant change < 80% (96.4 to 104.4%) in the measured concentrations at 48 hours and so the results are based on 0-Hour measured test concentrations only.
(5 There was no significant change < 80% (93 to 102 %) in the measured concentrations at 72 hours and so the results are based on 0-Hour measured test concentrations only.

In three OECD TG 203 fish studies performed with benalaxyl technical, benalaxyl Isomer R and benalaxyl Isomer S the 96-hour LC_{50} values were 4.8, 4.9 and 5.0 mg/L, respectively. The dose preparation included dispersion of nominal amounts of test item in test water with the aid of a propeller stirrer, filtration of undissolved test item and pooling the preparations to give the 100% v/v saturated solution test concentration which was used to give the test concentrations as v/v saturated solution. The results were based on mean measured concentrations.

For invertebrates there were four OECD TG 202 studies available. Three of the tests used a similar dose preparation technique, based on saturated concentrations, as in the fish studies. The 48-hour EC_{50} values for benalaxyl technical, benalaxyl Isomer R and benalaxyl Isomer S were 15, 13 and 17 mg/L, respectively. The results were based on initial measured concentrations.

The fourth invertebrate test was performed according to Part I of the OECD TG 202 (1984) with benalaxyl. The study was thought as screening test for the reproduction test on *Daphnia magna*. The 48-hour EC_{50} was 0.59 mg/L (nominal). Dose preparation in this test was based on using acetone as solvent. Control and solvent control using 100 mL acetone/L (used in the highest concentration of the test substance) were performed. There were deficiencies in reporting of the study conditions, but the validity criteria of the OECD TG 202 were fulfilled. However, as test concentrations were not measured, the study was included in the DAR as supportive information only. The DS considered the study to be reliable and used this study, showing the highest toxicity for aquatic organisms, as the basis for aquatic acute classification proposal.

The 72-hour E_rC_{50} values for algae, *Pseudokirchneriella subcapitata* were 3.5, 3.4 and 3.4 mg/L for to benalaxyl technical, benalaxyl Isomer R and benalaxyl Isomer S, respectively, in OECD TG 201 tests. The results are based on measured concentrations. All three tests used a similar dose preparation technique, based on saturated concentrations, as in the fish studies.

Chronic aquatic toxicity

***Table**: Summary of reliable information on chronic aquatic toxicity*

Method	Species	Test material	Results	Reference
Fish				
30 days (ELS; flow-through) OECD TG 210 (2013) GLP	*Danio rerio*	Benalaxyl Technical (98.4%)	NOEC = 0.079 mg/L (body weight) mm (>80% of nominal) DMF (1 used as solvent (*	RAR B.9.2.2.1 CA 8.2.2.1/02 Anonymous (2014)
Invertebrates				
21 days (flow though) OECD TG 202, Part II, (1984) GLP	*Daphnia magna*	Benalaxyl Technical (96.6%)	**NOEC = 0.03 mg/L** mm (78%±25% of nominal) acetone used as solvent (*	RAR B.9.2.5.1 CA 8.2.5.1/01 Anonymous (1992b)
28 days (spiked water) OECD TG 207 and BBA-Guideline proposal (1995) GLP	*Chironomus riparius*	Benalaxyl Technical (96.68% ± 0.95%)	NOEC = 3.13 mg/L (2 nominal acetone used as solvent (*	RAR B.9.2.5.3 CA 8.2.5.3/01 Anonymous (1998)
Algae				
72 hours (static) OECD TG 201 and EC C.3 GLP	*Pseudokirchneriella subcapitata*	Benalaxyl technical (98.4%)	E_rC_{10} = 0.33 mg/L NOEC = 0.066 mg/L (gmm 13-20% of nominal) no solvent used, saturated solutions	RAR B.9.2.6.1 CA 8.2.6.1/02 Anonymous (2014a)
72 hours (static) OECD TG 201 and EC C.3 GLP	*Pseudokirchneriella subcapitata*	Benalaxyl Isomer R (98.6 %)	E_rC_{10} = 0.49 mg/L NOEC = 0.35 mg/L (gmm 21-40% of nominal) no solvent used, saturated solutions	RAR B.9.2.6.1 CA 8.2.6.1/03 Anonymous (2014b)
72 hours (static) OECD TG 201 and EC C.3 GLP	*Pseudokirchneriella subcapitata*	Benalaxyl Isomer S (98.3 %)	E_rC_{10} = 0.12 mg/L (3NOEC < 0.042 mg/L measured	RAR B.9.2.6.1 CA 8.2.6.1/04 Anonymous

			no solvent used, saturated solutions	(2014c)

(*the details concerning the dose preparation technique were taken from the DAR 2018 Volume 3CA B-9
(1 dimethylformamide
(2 Results from the analysis of overlying water, pore water and sediment are reported in a separate study (DAR 2018, Volume 3 CA B9, study: Benalaxyl dissipates from the water and remains absorbed to sediment after 28 days)
(3 There was no significant change < 80% (93 to 102%) in the measured concentrations at 72 hours and so the results are based on 0-Hour measured test concentrations only.

There was one chronic study available on fish *Danio rerio* performed according to OECD TG 210 early-life stage test. Fertilised zebrafish eggs were exposed to the test item in a flow-through system to mean measured concentrations of 0.079, 0.26, 0.85, 2.68 and 8.33 mg/L, control, and a solvent control for a duration of 30 days after hatching. Dimethylformamide (DMF) was used as a solvent (100 µL/L) in dose preparation. The concentration of the solvent was in a range recommended in the test guideline. The eggs and larvae were observed daily for any sublethal effects and mortality. The NOEC for hatching success of zebrafish eggs was determined to be 8.33 mg/L. The NOEC values for survival, length and body weight (both wet- and dry-) were 2.68, 0.85, 0.079 mg/L respectively.

For invertebrates there were two studies available, one for *Daphnia* and one for *Chironomus riparius* (OECD TG 207, spiked water). Results from the analysis of overlying water, pore water and sediment of the *Chironomus* study showed that benalaxyl dissipates from the water and remains absorbed to sediment after 28 days.

In the OECD TG 202, Part II study toxicity of benalaxyl to *Daphnia magna* was determined over 21 days under flow-through conditions. *Daphnia* were exposed to nominal concentrations of 0.041, 0.122, 0.34, 1.1 and 3.3 mg/L. Acetone (165 µL/L) in the highest concentration of the test substance was used as the solubilising agent. The concentration of the solvent exceeded the highest concentration allowed in the current TG for chronic tests on daphnids. A control and solvent control were also prepared. The number of immobilised daphnids, observations of abnormal behaviour and the number of young daphnids (F1) were recorded on Days 1, 4, 7, 9, 11, 14, 16, 18 and 21. The samples for analytical control were taken at days 1, 11 and 21. The mean measured concentrations represented 78% ± 25% of nominal values for the test duration. Based on measured average concentrations, the 21-day EC_{50} (immobilisation, 21d) of 0.12 mg/L, EC_0 (immobilisation, 21d) of 0.03 mg/L, and EC_0 (reproduction, 21d) of 0.03 mg/L and an overall NOEC of 0.03 mg/L were established. The DS used this study, showing the highest toxicity for aquatic organisms, as the basis for aquatic chronic classification.

In addition, in the full study report available to the RAC, it was further explained that a test without the use of solubilising agent was performed. The stock solution was dispersed mechanically (ultrasonic bath). The nominal concentrations were 0.21, 1.04, 5.2 26 and 130 µg/L. No effects were seen in this test.

In the three algae studies (OECD TG 201) for benalaxyl technical, benalaxyl-R and benalaxyl-S 72-hour NOEC values of 0.066, 0.35 and <0.042 mg/L were, respectively, established based on measured concentrations. The corresponding E_rC_{10} values were 0.33, 0.49 and 0.12 mg/L, respectively. The dose preparation in each of the studies was

done by dispersing the test item with the aid of propeller stirring and removing the undissolved test item by filtration to get a 100% v/v saturated solution. A series of dilutions were made to give v/v stock solutions which were then inoculated to algal suspension to give the required test concentrations in v/v saturated solutions.

Comments received during consultation

The manufacturer supported retaining the classification Aquatic Acute 1, H400 and Aquatic Chronic 1, H410.

Comments were made by two MSCAs and one National Authority (NA). A MSCA thought that it was not clear why the aquatic acute classification was based on the study CA 8.2.4.1/01 (Daphnia, Part I of OECD TG 202) which was originally included in the DAR as supplementary information only. Due to lack of chemical analysis further explanation for identifying this study as a key study was requested. The DS gave no further explanation in response. Both MSCAs pointed out a mistake in Table 24 of the CLH report concerning nominal/measured concentrations in this study. The other MSCA supported the proposed classification with M-factor of 1 for both acute and chronic classification.

The NA also paid attention to the lack of analytical verification in the aquatic acute key study CA 8.2.4.1/01. They considered supporting information to be necessary to define the reliability of the *Daphnia magna* study. In their opinion the study could be accepted because the physico-chemical properties support that the substance would remain stable in the aquatic phase and because in other ecotoxicity studies the measured concentrations are generally within 80-120% of the initial measured concentrations. In addition, most of the study parameters were comparable to those recommended in OECD TG 202 (2004) and the control data met the validity criteria. The NA agreed to the proposed chronic classification. The DS thanked for the comments.

Assessment and comparison with the classification criteria

Comparison with the criteria

Degradation

RAC agrees with the DS conclusion that benalaxyl is not rapidly degradable:

- benalaxyl was not degraded in the OECD TG 301 F ready biodegradability test in 28 days.
- in the aerobic surface water simulation test (OECD TG 309) mineralisation of benalaxyl was negligible accounting for only 0.3% to 0.4% AR at the low- and high-test concentration, respectively. Benalaxyl did not undergo any significant biodegradation/mineralisation in natural waters within the study period of 62 days.
- In the OECD TG 111 hydrolysis study benalaxyl was stable to hydrolysis at pH 4 and 7. At pH 9 DT_{50}s of 157 days and 86 days at 20°C and 25ºC, respectively, were extrapolated from the results at the higher temperatures.
- In the water/sediment study benalaxyl degraded with SFO DT_{50}s of 141.9 to 199.4 days at 20°C in the total system. Mineralisation was negligible.

Bioaccumulation

RAC agrees with the DS conclusion that benalaxyl has a low potential for bioaccumulation.

The available fish bioconcentration study has been considered not reliable by the DS; RAC agrees with this assessment.

In the octanol/water-coefficient study using shake-flask method a log K_{OW} of 3.54 at 20°C and at pH=6.1 was measured. Benalaxyl can, however, be considered as a surface-active substance because of the surface tension of 47.0 mN/m and thus the shake-flask method is not applicable. The KOWWIN v1.68 estimated log K_{ow} of 3.69 is, however, in the same order of magnitude. The log K_{ow} values are below the classification cut-off value of 4.

Aquatic toxicity

RAC notes that when reviewing the aquatic toxicity studies, one possible explanation to the over one order of magnitude difference in toxicity study results for the same species might be the dose preparation technique used. This can, obviously, be noted only when there are studies on the same trophic level using different dose preparation techniques. The used techniques are either based on the use of a solvent or the use of stirring followed with filtration of undissolved test item to get saturated test solutions. Both techniques are seen appropriate for poorly soluble substances in the OECD Guidance Document 23.

RAC also used ECOSAR v1.11 QSAR model to estimate the toxicity of benalaxyl. The model used prediction for esters, amides and baseline toxicity but neither of the estimations fitted the test results available for classification. RAC concludes that QSAR predictions for toxicity are not relevant in this case.

Acute

In the three reliable fish studies saturated solutions were used for dose preparation. The lowest 96-hour LC_{50} was 4.8 mg/L (measured) for benalaxyl technical.

For invertebrates three of the four studies on *Daphnia magna* used saturated solutions for dose preparation. The tests are considered reliable by RAC. The lowest 48-hour EC_{50} was 13 mg/L (measured) for benalaxyl-R. The fourth test was performed using acetone as solvent and the 48-hour EC_{50} was 0.59 mg/L (nominal). Despite the lack of measured concentrations, RAC considers this study reliable based upon the evaluation of the full study report. The study was a screening test for the reproduction study and led to the choice of the tested concentrations according to the OECD TG 202 (1984). The physico-chemical profile of the substance does not indicate any potential mechanism that would cause the test concentrations decline during the test. No information on tests using solvent, and which had measured concentrations for 48-hours, was available, therefore no comparison with tests using saturated solution method was possible.

In the three reliable algae studies saturated solutions were used for dose preparation. The lowest 72-hour E_rC_{50} was 3.4 mg/L for benalaxyl-R and benalaxyl-S.

RAC considers that the lowest acute toxicity value is a 48-hour EC_{50} of 0.59 mg/L (nominal) for *Daphnia*. The EC_{50} is in the range of $0.1 < EC_{50} \leq 1$ and thus M-factor of 1 is warranted.

Chronic

There is only one reliable chronic fish test available. Dimethylformamide was used as solvent in the test which gave a 30-day NOEC of 0.079 mg/L (measured) for benalaxyl technical.

For invertebrates there were two chronic studies available. The full study report available to RAC provided more detailed information of the *Daphnia magna* study. After evaluating the full study report RAC agrees with the DS to consider the study valid and reliable. RAC notes that for dose preparation acetone was used as a solvent. The concentration of acetone used exceeded (165 µL/L) the highest concentration allowed in the current TG for chronic tests on daphnids (0.1 mL/L in OECD TG 211). The 21-day NOEC (body weight) for benalaxyl technical was 0.03 mg/L (measured).

The *Chironomus* study is not in this case seen relevant for classification since the conclusion of the additional study concluded that benalaxyl dissipates from the water and remains absorbed to sediment after 28 days. Therefore, the exposure route in the study cannot be confirmed.

There are three reliable chronic algae studies available for benalaxyl technical, benalaxyl-R and benalaxyl-S. The lowest NOEC and E_rC_{10} values are < 0.042 and 0.12 mg/L, respectively, for benalaxyl-S. EC_{10} values are preferred by RAC as these are statistically derived from the entire dataset, and less dependent on test design considerations than the NOEC.

RAC considers that the lowest chronic toxicity value is a 21-day NOEC of 0.03 mg/L (measured) for *Daphnia*. The NOEC is in the range of 0.01 < NOEC ≤ 0.1 and, for a not rapidly degradable substance, an M-factor of 1 is warranted.

RAC, thus, agrees with the DS proposal to classify benalaxyl with **Aquatic Acute 1, H400, M = 1 and Aquatic Chronic 1, H410, M = 1**.

RAC considers, however, that the classification of benalaxyl might have to be revisited in case

- new acute invertebrate toxicity data based on measured concentrations become available;
- toxicity data on blue-green algae (cyanobacteria) become available. As a fungicide benalaxyl can be considered to have anti-microbial activity and blue-green algae are potentially more sensitive than green-algae to anti-microbials. With the current difference in sensitivity between algae and daphnid of less than a factor of 10, this could influence the current classification;
- the reason for over one order of magnitude differences in aquatic toxicity studies on the same species find an explanation.

12 EVALUATION OF ADDITIONAL HAZARDS

12.1 Hazardous to the ozone layer

None

13 ADDITIONAL LABELLING

None

14 REFERENCES

14.1 Physico-chemical properties

Data point	Year	Title
CA 2.1/01 CA 2.2, CA 2.3, CA 2.5, CA 2.6 CA 2.7/01, CA 2.9/02, CA 2.9/11, CA 2.9/12, CA 2.9/13 CA 2.10	1995	Benalaxyl purified active substance: Physical and chemical properties of the purified active substance, BIOLAB, Project report No.: 94/1087.B, Company file No.: 108, GLP. Unpublished
CA 2.1/02	1995	Boiling point determination of benalaxyl purified active substance, EniChem I.G.D., Project report No.: 030/95, Company file No.: 110, GLP. Unpublished
CA 2.9/01	1993	Flammability (Solids) test substance: Benalaxyl., NATEC, Project report No.: NA 91 9628/2, Company file No: 102 GLP. Unpublished

14.2 Toxicology and metabolism

Data point	Year	Title
CA_B.6.1.2.1	1996a	Triolo, A.14C-Benalaxyl, Preliminary pharmacokinetics study in the rat after single oral administration RBM, Istituto di Ricerche Biomediche “A. Marxer”, Italy; RBM Exp. No. 950606. Company file No. 111, Not GLP/ Not published
CA_B.6.1.2.2	1996b	Triolo, A. 14C-Benalaxyl, Blood pharmacokinetics, excretion and tissue distribution of radioactivity in the rat after a single oral administration (final report) RBM, Istituto di Ricerche Biomediche “A. Marxer”, Italy; RBM Exp. No. 950607. Company file No. 112,GLP / Not published
CA_B.6.1.2.3	1996c	Triolo, A. 14C-Benalaxyl, Blood pharmacokinetics, excretion and tissue distribution of radioactivity in the rat after repeated oral administrations. RBM, Istituto di Ricerche Biomediche “A. Marxer”, Italy; RBM Exp. No. 950608. Company file No. 113, GLP / Not published
CA_B.6.1.2.4	1997	Castoldi, F.C., Pizzingrilli, G. Profiling of radiolabelled metabolites of 14C-Benalaxyl in urine and faeces of male rats after single and repeated oral administration, Isagro Ricerca, Italy – Report No. R/ABT.96.01. Company file No. 113/1, GLP /Not published
CA_B.6.1.2.5	2001	Kemp, L. [14C]-Benalaxyl Biliary excretion study in the rat, Huntingdon Life Sciences, Ltd, UK – Study No. IGA 001/12155. Company file No. 188, GLP Not published

Data point	Year	Title
CA_B.6.1.2.6	1981	Santi, R., Guarnieri, R. Absorption, metabolism and excretion of 14C-GALBEN in Albino rats (part 1) Farmoplant, Italy. Company file No. 59, Not GLP/Not published
CA_B.6.1.2.7	1983	Guarnieri, R., Pizzingrilli, G.F., Santi, R., Valcamonica, C.14C-GALBEN Metabolism in albino rats (part 2), Farmoplant, Italy. Company file No. 60, Not GLP /Not published
CA_B.6.1.3.1	1983	Pizzingrilli, G.F., Valcamonica, C. In vitro degradation of 14C-GALBEN with rat liver microsomes, supplementary study, Farmoplant, Italy. Company file No. 89, Not GLP /Not published
CA_B.6.1.3.5	2007	Jing Qiu Qiuxia Wang Wentao Zhu Guifang Jia Xinquan Wang Zhiqiang Zhou Stereoselective determination of benalaxyl in plasma by chiral high-performance liquid chromatography with diode array detector and application to pharmacokinetic study in rabbits Chirality. 2007 Jan;19(1):51-5.
CA_B.6.1.3.6	2011	Ping Zhang 1, Wentao Zhu, Ziheng Dang, Zhigang Shen, Xinyuan Xu, Ledan Huang, Zhiqiang Zhou Stereoselective Metabolism of Benalaxyl in Liver Microsomes From Rat and Rabbit Chirality. 2011 Feb; 23(2):93-8
CA_B.6.3.2.1	1985	Maltoni, C., Soffritti, M., Perino, G.et. al. Short-term range finding study of evaluation of chronic and oncogenic potential of Galben® in Swiss mice (Oral Dosing Study) Bentivoglio (BT) Experimental Laboratories of the Institute of Oncology “F. Addarii”, Italy; Study No. BT 5004 bis.Company file No. 14; Not GLP / Not published
CA_B.6.3.2.5	1982	Mondino, A., Verardi, A., et al., Study of the toxicity of repeated oral administration of product M 9834 (GALBEN) to Beagle dogs at the dosage levels of 10, 200 and 800 ppm for 52 weeks, RBM, Institute of Biomedical Research "Antoine Marxer", Italy; Experiment No. 1170. Company file No. 12, GLP / Not published
CA_B.6.4.1.3	1979a	De Carneri, I., et al, Microbiological mutagenesis study on CRA 109 (M 9834); Genetic mutation test in Salmonella typhimurium (Ames), Farmitalia Carlo Erba, Italy. Company file No. 18, Not GLP / Not published
CA_B.6.4.1.4	2002a	Wollny, H.E Salmonella Typhimurium reverse mutation assay with BENALAXYL RCC-CCR, Germany; Project No. 709401. Company file No. 207 GLP / Not published
CA_B.6.4.1.5	1979b	De Carneri, I., et al, Microbiological study of mutagenesis on CRA 109 (M 9834): DNA damage and repair test (Mitotic gene conversion in Saccharomyces cerevisiae D4), Farmitalia Carlo Erba, Italy. Company file No. 19, Not GLP /Not published
CA_B.6.4.1.6	1980	De Carneri, I., et al , Microbiological study of mutagenesis on CRA 109 (M 9834): In vitro gene mutation test in Schizosaccharomyces pombe P1, Farmitalia Carlo Erba, Italy. Company file No. 20, Not GLP / Not published
CA_B.6.4.1.7	2002a	Schulz, M In vitro chromosome aberration test in Chinese Hamster Ovary (CHO) Cells with benalaxyl, RCC-CCR, Germany; Project No. 709404. Company file No. 211, GLP / Not published
CA_B.6.4.1.8	1980	Mondino, A. Fumero, S., et al., “In vitro” study of the induction of chromosome aberrations by compound M 9834 in human lymphocyte cultures, RBM, Institute of Biomedical Research "Antoine Marxer", Italy. Company file No. 21, Not GLP / Not published
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Data point	Year	Title
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CA_B.6.4.2.3	1980	Mondino, A., In vivo" study of the induction of chromosome aberrations in the Chinese hamster by compound M 9834, administered orally RBM, Institute of Biomedical Research "Antoine Marxer", Italy; Experiment No. M 201; Company file No. 22, Not GLP / Not published
CA_B.6.5.1.1	1983	Becci, P.J., Thompson, S.W., Davidson, T.J., Lifetime oral dosing studies in rats: combined oncogenicity and chronic toxicity of GALBEN technical (M 9834), Food & Drug Research Laboratory, USA; FDRL Report No. 6568-11. Company file No. 13, GLP / Not published
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14.3 Environment

Data point	Year	Title
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CA 7.1.1.1/03	2015a	Degradation and Metabolism of ^{14}C-benalaxyl in four soils incubated under aerobic conditions, GLP, Unpublished
CA 7.2.2.2/01	2015b	Aerobic Mineralisation of ^{14}C-benalaxyl in Surface Water - Simulation Biodegradation Test, GLP, Unpublished
CA 7.1.2.2.1/01	1981	Degradation rate of GALBEN in the soil laboratory measurement + Appendix A: Analytical Method, Non-GLP, Unpublished
CA 7.1.2.1.1/03, CA 7.1.2.1.2/04	2009	Determination of modelling endpoints for benalaxyl and soil metabolites from laboratory degradation studies, using Modelmaker 4.0, according to FOCUS kinetics, Non-GLP, Unpublished
CA 7.1.3.1.1/01	1993	Adsorption/Desorption test substance: Benalaxyl, GLP, Unpublished
CA 7.1.3.1.1/02	2018	[^{14}C]Benalaxyl determination of adsorption/desorption behaviour in two soils.
CA 7.2.2.3/01	1997	[14C-U-Aniline Ring] benalaxyl: Degradation and Retention in Water-Sediment Systems, GLP, unpublished

Data point	Year	Title
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CA 8.2.1/02	1979b	Four-day static aquatic toxicity studies with M 9834 in guppy, Not GLP, Unpublished
CA 8.2.1/03	1979c	Four-day static aquatic toxicity studies with M 9834 in rainbow trout, Not GLP, Unpublished
CA 8.2.1/04	1980	Determination of the acute toxicity of M 9834, technical grade for Cyprinus carpio L, Not GLP, Unpublished
CA 8.2.1/05	1984	Bluegill sunfish 96 hour static acute toxicity test with GALBEN, GLP, Unpublished
CA 8.2.2.1/02	2014	Toxicity of Benalaxyl Technical to Zebrafish (Danio rerio) in an Early-Life Stage Test, GLP, Unpublished
CA 8.2.4.1/02	2014a	Benalaxyl technical: Daphnia sp., 48-Hour Acute Immobilization Test, GLP, Unpublished
CA 8.2.2.1/03	2014b	Benalaxyl isomer R: Daphnia sp., 48-Hour Acute Immobilization Test, GLP, Unpublished
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CA 8.2.5.3/01	1998	Assessment of side effects of benalaxyl technical on the larvae of the midge, Chironomus riparius with the laboratory test method, GLP, Unpublished
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CA 8.2.5.1/01	1992b	Benalaxyl reproduction test on Daphnia magna, Report No. 90 9455, GLP, Unpublished
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CA 8.2.1/06	2014a	Benalaxyl technical: Acute Toxicity to Rainbow Trout (Oncorhynchus mykiss), GLP, Unpublished
CA 8.2.1/07	2014b	Benalaxyl isomer R: Acute Toxicity to Rainbow Trout (Oncorhynchus mykiss), GLP, Unpublished
CA 8.2.1/08	2014c	Benalaxyl isomer S: Acute Toxicity to Rainbow Trout (Oncorhynchus mykiss), GLP, Unpublished
CA 8.2.6.1/01	1982	Toxicity study with technical Galben on Selenastrum capricornutum, Not GLP, Unpublished
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CA 8.2.6.1/03	2014b	Benalaxyl isomer R: Algal Growth Inhibition Test, Harlan Laboratories, Ltd., UK, Report No. 41206983, FMC Study No. 2014ETX-BEN0617, GLP, Unpublished
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15 ANNEXES

The five sections (below) of the revised Renewal Assessment Report (RAR, 2018) of the active substance Benalaxyl containing confidential data have been included as confidential attachments.

Benalaxyl_RAR_03_Vol-3CA_B-1_2018-08-revised.pdf

Benalaxyl_RAR_04_Vol_3CA_B-2_2018-08-revised.pdf

Benalaxyl_RAR_08_Vol-3CA_B-6_2018-10-revised.pdf

Benalaxyl_RAR_10_Vol-3CA_B 8_2018-10-revised.pdf

Benalaxyl_RAR_11_Vol-3CA_B-9_2018-10-revised.pdf