Committee for Risk Assessment

RAC

Opinion

proposing harmonised classification and labelling
at EU level of

dimethachlor (ISO); 2-chloro-*N*-(2,6-dimethylphenyl)-*N*-(2-methoxyethyl)acetamide

EC Number: 256-625-6
CAS Number: 50563-36-5

CLH-O-0000007432-78-01/F

Adopted

14 March 2024

14 March 2024

CLH-O-0000007432-78-01/F

OPINION OF THE COMMITTEE FOR RISK ASSESSMENT ON A DOSSIER PROPOSING HARMONISED CLASSIFICATION AND LABELLING AT EU LEVEL

In accordance with Article 37 (4) of Regulation (EC) No 1272/2008, the Classification, Labelling and Packaging (CLP) Regulation, the Committee for Risk Assessment (RAC) has adopted on **14 March 2024** by **consensus** an opinion on the proposal for harmonised classification and labelling (CLH) of:

Chemical name:	**dimethachlor (ISO); 2-chloro-*N*-(2,6-dimethylphenyl)-*N*-(2-methoxyethyl)acetamide**
EC Number:	**256-625-6**
CAS Number:	**50563-36-5**

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Rapporteur, appointed by RAC:	**Gerlienke Schuur**
Co-Rapporteur, appointed by RAC:	**Dania Esposito**

Administrative information on the opinion

Croatia has submitted on **4 May 2023** a CLH dossier containing a proposal together with the justification and background information documented in a CLH report.

The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/harmonised-classification-and-labelling-consultation/*** on **22 May 2023**.

Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **21 July 2023**.

The opinion takes into account the comments provided by MSCAs and concerned parties in accordance with Article 37(4) of the CLP Regulation and the comments received are compiled in Annex 2.

The following table provides a summary of the Current Annex VI entry, Dossier submitter proposal, RAC opinion and potential Annex VI entry, if agreed by the Commission.

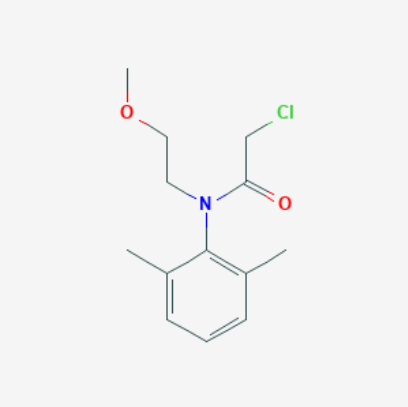
Classification and labelling in accordance with the CLP Regulation (Regulation (EC) 1272/2008)

	Index No	Chemical name	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATE	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	616-031-00-3	dimethachlor (ISO); 2-chloro-*N*-(2,6-dimethylphenyl)-*N*-(2-methoxyethyl)acetamide	256-625-6	50563-36-5	Acute Tox. 4* Skin Sens. 1 Aquatic Acute 1 Aquatic Chronic 1	H302 H317 H400 H410	GHS07 GHS09 Wng	H302 H317 H410			
Dossier submitters proposal	616-031-00-3	dimethachlor (ISO); 2-chloro-*N*-(2,6-dimethylphenyl)-*N*-(2-methoxyethyl)acetamide	256-625-6	50563-36-5	**Retain** Skin Sens. 1 Aquatic Acute 1 Aquatic Chronic 1 **Add** Carc. 2 **Modify** Acute Tox. 4	**Retain** H302 H317 H400 H410 **Add** H351	**Retain** GHS07 GHS09 Wng **Add** GHS08	**Retain** H302 H317 H410 **Add** H351		**Add** oral: ATE = 1600 mg/kg bw M = 10 M = 10	
RAC opinion	616-031-00-3	dimethachlor (ISO); 2-chloro-*N*-(2,6-dimethylphenyl)-*N*-(2-methoxyethyl)acetamide	256-625-6	50563-36-5	Carc. 2 Acute Tox. 4 Skin Sens. 1 Aquatic Acute 1 Aquatic Chronic 1	H351 H302 H317 H400 H410	GHS08 GHS07 GHS09 Wng	H351 H302 H317 H410		oral: ATE = 1600 mg/kg bw M = 10 M = 10	
Resulting Annex VI entry if agreed by COM	616-031-00-3	dimethachlor (ISO); 2-chloro-*N*-(2,6-dimethylphenyl)-*N*-(2-methoxyethyl)acetamide	256-625-6	50563-36-5	Carc. 2 Acute Tox. 4 Skin Sens. 1 Aquatic Acute 1 Aquatic Chronic 1	H351 H302 H317 H400 H410	GHS08 GHS07 GHS09 Wng	H351 H302 H317 H410		oral: ATE = 1600 mg/kg bw M = 10 M = 10	

GROUNDS FOR ADOPTION OF THE OPINION

RAC general comment

Dimethachlor is an approved active substance in the Plant Protection Products Regulation. A draft Renewal Assessment Report (DRAR) is prepared for the renewal of the approval. Dimethachlor is used as an herbicide.



The pure substance forms colourless crystals. Water solubility is 2.1 g/L at 20 °C and pH 6.5. Vapour pressure is 2.3 × 10^{-3} Pa at 20 °C. Log P_{ow} is 2.17 at 25 °C.

After oral administration to rats, dimethachlor is almost completely absorbed from the intestinal tract. Within 7 days 88-97 % of the dose is excreted. Dimethachlor was found in blood perfused organs such as lungs, heart, kidneys, liver and spleen. Half-lives of dimethachlor and/or its metabolites from tissues is about 2-3 weeks. Dimethachlor binds covalently to the rat haemoglobin molecule, which is unlikely for the human haemoglobin. This might be due to differences in the structure, where the rat haemoglobin contains a reactive cysteine residue available for the reaction with the activated carbon atom in the chloracetyl moiety of dimethachlor.

Metabolism of dimethachlor is extensive, and biotransformation follows two main routes. One pathway results in substitution of the chlorine via a glutathione pathway. The other pathway results from oxidation and glucuronidation following O-demethylation. Minor metabolic pathways include the reduction of the methylene chloride moiety (-CH_2Cl) giving rise to acetyl derivatives, the replacement of the chlorine by –OH and the subsequent oxidation to oxalic acid derivatives. *In vitro* metabolic profiling carried out in human, Wistar rat, CD1 mouse, New-Zealand rabbit, and Beagle dog by incubating liver microsomes with 10 µM of [phenyl-U-14C]-dimethachlor and a NADPH-regenerating system for 60 minutes at 37 °C, showed that the metabolism of dimethachlor in liver microsomes was qualitatively similar between human, rat, mouse, rabbit and dog; with no unique human metabolites observed (Thibaut, 2019).

RAC evaluation of physical hazards

Summary of the Dossier Submitter's proposal

Explosives

Dimethachlor was tested in the EEC A.14 test for all three parts (thermal sensitivity, mechanical sensitivity and differential scanning calorimetry) with a result concluding that the substance is

not an explosive. In the ASTM E537 (thermal stability via differential scanning calorimetry) it was shown that the exothermic decomposition energy is less than 500 J/g. Further, the performed calculation on the oxygen balance resulted in -206.5, which is below the trigger of -200 for explosive properties.

The Dossier Submitter (DS) proposed no classification based on the absence of functional groups associated with explosive properties in the molecule. Performed tests on thermal stability and exothermic decomposition energy support this.

Flammable solids

Both from the EEC A.10 and the UN Test N.1 it was concluded that dimethachlor was not considered highly flammable.

The DS proposed no classification, as a flame of a gas burner resulted in melting of the substance, it did not catch fire and combustion was not propagated.

Self-reactive substances

Dimethachlor was tested in EEC A.10 and A.16, as well as in IEC 60079-20-1. No self-ignition was shown up to 465±25 °C.

The DS proposed no classification based on that there are no functional groups associated with self-reactive properties present in the molecule.

Pyrophoric solid

Dimethachlor was tested in EEC A.10 and A.16, as well as in IEC 60079-20-1. No self-ignition was shown up to 465±25°C.

The DS proposed no classification based on these data, as well as relevant data over the years of use of dimethachlor.

Self-heating substances

Dimethachlor was tested in EEC A.10 and A.16, as well as in IEC 60079-20-1. No self-ignition was shown up to 465±25 °C. Screening criteria for non-classification, a melting point below 160°C (namely 45.8-46.7 °C), is in accordance with the data.

The DS proposed no classification.

Substances which in contact with water emit flammable gases

According to the Annex I part 2 point 2.12.4.1 of Regulation (EC) No. 1272/2008: "The classification procedure for this class need not be applied if: (a) the chemical structure of the substance or mixture does not contain metals or metalloids".

As there are no metals or metalloids present in the molecule, it can be concluded that dimethachlor is not to be considered as substance which in contact with water emit flammable gas.

The DS proposed no classification.

Oxidising solid

The tests EEC A.17 and UN Test O.2 (although relevant for oxidising liquids) performed result in the conclusion that dimethachlor is not considered an oxidising substance. Dimethachlor contains oxygen but it is bonded to carbon only.

Thus, according to the Annex I part 2 point 2.14.4.1 of Regulation (EC) No. 1272/2008: "For organic substances or mixtures the classification procedure for this class shall not apply if:

(a) the substance or mixture does not contain oxygen, fluorine or chlorine; or

(b) *the substance or mixture contains oxygen, fluorine or chlorine and these elements are chemically bonded only to carbon or hydrogen*."

As dimethachlor contains oxygen, which is chemically bonded only to carbon, the DS concluded that dimethachlor is not an oxidising substance.

Organic peroxides

Dimethachlor does not contain the organic peroxide -O-O- structure. The hazard class is not applicable as dimethachlor does not contain an organic peroxide.

The DS concluded no classification.

Corrosive to metals

There is no test available.

The DS proposed no classification based on all other relevant data.

Comments received during consultation

No comments were received regarding physical hazards.

Assessment and comparison with the classification criteria

Explosives

RAC concurs with the DS that no classification is warranted for dimethachlor for explosive properties based on the absence of functional groups associated with explosive properties in the molecule. Performed tests on thermal stability and exothermic decomposition energy support this. Further, the performed calculation on the oxygen balance resulted in -206.5, which is below the trigger of -200 for explosive properties. RAC concludes that the substance **does not warrant classification for explosive properties**.

Flammable solids

RAC concurs with the DS on no classification, as the result in the EEC A.10 and UN Test N.1 was "not highly flammable". RAC concludes that the substance **does not warrant classification for flammable solids properties**.

Self-reactive substances

RAC concurs with the DS on no classification, based on the fact that there are no functional groups associated with self-reactive properties present in the dimethachlor structure. RAC concludes that the substance **does not warrant classification for self-reactive properties**.

Pyrophoric solid

RAC concurs with the DS on no classification, based on the screening procedure, i.e. based on experience in the manufacturing or handling. RAC concludes that the substance **does not warrant classification for pyrophoric solid properties**.

Self-heating substances

RAC concurs with the DS on no classification, based on the results in an EEC A.16 test where no self-ignition was shown up to 465±25 °C. Screening criteria for non-classification, a melting point below 160 °C (namely 45.8-46.7 °C), is in accordance with the data. RAC concludes that the substance **does not warrant classification for self-heating properties**.

Substances which in contact with water emit flammable gases

RAC concurs with the DS on no classification based on the non-presence of metals or metalloids in the molecule. RAC concludes that the substance **does not warrant classification for this hazard class**.

Oxidising solid

RAC concurs with the DS on no classification, based on the fact that dimethachlor contains oxygen which is chemically bonded only to carbon (according to the Annex I part 2 point 2.14.4.1 of Regulation (EC) No. 1272/2008)). RAC concludes that the substance **does not warrant classification for oxidising properties**.

Organic peroxides

RAC concurs with the DS on no classification, based on the fact that dimethachlor does not contain the organic peroxide -O-O- structure. RAC concludes that the substance **does not warrant classification for this hazard class**.

Corrosive to metals

For Corrosive to metals, it is unclear which other data the DS is referring to. The melting point (45.8-46.7 °C) is below 55 °C and therefore the UN Test C.1 would be applicable for dimethachlor but is not available. RAC concludes that the substance **does not warrant classification as corrosive to metals**, due to lack of data.

HUMAN HEALTH HAZARD EVALUATION

RAC evaluation of acute toxicity

Summary of the Dossier Submitter's proposal

Acute oral toxicity

Two acute oral toxicity studies with dimethachlor are available.

An acute oral toxicity study (Anonymous, 1993a) was performed according to OECD TG 401 and GLP, with 5 rats per sex dosed by gavage at 2000 mg/kg bw, and 5 females at 1000 mg/kg bw dimethachlor (purity 96.8 % w/w). At 2000 mg/kg bw 2 females died. The LD_{50} was determined to be > 2000 mg/kg bw for males, > 1000 mg/kg bw and approximately 2000 mg/kg bw for females, and ≥ 2000 mg/kg bw for both sexes. No rat. Male or female, died at the other doses.

In a pre-guideline, pre-GLP acute oral toxicity study (Anonymous, 1973), rats (n = 5 per sex/group) were dosed by gavage at 1000, 1290, 1670, 2150 or 3170 mg/kg bw dimethachlor (purity not reported), followed by an observation period of 7 days. Mortalities were seen in 0/5, 1/5, 3/5, 2/5 and 5/5 males and 0/5, 2/5, 3/5, 5/5 and 5/5 females respectively. The LD_{50} was calculated as 1600 (95 % CI: 1250-2048) mg/kg bw for both sexes.

The DS proposed classification as Acute Tox. 4; H302 based on the acute oral LD_{50} of dimethachlor in female rats which was estimated to be less than the upper criterion of 2000 mg/kg bw, supported by the LD_{50} value of 1600 mg/kg bw determined in the earlier (pre-guideline and pre-GLP) study.

Acute dermal toxicity

No mortalities were found in an acute dermal toxicity study (Anonymous, 1993b) performed according to OECD TG 402 and GLP in rats (n = 5 per sex) exposed to the limit dose of 2000 mg/kg bw dimethachlor (purity 96.8 %) for 24 hours.

The DS proposed no classification as the LD_{50} is above the cut-off of 2000 mg/kg bw.

Acute inhalation toxicity

No mortalities were found in an acute inhalation toxicity study (Anonymous, 1994) performed according to OECD TG 403 and GLP in rats (n = 5 per sex) exposed nose-only to an aerosol (MMAD 1.2-2.2 µm; GSD 2.3-2.7) of analytical concentration of 4.45 mg/L dimethachlor (purity: 96.8 %) for 4 hours. The concentration of 4.45 mg/L was the maximum attainable concentration.

The DS proposed no classification as the 4-hour LC_{50} is > 4.45 mg/L, which is close to the classification threshold value of 5 mg/L and with no mortalities.

Comments received during consultation

No comments received.

Assessment and comparison with the classification criteria

RAC notes that the acute toxicity studies are very old (about 30-50 years), which introduces some uncertainty in the assessment.

Acute oral toxicity

The acute oral toxicity study from 1993a calculated the LD_{50} in females as approximately 2000 mg/kg bw. This is based on 0/5 mortalities at 1000 mg/kg bw and 2/5 at 2000 mg/kg bw. The other (pre-guideline, pre-GLP) study (1973) resulted in an LD_{50} of 1600 mg/kg bw, for both sexes. Based on both studies, RAC concludes (in agreement with the DS proposal) that dimethachlor **warrants classification as Acute Tox. 4; H302**. This confirms the current minimum classification.

The DS did not propose an ATE; however, RAC proposes an **ATE of 1600 mg/kg bw** based on the lowest LD_{50}.

Acute dermal toxicity

RAC concurs with the assessment of the DS that dimethachlor **warrants no classification for dermal acute toxicity**.

Acute inhalation toxicity

RAC concurs with the assessment of the DS that dimethachlor **warrants no classification for inhalation acute toxicity**.

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

Summary of the Dossier Submitter's proposal

The DS reported the clinical effects seen in acute and short-term animal studies. See Table below. The purity of dimethachlor was 96.8 % in all studies, except in Anonymous (1973) where it was not reported.

No human data are available.

Route/ species	Clinical signs	Mortality	Reference
Oral, rats (OECD TG 401, acute toxicity study, GLP)	1000 mg/kg bw: piloerection, hunched posture and dyspnoea. 2000 mg/kg bw: piloerection, hunched posture, dyspnoea and decreased locomotor activity.	1000 mg/kg bw: 0/5 ♀ 2000 mg/kg bw: 0/5 ♂ and 2/5 ♀	Anonymous, 1993a
Oral, rats (acute toxicity study)	All doses: sedation, dyspnoea, exophthalmos, curved position, trismus, tonic-clonic muscle spasms and ruffled fur	1000 mg/kg bw: none 1290 mg/kg bw: 1/5 ♂ and 2/5 ♀ 1670 mg/kg bw: 3/5 ♂ and 3/5 ♀ 2150 mg/kg bw: 2/5 ♂ and 5/5 ♀ 3170 mg/kg bw: 5/5 ♂ and 5/5 ♀	Anonymous, 1973
Oral, mice (OECD TG 474, micronucleus study, GLP)	600 mg/kg bw: Decreased spontaneous activity, eyelid closure, apathy and abdominal position. 800 mg/kg bw: Decreased spontaneous activity, eyelid closure, apathy, abdominal position and convulsions. 1000 mg/kg bw: Decreased spontaneous activity, eyelid closure, apathy and abdominal position.	60 mg/kg bw: none 180 mg/kg bw: none 600 mg/kg bw: preliminary test 0/2 ♂ and 0/2 ♀; main test 2/18 ♂ and 0/18 ♀ 800 mg/kg bw: 2/2 ♂ and 0/2 ♀ 1000 mg/kg bw:2/2 ♂ and 0/2 ♀	Anonymous, 1991
Oral, rats (OECD TG 407, 28-day repeated dose toxicity study, GLP)	750 mg/kg bw/d: creeping movement, hunched posture, hypoactivity and piloerection. Ataxia, convulsions, tremor, salivation and hypertension were observed in one female. All observations started on day 2.	750 mg/kg bw/d: 3/10 ♂ and 2/10 ♀ (2 ♂ and 1 ♀ died on day 2; 1 ♀ was sacrificed, 1 ♂ died on day 5d)	Anonymous, 1993
Oral, rabbits (OECD TG 414, PNDT, GLP)	There were no treatment-related clinical signs.	10 mg/kg bw/d: none 100 mg/kg bw/d: none or 1/20 on GD18# 350 mg/kg bw/d: 2/20 (2 died on GD18-20)	Anonymous, 1993

		600 mg/kg bw/d: 3/3 (1 died after one dose, 1 after two doses, 1 was killed after two doses; no further animals allocated to group)	

GD=gestation day

Information in the CLH report notes that there are no effects at 100 mg/kg bw/d, while the DRAR (2022) notes that one female was found dead on day 18 post-coitum at 100 mg/kg bw/d.

The DS noted that transient sedation effects were observed in rats at a non-lethal dose (1000 mg/kg bw) in an acute oral toxicity study (Anonymous, 1973). This sedation effect was however accompanied by more severe symptoms (dyspnoea, exophthalmus, curved position, trismus, tonic-clonic muscle spasms) indicating general toxicity. Furthermore, this study was a pre-GLP, pre-guideline study, with limited reporting. In the other oral acute toxicity study (Anonymous, 1993a), sedation was not observed, not even at the lethal dose of 2000 mg/kg bw.

The DS concluded, although transient sedation, apathy and decrease spontaneous activity were observed in rats and mice after single oral administration, they were accompanied with signs of general toxicity and lethality. Evidence is not strong enough for a classification for STOT SE 3 for narcotic effects. Further, as a single dose of dimethachlor did not have effects indicative of organ dysfunction, STOT SE 1 or 2 classification is also not warranted.

Comments received during consultation

No comments received.

Assessment and comparison with the classification criteria

No human data are available. The clinical effects reported in acute toxicity animal studies are summarised in the Table above.

With regards to STOT SE Category 3 for narcotic effects, in one acute oral toxicity study (Anonymous, 1973,) in the more recent GLP- and OECD TG-compliant study (Anonymous, 1993a), in the 28-day study (after 2 days of exposure), and in the mouse micronucleus assay, symptoms as sedation, ataxia and decreased spontaneous activity were seen. However, these effects were mostly seen at doses with mortalities (except for the 1000 mg/kg bw in the 1973 study). In the pre-natal developmental toxicity study (up to 350 mg/kg bw/d), no clinical signs were reported. Taken into account all the data, RAC concurs with the DS that **no classification is warranted for STOT SE Category 3 for narcotic effects**.

With regards to STOT SE Category 3 for respiratory tract irritation, dyspnoea was noted in some animals in the reported studies, however mostly at dose levels with mortality. There is no evidence available from human data, nor on histopathology in animals. RAC concurs with the DS that **no classification is warranted for STOT SE Category 3 for respiratory tract irritation**.

RAC also concurs with the DS that no organ dysfunction is found after a single dose with dimethachlor, and that **no classification for STOT SE Category 1 or 2 is warranted**.

RAC evaluation of skin corrosion/irritation

Summary of the Dossier Submitter's proposal

An acute dermal irritation test according to OECD TG 404 and GLP is available with dimethachlor (Anonymous, 1993a). 0.5 g of dimethachlor was applied to the shaved flank for 4 hours under a semi-occlusive dressing in three female rabbits. Individual mean scores for erythema (at 24, 48 and 72 hours) were 1.67, 1.33 and 2.33, and for oedema (at 24, 48 and 72 hours) were 1.33, 1.33 and 2.0 for the 3 rabbits. There were no signs of skin irritation at 7 days.

Individual scores	**24 hours**	**48 hours**	**72 hours**	**7 days**
Erythema	2, 2, 3	2, 1, 3	1, 1, 1s	-
Oedema	2, 2, 3	1, 1, 2	1, 1, 1	-
Individual mean scores (24-72 hours)	**rabbit**	**1**	**2**	**3**
Erythema		1.67	1.33	2.33
Oedema		1.33	1.33	2.0

s = scaling

Also, no irritation was observed in the available Guinea Pig Maximisation Test with 30 and 50 % dimethachlor in vaseline (Anonymous, 1993b).

The DS concluded that the data do not meet the criteria for classification (mean scores of ≥ 2.3 present in at least 2 out of 3 animals), so no classification warranted.

Comments received during consultation

No comments received.

Assessment and comparison with the classification criteria

RAC concurs with the assessment of the DS. Based on the available skin irritation study with mean scores for erythema and oedema at 24, 48 and 72 hours below the threshold for classification of 2.3 in 2 out of 3 animals, **no classification for skin corrosion/irritation is warranted**.

RAC evaluation of serious eye damage/irritation

Summary of the Dossier Submitter's proposal

An acute eye irritation test according to OECD TG 405 and GLP is available with dimethachlor (Anonymous, 1993b). 0.1 ml (0.66 g) of dimethachlor was applied to the eye in three female rabbits. Results are shown in the Table below. No signs of eye irritation were reported at 7 days.

Mean scores (24-72 hours)	**1**	**2**	**3**
Conjunctival redness	0.67	1	1
Chemosis	0	0.33	0.33
Iridial changes	0	0	1
Corneal opacity	0	0.33	0

To fulfil the CLP criteria for Eye damage/irritation Category 2, it is necessary to observe at least in 2 out of 3 tested animals a positive response of corneal opacity ≥ 1 and/or iritis ≥ 1, and/or conjunctival redness ≥ 2 and/or conjunctival oedema (chemosis) ≥ 2, calculated as the mean scores following grading at 24, 48 and 72 hours after installation of the test material, which fully reverses within an observation period of 21 days. The DS concluded that the data do not meet the criteria for classification, so no classification is warranted.

Comments received during consultation

No comments received.

Assessment and comparison with the classification criteria

RAC concurs with the assessment of the DS. **No classification for serious eye damage/irritation is warranted** based on the data from the available eye irritation study, as no mean scores were above the guidance values from the CLP criteria.

RAC evaluation of respiratory sensitisation

Summary of the Dossier Submitter's proposal

The DS noted that no recognised and validated animal tests for respiratory sensitisation exist. It was noted that dimethachlor is a skin sensitiser. From medical surveillance data of manufacturing plant personnel, no adverse effects are reported.

The DS concluded no classification.

Comments received during consultation

No comments received.

Assessment and comparison with the classification criteria

As no data are available, RAC concludes on **no classification** on **respiratory sensitisation based on lack of data**.

RAC evaluation of skin sensitisation

Summary of the Dossier Submitter's proposal

One Guinea Pig Maximisation Test (GPMT) according to OECD TG 405 and GLP is available (Anonymous, 1993c). Concentrations were selected on the basis of pre-tests in separate animals. In the test the intradermal induction was performed with 5 % dimethachlor (w/v) in oleum arachidis and 5 % dimethachlor in Freund's adjuvant/saline mixture (w/v) in week 1. In week 2 the epidermal induction was performed with 50 % (w/w) dimethachlor in vaseline. In week 5 the epidermal challenge was performed with 50 % (w/w) dimethachlor in vaseline. All test animals (N = 20) showed positive skin reactions at 24 and 48 hours after the challenge. No positive skin reactions were reported in the control animals or at the control sites of the test animals.

The test resulted in a positive outcome with 100 % of the animals sensitised after an intradermal induction dose of 5 %. That would result in a classification in Category 1B. However, subcategory 1A cannot be excluded, as no experiment with an intradermal dose ≤ 1 % is available. Therefore, the DS proposed classification for Skin Sens. 1, without sub-categorisation.

Comments received during consultation

No comments received.

Assessment and comparison with the classification criteria

RAC concurs with the assessment of the DS and concludes that **classification as Skin Sens. 1; H317 is warranted**. This is based on the positive result (100 % of the animals sensitised) in a GPMT test with 5 % intradermal induction dose.

As Category 1A cannot be excluded because no experiment is performed with an intradermal induction dose below 1 %, no subcategory is warranted.

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

Summary of the Dossier Submitter's proposal

Several subchronic toxicity studies with dimethachlor exposure via the diet are described by the DS in the CLH report (see Table 37), such as a 25/26-day study in rats, 28-day studies in mice, rats and dogs, and 90-day studies in mice, rats and dogs. Chronic (carcinogenicity) oral studies are available in mice (two studies) and rats.

The main target organ was liver with reported increased liver weights, as well as hepatocyte hypertrophy and sometimes liver cytoplasmic inclusion bodies. Next to liver, kidney weights were increased, accompanied with histopathologic findings such as renal chronic progressive nephropathy, renal tubular dilation, and renal cysts (especially in male mice). In the dog studies, decrease in thymus weight was accompanied by thymus cortical atrophy.

One dermal 28-day repeated dose study in rats is available, without any findings. Repeated dose inhalation studies were not available for dimethachlor.

The DS also reported on three reproductive toxicity studies (a 2-generation study in rats and developmental toxicity studies in rats and rabbits). No relevant effects on organs were reported.

Classification

The DS considered liver to be the target organ, as shown by altered clinical chemistry (decreases in globulin, total protein, AG ratio and cholesterol and increased activity of ALP and ɣ-GT). This was accompanied by increased relative liver weights and hepatocyte hypertrophy. The DS noted that there is no evidence of organ disfunction. The dose levels at which effects are observed were well above the guidance values for STOT RE Category 2, or were considered not to be severe enough (minimal hepatocellular cytoplasmic vacuolisation in the 3-month dog study, in the absence of clinical chemistry).

The DS considered the effects on red cell parameters in one 90-day dog study (1994a) not relevant for classification, as no evidence of severe anaemia was found in the histopathological findings.

The DS considered the atrophy of the thymus (observed in two dog studies), associated with body weight loss, secondary to physiological stress at a toxic dose.

Increased kidney weights, associated with microscopic pathology findings in three mice studies, were found above the guidance values for classification.

Thus, the DS considered classification for STOT RE not warranted.

Comments received during consultation

No comments received.

Additional key elements

An overview of available studies is provided in the Table below, with regards to the adverse effects related to liver and kidney in the different repeated toxicity studies (with different species and exposure durations) in comparison with the equivalent Guidance Values for STOT RE Category 2 for the relevant exposure time based on the CLP guidance. Studies were performed with dimethachlor of 96.8 % purity, except for the 90-day dog study which used dimethachlor of 93.8 % purity.

Study	Dosing	Effects (relevant for classification and at lowest dose level)	GV for STOT RE Cat. 2 mg/kg bw/d (equivalent for relevant exposure duration)	Reference
28-day, dogs, diet	0, 15.28, 62.95 and 119.84 mg/kg bw/d in males; 0, 18.05, 70.24 and 118.59 mg/kg bw/day in females	119.8/118.6 mg/kg bw/d: Increased liver weight (relative; 26.8 %♂, 41.8 %♀), hepatocyte hypertrophy (2/2♂; 1/2♀) and liver inflammatory cell infiltration (2/2♂; 2/2♀) 63/70.2 mg/kg bw/day: Increased liver weight (relative; 22.6 %♂, 24.7 %♀), hepatocyte hypertrophy (1/2♂) and liver inflammatory cell infiltration (1/2♂)		Anonymous, 1993 *(considered as supplementary information only)*
25/26-day, rats, diet	0, 9.5, 67, 294.8 and 487.9 mg/kg bw/d in males; 0, 10.0, 68.3, 304 and 485.2 mg/kg bw/d in females	294.8/304 mg/kg bw/d: Increased liver weight (relative; 13.4 %♂, 14.4 %♀), hepatocyte hypertrophy (8/10♂, 10/10♀, vs 0/10 controls)	about ≤ 300	Anonymous, 1992a
28-day, mice, diet	0, 20.9, 204.4, 623.7, and 1493.3 mg/kg bw/d in males; 0, 23.2, 232.3, 715.2 and 1783.7 mg/kg bw/d	623.7/715.2 mg/kg bw/d: Increased liver weight (relative; 20.9 %♂, 14.2 %♀), increased kidney weight (relative; 19.6 %♂), hepatocellular hypertrophy (8/10♂, 10/10♀, vs 0/10 controls)	≤ 300	Anonymous, 1992b
28-day, rats, gavage	0, 30, 150, and 750/350 mg/kg bw/d	350 mg/kg bw/d: Increased liver weight (relative; 27.1 %♂, 19.8 %♀), centrilobular hepatocyte hypertrophy (4/10♂, 8/10♀, vs 0/10 controls)	≤ 300	Anonymous, 1993

90-day, dogs, diet	0, 3.4, 10.1 and 35.4 mg/kg bw/d in males; 0, 3.1, 10.4, 45.4 mg/kg bw/d in females	35.4/45.5 mg/kg bw/d: Increased liver weight (absolute; 23.1 %♀), liver concentric laminated cytoplasmic inclusions in hepatocytes (4/4♂; 2/4♀; 0/4 controls)		Anonymous, 1974 (no OECD TG, no GLP)
90-day, dogs, diet	0, 9.96, 32.28 and 104.3 mg/kg bw/d in males; 0, 10.81, 35.95 and 102.8 mg/kg bw/d in females	104.3/102.8 mg/kg bw/d: Increased liver weight (44.6 %♂, 28.3 %♀), hepatocyte hypertrophy (3/4♂, 4/4♀, vs 0/4 controls), hepatocellular cytoplasmic vacuolisation (2/4♀, vs 0/4 controls) 32.38/35.95 mg/kg bw/d: Increased liver weight (17.2 %♂, 18.7 %♀), hepatocyte hypertrophy (1/4♂, 1/4♀, vs 0/4 controls), hepatocellular cytoplasmic vacuolisation (2/4♀, vs 1/4 controls)		Anonymous, 1994/1994a
90-day, rats, diet	0, 2.21, 71.7 and 449.4 mg/kg bw/d in males; 0, 2.21, 76.0 and 457.4 mg/kg bw/d in females	449.4/457.4 mg/kg bw/d: - Increased liver weight (relative; 13.1 %♂, 20.6 %♀), hepatocyte hypertrophy (5/10♂, 3/10♀, vs 0/10 controls) - Increased kidney weight (relative; 15.7 %♂, 8.6 %♀)	≤ 100	Anonymous, 1994
90-day, mice, diet	0, 17.5, 175, 614 and 1228 mg/kg bw/d in males; 0, 18.5, 185, 648 and 1296 mg/kg bw/d in females	614/648 mg/kg bw/d: - Increased liver weight (relative; 17.1 %♂, 10.3 %♀), hepatocyte hypertrophy (7/10♂, 9/10♀, vs 3/10 controls both sexes) - Increased kidney weight (relative; 28.3 %♂, 9.4 %♀), kidney acute tubular lesions (5/10♂ vs 0/10 controls) 175/185 mg/kg bw/d: - Increased liver weight (relative; 12.3 %♂), hepatocyte	≤ 100	Anonymous, 1999

		hypertrophy (7/10♂, vs 3/10 controls) - Increased kidney weight (relative; 11.9 %♂, 11.0 %♀)		
18-month, mice, diet	0, 2.25, 32.3 and 488.1 mg/kg bw/d in males; 0, 2.17, 31.2 and 450.9 mg/kg bw/d in females	488.1/450.9 mg/kg bw/d: - Increased liver weight (relative; 17.1 % ♂, 22.2 % ♀), hepatocyte hypertrophy (45/50 ♂ vs 25/50 controls, 46/50 ♀ vs 27/50 controls), - Increased kidney weight (relative; 41 % ♂, 20.92 % ♀), renal chronic progressive nephropathy (25/50 ♂ vs 3/50 controls), renal tubular dilatation (16/50 ♂ vs 2/50 controls), renal cysts (33/50 ♂ vs 16/50 controls)	≤ 16	Anonymous, 1995
18-month, mice, diet	0, 2.54, 34.3, 184 and 511 mg/kg bw/d in males; 0, 2.25, 31.4, 162 and 454 mg/kg bw/d in females	34.3/31.4 mg/kg bw/d: - Nephropathy and renal tubular atrophy (35/50 ♂ vs 25/50 controls; 21/50 ♀ vs 16/50 controls), increase kidney weights (relative; 8 % ♂)	≤ 16	Anonymous, 2001
2-year, rats, diet	0, 0.765, 11.1 and 157.3 mg/kg bw/d in males; 0, 0.892, 12.9, and 182.6 mg/kg bw/d in females	157.3/182.6 mg/kg bw/d: - Increased liver weight (relative; 9.4 % ♂, 19.5 % ♀), hepatocyte hypertrophy (24/60 ♂, 17/60 ♀ (0/60 controls), cytoplasmatic inclusion bodies (27/60 ♂ vs 0/60 controls) - Increased kidney weight (relative; 22.2 % ♂, 14.2 % ♀)	≤ 12.5	Anonymous & Anonymous, 1995
Dermal repeated dose 28-day, rats	0, 10, 100 and 1000 mg/kg bw/d exposure 6 hours per day, 5 days per week	No effects	≤ 600	Anonymous, 1993d
2-generation study rats	0, 1.33, 20, 133 and 267 mg/kg bw/d	No relevant effects on organs		Anonymous, 1994
PNDT rats	0, 50, 350, 700 mg/kg bw/d by	No relevant effects on organs; 5/25 deaths		Anonymous, 1994

	oral gavage on GD 6-15	(GD9-14) at 700 mg/kg bw/d		
PNDT rabbits	0, 10, 100, 350, 600 mg/kg bw/d by oral gavage on GD 6-18	No relevant effects on organs; 3/3 deaths (after 2 doses) at 600 mg/kg bw/d (no further animals), 2/20 deaths at 350 mg/kg bw (GD18-20) considered incidental		Anonymous, 1993

Assessment and comparison with the classification criteria

Clearly, the liver is the target organ after exposure to dimethachlor as shown in all repeated dose studies with rats, mice and dogs. Liver weights are increased, histopathology showed effects as hepatocellular hypertrophy and degeneration, and this is combined with effects on clinical blood chemistry. In rats and mice, the effects (liver weight increase in combination with hepatocyte hypertrophy) are found above the (equivalent) guidance value for STOT RE Category 2. The 28-day dog study was assessed as not reliable. In the two 90-day dog studies (one performed before OECD guidelines and GLP was available), effects were found at about 35 mg/kg bw/d, such as liver weight increase (about 20 %), hepatocyte hypertrophy (incidences 1/4♂, 1/4♀, vs 0/4 controls) and hepatocellular cytoplasmic vacuolisation (2/4♀, vs 0/4 controls) or concentric laminated cytoplasmic inclusions in hepatocytes (4/4♂; 2/4♀; vs 0/4 controls).

Further, some changes reported in the clinical chemistry are associated with liver effects, such as decreases in globulin, total protein, A/G ratio, cholesterol and increased activity of ALP and ɣ-GT). Alkaline phosphatase (ALP) is a well-known marker for the clinical chemistry of hepatobiliary damage in humans and animals; however, some doubt is raised about the adversity of increased ALP in dogs (Yokoyama et al., 2021). Hall et al. (2012) considered *"increases in circulating ALP activity in the dog, with associated increased liver weight and histological hepatocellular hypertrophy but without hepatocellular degeneration as an adaptive, rather than an adverse response".* ɣ-GT is common to several tissues but is also a key marker in liver function tests. Serum levels of ɣ-GT increase markedly with bile duct damage, and it is recognised as a marker of cholestasis. Hall et al. (2012) noted that ɣ-GT (biologically significant) changes could be used in a weight-of-evidence approach, in the absence of histopathological changes.

Histopathology evidence in the dog studies did show hepatocyte hypertrophy, but there is not any structural degeneration or necrotic changes reported, the latter considered adverse as reviewed by Hall et al. (2012).

In conclusion, in a weight-of-evidence approach, there is no need for classification for STOT RE for liver, as significant effects (liver weight increases accompanied with hepatocyte hypertrophy) are found at levels above the guidance value of STOT RE Category 2 (in mice and rat repeated dose studies), or dimethachlor is not producing significant toxic effects (liver hypertrophy, but not degeneration or necrosis, as relevant for the dog 90-day studies).

With respect to effects of dimethachlor on kidney, such as increased organ weight found in three mice studies combined with increased incidence of renal acute tubular lesions in one of them. In the carcinogenicity studies in mice, also histopathological findings in kidney were reported (chronic nephropathy, tubular dilatation or atrophy). All these effects were found at doses above the guidance value for STOT RE Category 2, and therefore not relevant for classification.

Overall, RAC concludes that **no classification for STOT RE is warranted** for dimethachlor.

RAC evaluation of germ cell mutagenicity

Summary of the Dossier Submitter's proposal

Nine genotoxicity tests on dimethachlor (purity ≥ 95.5 %) are available. Seven *in vitro* studies were reported, gene mutation assays in bacteria and mammalian cells, an unscheduled DNA synthesis test, a chromosome aberration test, and an *in vitro* micronucleus test. All *in vitro* tests resulted in a negative outcome, except for the *in vitro* micronucleus assay. The *in vivo* micronucleus tests resulted in no increase in micronuclei, while in the second micronucleus test (2020) cytotoxicity was shown.

Study/method	Information	Results	Reference
In vitro tests			
Reverse mutation test in bacteria *in vitro* OECD TG 471/GLP	*Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, TA 1538 Concentrations: 10.0, 33.3, 100.0, 333.3, 1000.0 and 5000 µg/plate	Negative Not mutagenic with or without metabolic activation. No cytotoxicity or precipitation at highest concentration tested. Positive controls included.	Poth (1991)
Reverse mutation test in bacteria *in vitro* OECD TG 471/GLP	*Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, *E. coli* WP2, WP2urA Concentrations: 10.0, 33.3, 100.0, 333.3, 1000.0 and 5000 µg/plate	Negative Not mutagenic with or without metabolic activation. No cytotoxicity or precipitation at highest concentration tested. Positive controls included.	Chang (2019)
Gene Mutation Assay in Chinese Hamster V79 Cells *in vitro* (V79/HPRT) OECD TG 476/GLP	Chinese hamster V79 cells. Concentrations: 2.0 to 50.0 µg/mL with and without metabolic activation.	Negative No indication of a mutagenic activity with or without metabolic activation. Cytotoxicity at 50 µg/mL without activation. Borderline cytotoxicity at 30 µg/mL without activation and at 50 µg/mL with activation. Positive controls included.	Müllerschön (1992)
Gene Mutation Assay in Chinese Hamster V79 cells *in vitro* (V79/HPRT) OECD TG 476/GLP	Chinese hamster V79 cells Concentrations: 32.7, 65.4, 130.9, 261.8, 523.5, 1047.0 and 2094.0 µg/mL	Negative No indication of a mutagenic activity with or without metabolic activation. Cytotoxicity was noted at 130.9 µg/mL with and without activation. Positive controls included.	Sokolowski (2019)
DNA damage and repair, Unscheduled DNA Synthesis in mammalian cells *in vitro* OECD TG 482/GLP	Rat hepatocytes (adult male if:RAIf) Concentrations: 0.14, 0.42, 1.25, 3.75, 7.5 and 15.0 µg/mL	Negative. No indication of DNA damage. Cytotoxicity at 15 µg/mL in both experiments. Positive controls included.	Hertner (1992)
In vitro Mammalian Chromosome Aberration Test OECD TG 473/GLP	Chinese hamster ovary cells (ATCC CCL61) Concentrations: Experiment. 1: 2.93, 5.86 and 11.72 µg/mL. Experiment 3: 5.86, 11.72 and 23.44 µg/mL With metabolic activation Experiments 2 and 4: 46.88, 93.75 and 187.50 µg/mL	Negative No increase in chromosome aberrations and no evidence of a clastogenic effect with or without metabolic activation. Concentrations tested limited by cytotoxicity. Positive controls included.	Hertner (1994)

In vitro Micronucleus Test in human lymphocytes OECD TG 476/GLP	Human lymphocytes Concentrations: 13.6, 23.8, 41.7, 72.9, 128, 223, 391, 684, 1197, 2094 µg/mL	Positive Clear cytotoxicity Value of micronucleated cells was statistically significantly increased. Positive controls included.	Naumann, 2019
In vivo tests			
Micronucleus assay in bone marrow cells OECD TG 474/GLP	NMRI mice (N = 5/sex/group) Single doses by gavage of 60, 180, and 600 mg/kg bw Sacrifice of control and high dose animals at 24, 48 and 72 hours, other doses at 24 hours.	Negative No significant increase in the incidence of micronucleated PCE was noted at any of the doses or time points. Cytotoxicity in bone marrow not shown: PCE/NCE not decreased in treated mice (no proof of bone marrow exposure) Two males dosed with 600 mg/kg bw died. Positive controls included.	Anonymous, 1991
Micronucleus assay in bone marrow cells OECD TG 474/GLP	Crl: CD1 mice. Single# doses by gavage of 200, 400, 800 mg/kg bw/d (males), 500, 1000, 2000 mg/kg bw/d (females)	Negative No increased incidence of micronuclei. Indirect proof of bone marrow exposure was performed, in preliminary toxicity assay.	Anonymous, 2020

CLH report notes single doses, while the DRAR (2022) notes "dosing was by oral (gavage) administration twice, approximately 24 hours apart".

The DS concluded that there is no evidence that dimethachlor is genotoxic as shown in a range of *in vitro* and *in vivo* assays. No classification was proposed.

Comments received during consultation

No comments were received.

Assessment and comparison with the classification criteria

Based on the results in the available *in vitro* and *in vivo* assays, RAC concurs with the DS that no **classification for germ cell mutagenicity is warranted**.

RAC evaluation of carcinogenicity

Summary of the Dossier Submitter's proposal

24-month repeated dose dietary study in rats (Anonymous & Anonymous, 1995) according to OECD TG 453 and GLP

Tif:RAIf rats (N = 60/sex/group) were exposed via the diet to 0, 0.765, 11.1, 157.3 mg/kg bw/d in males, and 0, 0.892, 12.9, 182.6 mg/kg bw/d in females. Survival was 72 % in both sexes after 104 weeks in the high dose group and 48 % in males and 52 % in females of the control group. Some decrease was seen in body weight gain and food consumption in males and females in the high dose group. No overall increase in tumour incidence was found. Extensive histopathological examination at 4 different levels of the respiratory epithelium of the nasopharynx resulted in a slightly increased incidence of adenoma of the respiratory epithelium in 3 high dose animals (3/60 vs 0/60 in controls).

18-month carcinogenicity dietary study in mice (Anonymous, 1995) according to OECD TG 451 and GLP

Tif: MAGf mice (N = 50/sex/group) were exposed via the diet to 0, 2.25, 32.3 and 488.1 mg/kg bw/d in males, and 0, 2.17, 31.2 and 450.9 mg/kg bw/d in females. Body weights were slightly decreased in males (9.7 %) and females (10 %) at the highest dose in week 77. Incidences of some tumour types were increased:

- hepatocellular tumours, high dose, males
    - benign hepatoma (within historical control data; HCD), high dose
    - hepatocellular carcinoma (not statistically significant, above HCD range in top dose) in low and top dose males, without a dose-response
- pulmonary adenomas (mid dose males, all female groups), without dose-response and within HCD range (or just slightly above in case of mid dose males).
- pulmonary carcinomas (top dose males), without clear dose-response, control and high dose group above HCD range

18-month carcinogenicity dietary study in mice (Anonymous, 2001) according to OECD TG 451 and GLP

ICO:CD1 (Crl) mice (N = 50/sex/group) were exposed via the diet to 0, 2.54, 34.3, 184 and 511 mg/kg bw/d in males, and 0, 2.25, 31.4, 162 and 454 mg/kg bw/d in females. Body weight was slightly decreased (8 and 6 %) in males in the two highest dose groups. No increase in tumour incidences were found.

Human data are not available for dimethachlor.

Mechanistic studies

In the CLH report, a comparison is made with acetochlor, another chloroacetanilide herbicide. Acetochlor is proposed (Green et al., 2000) to be metabolised via two routes; 1. Via the formation of aniline and subsequent p-hydroxylation, and 2. Via the formation of sulfoxide and *para*-hydroxysulfoxide. Both pathways lead to a reactive quinone-imine and quinone-imine sulfoxide, which bind to tissue proteins and other nucleophiles such as glutathione (see Figure 2.6.5-1 in the CLH report).

There is however a difference between dimethachlor (substituent has an alkylamine functional group – level of oxidation = alcohol) versus acetochlor (substituent has an hemiaminal functional group – level of oxidation = aldehyde). After cleavage of the ether bridge in the alkyl group, alachlor (and acetochlor) will spontaneously form the corresponding anilines, whereas for dimethachlor stable alcohol derivatives are formed, which actually represent the major individual metabolites in rats.

Biotransformation, starting with sulfoxide metabolites was studied in rat and human liver and nasal microsomes *in vitro*. *Para*-hydroxylation of acetochlor sulfoxide and dimethachlor sulfoxide was observed in rat nasal and rat liver (acetochlor sulfoxide only) and human liver microsomes *in vitro*. *Para*-hydroxylation was not observed in human nasal microsomes for both metabolites. It is hypothesised that the nasopharyngeal adenoma is not relevant for humans (Knowles et al., 2020).

Further, exposure to chloroacetanilides such as alachlor, acetochlor and dimethachlor resulted in nasal tumours (see overview Table below), although with a difference in the incidences.

Classification

The DS considered the increased incidences of liver and pulmonary tumours in the mice study as not relevant, since the changes were not dose-related and/or within the HCD. It should be noted that nasal cavity was not examined in the first mice study. The second mice study did not show

any tumours. In the rat carcinogenicity study, the nasopharyngeal adenomas arising from nasal respiratory tract epithelium in male rats appear to be treatment-related. Relevance of the nasal adenomas originating from respiratory nasal epithelia for humans is discussed. The sulfoxide pathway is concluded to be not human relevant as shown in the olfactory nasal microsomes. This pathway is proposed to be responsible for nasal adenomas. N-dealkylation is only minor route for dimethachlor. The DS notes that other mechanisms cannot be ruled out (such as binding to chromatin protein), as well as their possible relevance for humans.

Nasopharyngeal adenomas are only found in one species, one sex and one study. The incidence is low (3/60) and the tumours are benign. Further, dimethachlor is not shown to be genotoxic. Therefore, the DS proposed classification for carcinogenicity Category 2 based on limited evidence of carcinogenicity.

Comments received during consultation

One National Authority requested the inclusion of detailed histopathological findings in the nasal passage from available repeated dose toxicity studies with dimethachlor. Another request was to include some comment on the known difference in enterohepatic circulation cut-offs in rodents and humans and the relative bioavailability's of the critical sulfur containing metabolites.

The DS responded that limited data is available on potential preneoplastic changes in the nasal cavity in repeated dose toxicity studies. In two short-term studies, no preneoplastic or hyperplastic findings were observed in the nasal turbinates. In the other studies, nasal cavity was not on the list for histopathological examination. With regards to data on bile excretion and enterohepatic circulation of dimethachlor, the DS responded that potential toxicokinetic differences between species could only be speculated. The DS also noted that the critical sulfur containing metabolites could be enzymatically formed on site (in nasal mucosa), and further, that other potential mechanisms could be responsible for nasopharyngeal adenoma occurrence.

One Member State Competent Authority (MSCA) supported Category 2. The MSCA noted two aspects to be taken into consideration:

a. Despite the results of the newer toxicokinetic study (Anonymous & Anonymous, 2018), an N-dealkylated metabolite was detected in Anonymous (1996), albeit at low levels. N-dealkylation of the alpha-carbon atom is a key step resulting in protein adduct formation.
b. S-metolachlor was excluded from the argumentation in the CLH report. This is more structurally related (at key position, namely the beta rather than the alpha carbon). In the recent RAC opinion for S-metolachlor (2022), RAC stated "nasal olphactory tumours induced by acetochlor were determined to be secondary to local cytotoxicity due to the formation of quinone-imine. These tumours were considered relevant to humans, although rats appeared to be more sensitive than humans." The increased incidences of nasal turbinate adenocarcinomas in male rats (2/69) were deemed to be of concern as it is a rare tumour type.
c. An increase in kidney lipoma is noted (0, 1, 1, 2) in males, the latter outside the HCD. As kidney is also a target organ, some discussion would be appreciated.

Further, the MSCA asked three questions:

1. In the mechanistic study by Knowles et al. (2020), from which epithelium (respiratory or olfactory) originated the nasal microsomes?
2. Were the animals with nasopharyngeal adenomas detected (3/60) animals who survived till the end, or died before study termination (in the light of the lower male survival rate)?
3. Request for clarification with regards to a contradiction between two statements in the DRAR/CLH report with regards to analysis of nasal (muzzle) tissue from all animals.

The DS responded:

b. The DS agreed that nasal tumours observed in the study with S-metolachlor should be discussed as well, taking into account tumour type (for dimethachlor only nasopharyngeal adenomas were observed, while for S-metolachlor nasal adenocarcinomas were found in male rats).
c. Kidney lipomas are expansile lesions, often well-circumscribed, which continue to grow over time and generally affect the architecture of surrounding cells. They are rather rare renal tumours, which could arise as spontaneous lesions, especially in aging rats, but could be occasionally noted with increased frequency after xenobiotic treatment, especially that which affects lipid metabolism. However, the increase in the incidence over the dose levels applied in the study was small (0, 1, 1, 2).

1. Mixed microsomes from olfactory and respiratory nasal epithelium were used, both from humans and rats.
2. Nasopharyngeal adenomas were observed at the highest dose in one male at terminal sacrifice (732nd day) and in two males found dead near the end of the study (day 667 and 624).
3. Although only limited number of tissue samples was examined in animals in the low and intermediate dose carcinogenicity sub-groups, the muzzle was among the organs/tissues which were examined histopathologically (as stated in DRAR and CLH Report: "In contrast to top dose and control group, only limited number of tissue samples was examined in animals in the low and intermediate dose carcinogenicity sub-groups (lung, liver, kidney, testis, epididymis, muzzle, and all gross lesions)."

One company considered the classification in Category 2 not justified. In a detailed statement information is provided on the weak carcinogenic effect of dimethachlor in the nasal tissue of the high dose rats being not relevant for humans. Support is provided from two *in vitro* metabolism studies, to support the mode of action (MoA) for nasal tumour formation.

The DS replied that in their opinion the mechanistic studies do not provide evidence that local cytotoxicity secondary to quinone-imine formation is responsible for nasopharyngeal adenomas arising from nasal respiratory epithelium in male rats. The DS considered that in the case this MoA is responsible for nasal adenoma formation in the rat, the adenomas would be of olfactory rather than of respiratory origin. It is unclear which MoA(s) is responsible for the hyperplastic reaction of nasal respiratory epithelia in the case of exposure to chloroacetanilides. The DS also noted that dimethachlor seems to be of lower carcinogenic potency compared to other chloroacetanilides (such as acetochlor), and it should be discussed whether the low incidence (3/60, i.e., 5 %) warrants classification.

In appendix 1 are reported additional key elements relevant in the assessment of the proposal.

Assessment and comparison with the classification criteria

Since no human data are available, Category 1A is not applicable.

Two carcinogenicity studies in mice are available. The study with CD1 mice dosed up to 511/454 mg/kg bw/d resulted in no tumours (Anonymous, 2001). The study with MAGf mice did show an increased incidence of liver carcinomas in the low and high dose, above the HCD range, but with no dose-response (Anonymous, 1995). The dose spacing is large (about 15-fold differences between the doses), and not according to the OECD guidance (OECD GD 116). The incidence of lung carcinomas (18 %) was statistically significant increased, above the HCD in the highest dose of 488.1 mg/kg bw/d in male mice, but without a clear dose-response.

In the rat carcinogenicity study, the only treatment-related neoplastic change is considered to be the occurrence of nasopharyngeal adenoma arising from respiratory epithelium in three males at 157.3 mg dimethachlor/kg bw/d (Anonymous & Anonymous, 1995). The increase is not statistically significant, no HCD are available; however it is a rare tumour of concern. In

comparison with the other chloroacetanilides causing this type of tumour, the incidence is low and only adenomas are found (no carcinomas). As possible MoA, the formation of protein adducts via quinone-imine formation, is proposed. From chloroacetanilides, these can be formed via two pathways. When looking at the metabolism of dimethachlor, it is noted that the N-dealkylated metabolite is not formed (Anonymous & Anonymous, 2018) or formed to a minor extent (Anonymous, 1996). The sulfoxide pathway however can also lead to quinone-imine precursors. It is postulated that this pathway is not relevant in human nasal tissues, as shown in differences in rat and human nasal microsomes in an *in vitro* test. Other MoAs cannot be totally excluded.

Further, the incidences (0, 1, 1, 2 with increasing dose) of kidney lipoma in male rats might add to the concern. Increases are not statistically significant; however, this type of tumour is also rare, and could be of concern. HCD for kidney lipoma in male rats (same laboratory, ±5 years range) is 0 (0-1.7), N = 9/629 (in male rats).

All in all, RAC considers appropriate classification as Category 2, based on the increased incidence of nasopharyngeal adenomas (3/60) in the respiratory epithelium in male rats. As this was shown in one sex (male) and one species (rat), Category 1B is not justified.

Although RAC acknowledges that the level of available evidence is limited (incidence is low, the tumour is rare, and only adenomas and not carcinomas are found), RAC still considers that this warrants classification as Category 2.

A similar tumour type is also shown to occur after exposure to other chloroacetanilides, such as acetochlor, alachlor, and butachlor (see RAC opinion Acetochlor, 2014). For the MoA, two metabolism pathways could form quinone-imines able to form protein-adduct. The N-dealkylation (via aniline) pathway is a minor one, the sulfoxide pathway might be less relevant in humans. However, other mechanisms cannot be excluded, and human relevance cannot be ruled out.

Therefore, RAC concludes that **dimethachlor warrants a classification a Carcinogen Category 2, H351.**

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

Three studies are available with regards to reproductive toxicity.

Two-generation study in rats (Anonymous, 1994) according to OECD TG 416 and GLP

Sprague-Dawley Crl:CD (SD)BR rats (N = 25/sex/group) were exposed via the diet to 0, 20, 300, 2000, 4000 ppm (0, 1.33, 20, 133 and 267 mg/kg bw/d). The study pre-dates the current OECD TG 416 (2001); therefore, parameters such as oestrus cyclicity, sperm motility and morphology are missing.

The DS considered that there was parental toxicity at the highest dose level, namely reductions in body weight and food consumption. No effects on fertility were found. The livebirth index was 95-100 %, the viability index (number alive PND4/number liveborn) ranged from 91-96 %, with no statistically significant differences between the dose groups. Weaning index (number alive at weaning/number alive PND4) decreased (47, 51, 56, 38 and 43 % in the control, 20, 300, 2000 and 4000 ppm group respectively). This seemed not treatment related, no clear dose-response, no plausible explanation identified. In the study, a second mating of the P generation was done, to allow the rearing of the F1b offspring. Livebirth index ranged from 92-100 %, viability index from 89-99 % and weaning index from 85-99 % and were comparable between the dose groups.

Systemic toxicity was observed in the pups, as body weights were decreased, evident at weaning as a consequence of direct consumption of the diet.

Developmental toxicity study in rats (Anonymous, 1994) according to OECD TG 414 and GLP

Pregnant female Tif:RAIf rats (N = 25/group) were exposed by oral gavage to 0, 50, 350, 700 mg dimethachlor/kg bw/d on gestation days (GD) 6-15. This is a deviation from OECD TG 414 as dosing time is shorter. At the highest dose, 5 animals died during GD 9-14. At this dose, also decreases in body weight gain and food consumption were reported. Incidences of total number of skeletal anomalies and skeletal variations were higher in the mid and high dose foetuses, compared to HC range. They comprised poor or absent ossification and indicate developmental delay in affected pups. These variations/anomalies were considered treatment-related at the high dose (absent ossification of metatarsal 1 and proximal phalanx of anterior and posterior digits; poor ossification of proximal phalanx of anterior and posterior digit; irregular ossification of occipital bone; wide fontanel; bipartite occipital bone) and the mid dose (poor ossification of proximal phalanx of anterior digit; absent and/or poor ossification of proximal phalanx of posterior digits; irregular ossification of occipital bone; wide fontanel). The DS considered these effects secondary to maternal toxicity. Clear maternal toxic signs were reported in the high dose (mortality, decreases in body weight gain and food consumption). In the mid dose, only the food consumption was statistically significantly changed.

Developmental toxicity study in rabbits (Anonymous, 1993) according to OECD TG 414 and GLP

Pregnant female NZW rabbits (N = 20/group) were exposed by oral gavage to 0, 10, 100, 350, and 600 mg dimethachlor/kg bw/d on GD 6-18. At the highest dose, 3 animals died, and no further animals were allocated to this group. At 350 mg/kg bw/d, 2/20 animals died at GD 18-20, and at 100 mg/kg bw/d 1/20 died at GD 18, all considered incidental by the study authors. At 350 mg/kg bw/d, the body weight gain was increased (35 % over GD6-19). At this dose, pre-implantation loss was increased (43 % versus 23.5 % in controls), number of liver foetuses decreased (4.4. vs 8.4 in controls) and the litter weight was smaller due to fewer foetuses (35 %). A high incidence of malformations was reported, in all groups, including the control.

Classification

The DS concluded that dimethachlor had no adverse effects on sexual function and fertility, as no effects were found in the 2-generation study (although it was noted that this study was performed in line with the previous version of the OECD TG 416).

The DS concluded on no adverse effects on development after treatment. Minor effects in developmental delay (delayed ossification) were seen only in the rat at the dose of ≥ 350 mg/kg bw/d, and were considered transient in nature, secondary to maternal toxicity and correlated with decreased food intake. With regards to lactation, no evidence of impaired nursing behaviour or decreased pup viability during lactation is present. Reduction in the pup weight manifested after the second week postpartum, when pups start to eat also food. So, no classification for adverse effects on development was proposed.

The DS concluded that no evidence is present in the 2-generation study of impaired nursing behaviour or decrease pup viability during lactation. The reduction in postnatal pup growth was only seen after the first and even more in the second week post-partum, when pups start to nibble food. So, no classification for adverse effects on or via lactation was proposed.

No human data are available.

Therefore, the DS concluded that the present data do not indicate a need to revise the no classification for dimethachlor for reproductive toxicity including lactation.

Comments received during consultation

No comments were received.

Assessment and comparison with the classification criteria

Fertility and sexual development

In the available studies, no treatment-related effects on female and male reproductive systems, fertility and reproductive outcome were found. RAC concurs with the DS that **no classification for adverse effect on sexual function and fertility is warranted**.

Developmental toxicity

Skeletal anomalies (poorly or non-ossified bones) as observed in the rat prenatal developmental toxicity study are indicating a developmental delay, considered related to maternal toxicity. The effects in the high dose animals are found at marked maternal toxicity, 5 animals died (25 %, above the 10 % noted in the CLP guidance), and are thus not relevant for classification. The effects at the mid dose (350 mg/kg bw/d) are found in the absence of clear maternal toxicity. Only a slight (11 %), statistically significant decrease in food consumption was found from GD6-11, not during the other periods, and in the absence of an effect on maternal body weight (gain). The only effect found is retarded ossification, no other effects are found which are relevant for development.

In the rabbit study, the body weight gain and food consumption are reported to be slightly decreased in the highest dose. From 20 inseminated rabbits, at the highest dose, 2 were found dead (study authors noted that this was not treatment-related) and one aborted; only 11 does with live foetuses were included. There seems to be a dose-dependent effect on corpora lutea/doe, implants/doe, percentage pre-implantation loss, live foetuses/doe, and litter weight. Only the latter was statistically significant decreased. All in all, some effects are noted at the highest dose, which is not without (slight) maternal toxicity.

Considering this, RAC concurs with the DS that **no classification for adverse effects on development is warranted**.

Lactation

No information is available on the presence of dimethachlor in milk. Effects on the pup body weights in the 2-generation study is present (statistically significant) from PND14 and later. It cannot be excluded that pups also ate from the solid food. RAC concurs with the DS that **no classification for effects on or via lactation is warranted**.

RAC evaluation of aspiration toxicity

Summary of the Dossier Submitter's proposal

The DS considered aspiration toxicity not relevant as dimethachlor is a solid.

Comments received during consultation

No comments were received.

Assessment and comparison with the classification criteria

RAC concurs with the DS that **no classification is warranted for aspiration toxicity**, as dimethachlor is a solid.

ENVIRONMENTAL HAZARD EVALUATION

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier Submitter's proposal

The substance is currently listed in Annex VI of Regulation (EC) No 1272/2008 with a classification for environment hazard as indicated above. The DS reported evidence that dimethachlor is not rapidly degradable and has a low potential for bioaccumulation. Based on re-evaluation of all relevant information provided for the renewal of the approval of the dimethachlor, the DS proposed to classify the substance as Aquatic Acute 1 (H400), adding an M-factor of 10, and Aquatic Chronic 1 (H410) adding an M-factor of 10.

Environmental Degradation

Ready Biodegradability

A 29-day study compliant with OECD TG 301B and GLP was performed incubating 29.1 and 30.3 mg/L technical grade dimethachlor (corresponding to 17.8 and 18.5 ThOC/L) at 22 ± 2 °C in a mineral medium treated with activated sludge from STP (Weinstock, 1994). The reference substance Sodium Benzoate was tested in parallel at 15 mg DOC/L. The percentage of biodegradation was calculated at each selected interval (0, 3, 7, 10, 13, 17, 20, 24, 28 and 29 days) by comparing the quantity of produced CO_2 with the theoretical carbon content. No biodegradation (0 % of the theoretical value) of dimethachlor was observed within 29 days, while 80 % degradation of the reference substance was observed in a 10-day interval.

Hydrolysis

In a 28-day study performed by Burkhard (1974) according to a non-guideline method, dimethachlor was tested at a concentration of 100 mg/L under different conditions of temperature and pH, notably at 30°C, 50°C and 70°C for pH 1, 5, 7 and 9, and at 5 °C, 30 °C and 50 °C for pH 13. Additional experiments were conducted at pH 1 and 13 with phenyl-U-^{14}C-labelled dimethachlor (0.69 MBq/mg) to identify hydrolysis products. Based on Arrhenius parameters derived using rate constants (first order kinetics), a half-life > 200 days was calculated for pH 1, 5, 7 and 9 at 20 °C; a ready hydrolysis, on the other hand, occurred at pH 13 (half-life of 9.13 days at 20 °C). At pH 1 and 13, the degradation product 2,6-dimethyl-N-(methoxyethyl)-hydroxyacetanilide (CGA39981) was detected.

An OECD TG 111 (GLP) study investigated the hydrolysis of Phenyl-U-^{14}C labelled dimethachlor at a concentration of 5 mg/L in sterile aqueous solutions buffered to pH 1, 5, 7 and 9 (Kirkpatrick, 1995a). A 5-day pre-test was run at 50 °C, resulting in < 10 % degradation of the test substance. The main test was performed for 30 days at 20 °C with samples taken at 0, 5, 10, 14, 18, 22, 26 and 30 days. The substance was hydrolytically stable at all pH values, with percentage of recoveries between 95.1-97.7 % of the solution radioactivity at all sampling times.

The degradation products CGA50266, CGA354742 and CGA42443 were individually tested according to OECD TG 111 and GLP; no degradation was observed after incubation for 7 days at 2 mg/L and 49.5 °C in aqueous solutions buffered to pH 4, 5, 7 and 9.

Aquatic simulation tests

Four studies were presented by the DS on the degradation of dimethachlor in simulated water or water-sediment systems.

The mineralisation and degradation route of [^{14}C]-dimethachlor was assessed by McLaughlin (2015) according to OECD TG 309 and GLP in natural freshwater collected from White's Pond (Plymouth, MA, US). Dimethachlor was tested up to 62 days at 10 and 95 µg/L in the dark at 20 ± 2 °C. Separate samples treated with the reference material sodium ^{14}C-benzoate at 10 µg/L were run at the same conditions to check the microbial population viability. No degradation of the parent compound was observed at 62 days after treatment (DAT) (102.6 and 102.5 % AR at 10 and 95 µg/L). The reference material was readily mineralised to $^{14}CO_2$ (89.6 and 91.2 % AR at 14 and 21 DAT), whereas the mineralisation of the test substance was very small, accounting for 0.8 and 1.3 % AR at 10 and 95 µg/L, respectively.

In a study conducted by Keller (1976) according to a non-guideline protocol, the surface of a pond near Les Evouettes (VS, Switzerland) was sprayed with dimethachlor formulated as EC 400 (Teridox®) in order to obtain a concentration of 1 mg/L. Samples were taken at 1 and 5 hours, and 3, 7, 14, 30, 45 and 63 days. The test substance disappeared in pond water with a half-life of approximately 7 days. At day 63, the residues level in water was 0.03 mg/L, while levels < LOD (0.02 mg/kg) were detected in sediment.

The degradation of phenyl-U-^{14}C labelled dimethachlor was investigated by Flückiger (1995) and (re-evaluated by) Flückiger and Sägesser (1995) according to the Dutch Registration Guideline BBA IV:5-1 in equilibrated water/sediment systems from a river (Rhine, Switzerland) and a pond (Anwiler-Teich, Switzerland). The substance was tested at nominal concentration of 0.497 mg/L for 182 days at 20 ± 1 °C in the dark; water and sediments were sampled at 0, 1, 3, 7, 14, 21, 28, 56, 84, 112 and 182 days. At the end of the study period, dimethachlor had nearly disappeared in both systems (0.1 % AR in the water phases, 0.1-0.3 % AR in the sediments). The total system DT_{50} values were 9 days (River) and 23 days (Pond), while the water phase DT_{50} values were 6 days (River) and 16 days (Pond). The sediment dissipation DT_{50} values were 1.5 days (River) and 16 days (Pond). High amounts of non-extractable radioactivity were identified at the end of the study (river: 56.7 %; pond: 50.9 %), suggesting that the disappearance of ^{14}C-dimethachlor was mainly due to the formation of strongly bound residues. At least 11 fractions were detected using LC/MS in water and sediments from the two systems. The two major fractions were the oxalic acid derivative CGA50266, with a maximum of 13.0 % AR in the water (182 DAT) and 4.2 % AR in the sediment (112 DAT) in the pond system, and the dichlorination product CGA42443, which showed maximum levels of 5.5 % AR in water (112 DAT) and 4.5 % AR in sediment (112 DAT) in the pond system. The test compound mineralisation was low in both test systems, with $^{14}CO_2$ accounting for 3.5 % and 3.1 % AR in the river and pond systems, respectively.

The aerobic transformation of dimethachlor was tested according to OECD TG 308 in two water-sediment systems from two US (MA): Taunton River (sandy loam) and Weweantic River (sand) (Connor, 2016b). The test was performed by adding ^{14}C-labeled dimethachlor to water at a concentration of 0.33 µg/mL. The system was maintained at 20 ± 2 °C in the dark and samples analysed at 0, 1, 3, 6, 14, 29, 63 and 99 days. Using first order kinetics, degradation half-lives of 22 and 45 days was calculated for the Taunton River and Weweantic River (total) systems, respectively. In the water phase, the dissipation DT_{50} values were 17 days in Taunton and 35

days in Weweantic River. The major degradation products CGA50266 was detected with maximum values of 18.4 % and 4.6 % AR respectively in water and sediments from Taunton river, respectively (Day 63). CGA42443 was also detected with maximum levels of 5.5 % (day 99) and 4.3 % (day 29) AR in Taunton river's water and sediments, respectively. Mineralisation to CO_2 was a minor route of degradation, accounting for 3.0 % to 3.2 % AR for the Weweantic and Taunton River systems.

Photochemical degradation

A study conducted according to OECD TG 101 and GLP assessed the direct photodegradation of dimethachlor using (spectrophotometrically) measured UV/VIS spectra and the program GCSOLAR to calculate the half-lives ($t_{1/2}$) for direct photolysis in surface water at 40 °N and 50 °N in summer (Schmidt, 2001). Calculated $t_{1/2}$ were 4520 and 8620 days for 40 °N and 50 °N, indicating that direct photo-transformation is not a relevant degradation process for dimethachlor in the environment.

In a second study (Kirkpatrick, 1995b), the photolysis of [U-^{14}C- phenyl] dimethachlor was evaluated according to US EPA N 161-2 and GLP. The substance was tested at a final concentration of 5 mg/L in aqueous buffer solution at pH 7. The test system was continuously irradiated with a xenon arc lamp for up to 15 days at a temperature of 25 °C. A dark (non-irradiated) control was run in parallel at the same experimental conditions. No degradation occurred in both irradiated and non-irradiated test systems, confirming that dimethachlor is not likely to be significantly photo-degraded in natural systems.

Conclusion on the rapidly degradability

The DS noted that dimethachlor is not readily biodegradable and not rapidly degradable via hydrolysis or photolysis in the aquatic environment. In surface water simulations, there was no evidence of significant degradation of dimethachlor. In water-sediment systems, dimethachlor rapidly dissipated in water, and produced high amounts of non-extractable radioactivity in sediment, suggesting that the formation of strongly bound residues was a major pathway for the disappearance from the tested systems. Based on the above considerations, the DS considered dimethachlor as not rapidly degradable for the purpose of classification and labelling.

Bioaccumulation

A Log K_{ow} of 2.17 at 25 °C was experimentally derived for dimethachlor (Stulz, 1994b) according to OECD TG 117 indicating a low potential to bioaccumulate in fish. No bioconcentration studies were available. The DS concluded that dimethachlor has low potential to bioaccumulate in aquatic species according to the criteria specified under the Regulation (EC) No. 1272/2008 (trigger value of Log K_{ow} > 4).

Aquatic toxicity

Acute aquatic toxicity

The relevant acute aquatic toxicity data presented by the DS for dimethachlor and formulations (A5089F, A5089H) are displayed in the table below. All reported studies are GLP-compliant.

Species / Method	Test material	Results (mg/L)	Reference
Fish			
Oncorhynchus mykiss; OECD TG 203; flow-through	dimethachlor (96.8 %)	96 h-LC_{50} = 5.9 (mm)	(1993, Vol. 3B.9 - KCA 8.2.1. / 01)
Cyprinus carpio; OECD TG 203; flow-through	dimethachlor (96.8 %)	96 h-LC_{50} = 7.6 (mm)	(1993a, Vol. 3B.9 - KCA 8.2.1. / 02)
Oncorhynchus mykiss; OECD TG 203; flow-through	A5089F (497 g a.s./L)	96 h-LC_{50} = 9.5 (mm) (4.72 mg a.s./L)	(1996) CGA17020/0377
Aquatic invertebrates			
***Daphnia magna*; OECD TG 202; static**	dimethachlor (96.8 %)	48h-LC_{50} = 24 (nom)	Grade (1994a, Vol. 3B.9 - KCA 8.2.5.1. / 01)
***Daphnia magna*; OECD TG 202; static**	A5089F (497 g a.s./L)	48 h-LC_{50} = 18.1 (nom) (9 mg a.s./L)	Neumann (1996) CGA17020/0382
Algae and aquatic plants			
Desmodesmus subspicatus; OECD TG 201; static	dimethachlor (96.8 %)	72 h-ErC_{50} = 0.072 (nom)	Grade (1993c, Vol. 3B.9 - KCA 8.2.6.1. / 01); Schuster (2018, stats re-analysis) - Vol. 3-B.9. KCA 8.2.6.1. / 02)
Anabaena flos-aquae; OECD TG 201; static	dimethachlor (99.5 %)	96 h-ErC_{50} > 100 (mm); 72 h-ErC_{50} > 100 (mm)	Falk (2016, Vol. 3B.9 - KCA 8.2.6.1. / 02)
***Pseudokirchneriella subcapitata*; OECD TG 201; static**	A5089H (494 g a.s./L)	96 h-ErC_{50} = 0.029 (nom); 72 h-ErC_{50} = 0.034 (nom) (0.017 mg a.s./L)	Volz (2006) CGA17020/0778
Lemna gibba; OECD TG 221; semi-static	dimethachlor (97.2 %)	**7 d-ErC_{50} = 0.0658 (nom)**	Memmert (1999, Vol. 3B.9 - KCA 8.2.7. / 01)
***Lemna gibba*; OECD TG 221; static**	A5089H (495 g a.s./L)	7 d-ErC_{50} = 0.063 (frond no.); 7 d-ErC_{50} > 0.12 (dry weight)	Liedtke (2011) A5089H_10003
***Lemna gibba*; OECD TG 221; static**	A5089F (497 g a.s./L)	7 d-ErC_{50} = 0.048 (nom; frond no.)	Grade (2002) CGA17020/0591

mm – mean measured concentration; nom - nominal concentration; a.s. – active substance (dimethachlor)

Short-term toxicity values are available for three trophic levels (fish, invertebrates and algae/aquatic plants) for both dimethachlor and formulations. The lowest acute toxicity value for dimethachlor is the ErC_{50} of 0.0658 mg/L for the aquatic plant *Lemna gibba* (growth rate inhibition). Lower ErC_{50} values, but in the same order of magnitude as for dimethachlor, were reported for the formulations A5089H and A5089F (0.063 and 0.048 mg/L, respectively). The DS considered that even though these data are not strictly suitable for classification, which should be based on tests conducted on the active ingredient only, they provide useful supporting information. Based on the above considerations, the DS proposed to classify dimethachlor as Aquatic Acute 1, H400, with an M-factor of 10, based on the ErC_{50} of 0.0658 mg/L derived in *Lemna gibba.*

Chronic aquatic toxicity

The relevant chronic toxicity data presented by the DS are displayed in the table below.

Species / Method	Test material	Results (mg/L)	Reference
Fish			
Oncorhynchus mykiss; OECD TG 204; flow-through	dimethachlor (96.8 %)	21d-NOEC > 0.85 (mm)	(1993d, Vol. 3B.9 - KCA 8.2.2 / 01)
Danio rerio; OECD TG 210; flow-through	dimethachlor (98.5 %)	30d-NOEC = 1.0 (nom; post-hatch)	(2018, Vol. 3B.9 - KCA 8.2.2.1 / 01)
Aquatic invertebrates			
Daphnia magna; OECD TG 211; static-renewal	dimethachlor (96.8 %)	22d-NOEC = 2.3 22d-EC_{10} = 2.18 (mm; no. offspring per live female)	Grade (1994b) CGA17020/0288 Kümmich (2019, stat re-analysis) CGA17020_10346
Daphnia magna; OECD TG 211; static-renewal	A5089F (502 g a.s./L)	21d-NOEC = 1.0 (0.5 mg a.s./L) (nom; reproduction)	Peither (2000) CGA17020/0579
Algae and aquatic plants			
Desmodesmus subspicatus; OECD TG 201; static	dimethachlor (96.8 %)	ErC_{10} = 0.0263 (mm)	Grade (1993c, Vol. 3B.9 - KCA 8.2.6.1. / 01); Schuster (2018, stats re-analysis) - Vol. 3-B.9. KCA 8.2.6.1. / 02)
Lemna gibba; OECD TG 221; semi-static	dimethachlor (97.2 %)	**7d-NOE_rC = 0.005** (nom; frond no.)	Memmert (1999, Vol. 3B.9 - KCA 8.2.7. / 01) CGA17020/0528 Kümmich (2018, stat re-analysis) CGA017020_10283
***Lemna gibba*; OECD TG 221; static**	A5089F (495 g a.s./L)	7d-NOE_rC = 0.008 (nom, frond no.)	Grade (2002) CGA17020/0591 Kümmich (2018, stat re-analysis) A5089F_10022

mm – mean measured concentration
nom - nominal concentration
a.s. – active substance (dimethachlor)

Data on chronic toxicity were obtained from all three trophic levels. Aquatic plants showed the lowest chronic end points. A study on *Lemna gibba* showed a NOErC of 0.005 mg/L (frond number) and this value was supported by a study with the formulation A5089F (NOErC = 0.008 mg/L, frond number). Based on these results, the DS proposed to classify dimethachlor as Aquatic Chronic 1, H410, with an M factor of 10.

Comments received during consultation

Two MSCAs commented the CLH report during public consultation.

The first MSCA expressed general agreement with the classification proposal and suggested to modify tables 91 and 95 by indicating as "key studies" only those yielding the lowest reliable toxicity endpoint per taxonomic group. Moreover, a recommendation was given to clarify whether the endpoints for formulated products are given as mg a.s./L or mg product/L in aquatic section tables. The DS agreed with the suggested changes and replied that tables will be amended accordingly.

The second MSCA expressed general agreement with the DS's classification proposal.

Assessment and comparison with the classification criteria

Comparison with the criteria

Degradation

Based on the information reported in the CLH dossier, RAC considers that:

- the substance was not readily biodegradable according to OECD TG 301B test guideline;
- the substance and its main degradation products were hydrolytically stable at environmentally relevant conditions of temperature and pH;
- no degradation was observed in photodegradation studies;
- no evidence of ultimate degradation was noted in aquatic simulation and water/sediment studies.

Therefore, RAC concludes to consider dimethachlor as **not rapidly degradable.**

Bioaccumulation

RAC agrees with the DS that dimethachlor has a low potential for bioaccumulation in aquatic organisms based on the experimentally derived (OECD TG 117) Log K_{ow} of 2.17.

Aquatic toxicity

Acute toxicity

Reliable acute toxicity data on dimethachlor are available for the three trophic levels. The lowest short-term toxicity value is a 7-day ErC_{50} = 0.0658 mg/L for the duckweed *Lemna gibba*. This value has been derived in a study compliant with OECD TG 221 and GLP showing no deviations from the test guideline validity criteria. RAC noted that mean measured concentrations were in the range of 95-115 % of nominal values, therefore results were based on nominal concentrations. Similar acute effects were induced by dimethachlor in the green algae *Desmodesmus subspicatus* (OECD TG 201, GLP), with an ErC_{50} = 0.0721 mg/L, and further supporting evidence were reported for *Lemna gibba* exposed to dimethachlor-based formulations, which resulted in ErC_{50} values in the same order of magnitude (0.01-0.1 mg/L). Overall, based on the key (ErC_{50}) value of 0.0658, and in line with the DS proposal, RAC concludes that dimethachlor **warrants classification as Aquatic Acute 1, with an M-factor of 10** ($0.01 < L/EC_{50} \leq 0.1$ mg/L).

Chronic toxicity

The chronic toxicity of dimethachlor was evaluated on all three trophic levels and the lowest long-term toxicity value was a 7 days NOE_rC of 0.005 mg/L obtained in a reliable test on the aquatic plant *Lemna gibba* (OECD TG 221, GLP). This value was supported by a study performed with a dimethachlor based formulation.

Based on the chronic key study result that falls in the range $0.001 < NOEC \leq 0.01$ mg/L and considering that the active substance is not rapidly degradable, RAC concludes that dimethachlor **warrants classification** as **Aquatic Chronic 1, H410, with an M-factor of 10.**

RAC evaluation of hazards to the ozone layer

Summary of the Dossier Submitter's proposal

Dimethachlor has a vapour pressure of 2.3 × 10^{-3} Pa at 20 °C and a Henry's Law constant of 2.8 × 10^{-4} Pa · m^3/mol at 20 °C. According to the experimental evidence presented in the CLH dossier, only 0.14 % of the Substance was lost to the air compartment after 24 hours of testing in laboratory-controlled air flow conditions. The low volatilisation was confirmed in non-guideline wind tunnel study, which resulted in non-detectable (< LOD) air concentrations at 6 and 24 h and a very low deposition into water samples placed between 1 and 20 m from the applied area.

Calculations using the method of Atkinson for indirect photo-oxidation through reaction with OH-radicals resulted in an atmospheric half-life of 3.2 hours under average atmospheric conditions. This indicates that the small proportion of dimethachlor that volatilise would be unlikely to be subject to long range atmospheric transport.

Considering the above findings and the fact that dimethachlor is not listed in Annex I to Regulation (EC) No 1005/2009, the DS proposed no classification for this hazard class based on conclusive data.

Comments received during consultation

None

Assessment and comparison with the classification criteria

Dimethachlor is not listed in Annex I to Regulation (EC) No 1005/2009. Although the Substance has a Henry's Law constant of 2.8 × 10^{-4} Pa · m^3/mol at 20 °C, suggesting the potential for some volatilisation, experimental evidence presented in the CLH dossier indicate a quick degradation in atmosphere (half-life of 3.2 hours). Overall, RAC agrees with the DS that the **dimethachlor does not warrant classification as hazardous to the ozone layer** based on conclusive data.

Additional references

Hall et al. 2012. Liver hypertrophy: a review of adaptive (adverse and non-adverse) changes Conclusions from the 3rd International ESTP Expert Workshop. Toxicologic Pathology 40, 971-994. DOI: 10.1177/0192623312448935

OECD 2012. Guidance document 116 on the conduct and design of chronic toxicity and carcinogenicity studies, supporting test guidelines 451, 452 and 453

Yokoyama et al. 2021. Refinement of decision tree to assess the consequences of increased serum ALP in dogs: additional analysis on toxicity studies of pesticides evaluated recently in Japan. Reg. Tox. & Pharm 124, 104963 https://doi.org/10.1016/j.yrtph.2021.104963

Appendices

RAC evaluation of carcinogenicity: Additional key elements

In the DRAR (2022), the neoplastic findings in the 24-month carcinogenicity study in rats (Anonymous & Anonymous, 1995) are provided. See a copy of the Table below.

	Males				Females			
Dose [ppm]	**0**	**20**	**300**	**4000**	**0**	**20**	**300**	**4000**
Animals examined	60	60	60	60	60	60	60	60
Adenohypophysis								
Carcinoma					0	0	1	0
Adenoma	16	3	8	13	18	16	6	10
Adrenal cortex								
Carcinoma, N (%)	1 (1.7)	0	0	2 (3.3)				
Adenoma, N (%)	2 (3.3)	0	0	4 (6.7)	1	1	1	0
Myelolipoma, N (%)	0	0	1	0	0	1	0	0
Adrenal medulla								
Medullary tumour, benign	4	5	3	3	1	1	0	0
Medullary tumour, malignant	1	1	0	0				
Bone								
Osteogenic sarcoma	0	1	0	0				
Brain								
Astrocytic glioma, malignant	1	1	0	0				
Oligodendroglioma	1	1	0	0				
Cerebral meninges								
Granular cell tumour, benign	2 (3.3)	0	1 (1.7)	4 (6.7)	1	0	0	0
Cranial nerve								
Neurinoma, malignant	0	1	0	0				
Haematopoietic tissue								
Lymphatic leukaemia	1	1	0	0	0	0	0	1
Myeloid leukaemia	2	0	0	0				
Heart								
Neurinoma	0	0	2	0				
Kidney								
Lipoma *	0	1 (1.7)	1 (1.7)	2 (3.3)				
Liver								
Benign hepatoma	0	0	0	1	0	1	1	1
Hepatocellular carcinoma	1	0	0	0				
Lung								
Squamous cell carcinoma	0	0	1	0				
Lymph node, mesenteric								
Haemangioma	2	1	2	1	0	1	0	0
Angiosarcoma					0	0	0	1
Lymphoreticular tissue								
Lymphoma, malignant	1	0	1	1				
Mammary gland								
Carcinoma	0	0	2 (3.3)	0	10	6	3	2
Adenoma	1 (1.7)	0	0	0	11	5	2	5
Fibroadenoma	1 (1.7)	2 (3.3)	3 (5.0)	4 (6.7)	12	15	24	15
Mouth								
Squamous cell papilloma	0	0	0	2				
Squamous cell carcinoma	1	0	0	1	0	1	0	0
Odontogenic tumour, benign								
Nasopharynx								
Adenoma #	0	0	0	3 (5.0)				
Ovary								
Tubular adenoma					2	0	0	0
Cystadenoma					1	0	0	0
Granulosa/theca cell tumour, malig.					0	0	2	1
Pancreas, exocrine Adenoma	1	0	1	1				
Prostate								
Carcinoma	0	1	0	0				

Skin								
Keratoacanthoma	3	5	1	2				
Basosquamous carcinoma	0	0	0	1				
Trichoepithelioma, benign	0	0	0	1	0	1	0	0
Epithelioma, calcifying								
Small intestine								
Carcinoma	1	0	0	0				
Spinal cord								
Astrocytic glioma, malignant					0	0	0	1
Spinal nerve root								
Neurinoma, malignant	0	0	0	1				
Spleen								
Haemangioma	0	1	0	0				
Subcutaneous tissue								
Fibroma	9	7	8	8	2	2	1	0
Fibrosarcoma	0	1	0	0				
Fibrous histiocytoma, benign	2	5	5	2	0	0	0	1
Fibrous histiocytoma, malignant					0	1	0	0
Lipoma	0	0	2	0	1	1	0	0
Angiosarcoma	1	0	0	0				
Osteogenic sarcoma	0	0	1	0				
Neurinoma, malignant	0	0	0	1				
Sarcoma, not otherwise specified	0	1	0	0				
Tumour, unclassified, malignant					0	0	1	0
Urinary bladder								
Transitional cell papilloma	0	0	1	0				
Thyroid gland								
Carcinoma	2 (3.3)	0	0	1 (1.7)	2	1	0	0
Adenoma	2 (3.3)	0	0	2 (3.3)	0	0	0	1
C-cell adenoma	1 (1.7)	1 (1.7)	1 (1.7)	3 (5.0)	2	0	1	1
Parathyroid gland								
Haemangioma	0	0	1	0				
Pancreatic islet								
Carcinoma	1 (1.7)	1 (1.7)	1 (1.7)	3 (5.0)				
Adenoma	2 (3.3)	1 (1.7)	0	3 (5.0)	2	0	0	1
Peritoneum								
Mesothelioma, malignant	0	0	0	1				
Thymus								
Thymoma, benign	0	0	0	2				
Uterus								
Leiomyosarcoma					0	0	1	0
Zymbal gland								
Squamous cell papilloma	0	1	0	0				
Primary site uncertain								
Haemangioma					0	1	0	0
Histiocytic sarcoma					0	1	0	0
Animals with **benign** tumours	33	28	31	41 +	39	36	28	25 -
Animals with **primary malignant** tumours	14	7	6	13	12	9	8	6
Animals with **metastatic** tumours	5	3	2	6	0	2	2	1
Animals with **any tumour**	40	31	35	46 +	44	41	35	27--

+/- significant trend with $p < 0.05$; ++/-- significant trend with $p < 0.001$

* Historical control data originate from 2-year studies for the same strain of rats (Tif:RAIf (SPF) rats), from the same test laboratory, and were generated within a 5-year period (±5 years; i.e., 1989-1995) with regard to the time period in which the study was performed (1992-1994); study incidences are presented as median (range), N = number of studies/number of animals examined: - Males: 0 (0-1.7), N = 9/629; - Females: 0.7 (0-1.7), N = 4/250

Since survival-adjusted Peto analysis was not performed by the study authors for individual tumour types, the RMS performed Poly-k (i.e. Poly-3) survival-adjustment, followed by Cochran-Armitage test for trend or Fisher's exact test for pairwise comparison (top dose group versus control) for each individual tumour type17. Only nasopharyngeal adenoma showed statistically significant increase in the trend test18, both with the nominal dose level scores (Chi2 for trend = 7.512, p = 0.0061, slope = 1.6e-05) and with the ordinal group-number scores (Chi2

for trend = 4.880, p= 0.0272, slope = 0.020).

Information on the survival in the rat carcinogenicity study is provided below. The mortality in high dose males was statistically significant decreased. As noted by the study authors, this may affect the results; however, when the lesion under analysis is not considered a fatal lesion, the results of the incidental analysis could be used.

	Males				**Females**			
Dose (ppm)	**0**	**20**	**300**	**4000**	**0**	**20**	**300**	**4000**
Treatment ended in observation period, selected	80	80	80	80	80	80	80	80
Advanced autolysis, no samples taken					1			
Examined macroscopically								
Scheduled sacrifice S1	10	10	10	10	10	9	10	7
Scheduled sacrifice S2	36	39	37	55	38	41	48	45
Moribund sacrifice	12	9	9	6	17	21	13	17
Found dead	22	22	25	10	14	9	9	11
Examined microscopically	60	60	60	60	59	60	60	60

More specific information on the male rats with kidney lipomas or nasopharynx adenoma and their day of sacrifice.

Male nr.	**Sacrifice**	**Tumour**
4000 ppm		
241	Scheduled S2, day 732	Kidney lipoma unilateral
242	Found dead, day 667	Nasopharynx adenoma
255	Scheduled S2, day 732	Nasopharynx adenoma
275	Scheduled S2, day 732	Kidney lipoma unilateral
290	Found dead, day 624	Nasopharynx adenoma
300 ppm		
178	Scheduled S2, day 733	Kidney lipoma unilateral
20 ppm		
119	Moribund sacrifice, day 585	Kidney lipoma unilateral

Tumour incidences with the concurrent historical control ranges (HC) for the different tumours in the mice carcinogenicity study (Anonymous, 1995), as presented in the CLH report (Table 44).

Dose dimethachlor mg/kg bw/d	**0**	**2.25**	**32.3**	**488.1**	**0**	**2.17**	**31.2**	**450.9**
	MALES				**FEMALES**			
Examined	50	50	49	50	50	50	50	50
Hepatocellular tumours								
No. of animals with hepatoma (% of examined)	12 (24)	13 (26)	5 (10)	17 (34)				
	HC 24 % (12-46 %)							
No. of animals with carcinoma (% of examined)	4 (8)	12 (24)	7 (14)	12 (24)				
	HC 11 % (0-16 %)							
No. of animals with hepatoma or carcinoma (% of examined)	15 (30)	18 (36)	8 (16)	23 (46)				
	HC 32 % (12-48 %)							
Pulmonary tumours								
No. of animals with adenomas (% of examined)	8 (16)	3 (6)	14 (29)	8 (16)	0 (0)	2 (4)	3 (6)	4 (8)
	HC 20 % (8-28 %)				HC 6 % (2-13 %)			
No. of animals with carcinoma (% of examined)	6 (12)	2 (4)	4 (8)	9 (18)*	3 (6)	1 (2)	1 (2)	1 (2)
	HC 5 % (2-9 %)				HC 4 % (2-7 %)			
No. of animals with adenoma or carcinoma (% of examined)	13 (26)	5 (10)	16 (33)	15 (30)	3 (6)	3 (6)	4 (8)	5 (10)
	HC 25 % (12-33 %)				HC 11 % (5-18 %)			

* Statistical significance (trend test)

Mechanistic data

The MoA proposed for carcinogenicity for the chloroacetanilides is protein-adduct formation with reactive quinone-imine and quinone-imine sulfoxide.

For acetochlor, two metabolism pathways are provided, 1) via free aniline, and 2) via sulfoxide. For dimethachlor, the N-dealkylated metabolite was detected (at low levels) in one *in vivo* toxicokinetic study (Anonymous, 1996), and not in another (Anonymous & Anonymous, 2018). The dimethachlor sulfoxide is detected in both *in vivo* studies.

An *in vitro* metabolism study (Anonymous et al., 2020) was performed to investigate human relevance of nasal adenomas. Metabolism of dimethachlor sulfoxide, one of the metabolites, was investigated in rat and human liver and nasal microsomes. Acetochlor sulfoxide was also included. *Para*-hydroxylation of dimethachlor sulfoxide was found in the rat nasal and human liver microsomes (see Table below). Acetochlor sulfoxide was also included in the study, which was shown to be *para*-hydroxylated by all microsomes, except in human nasal microsomes and rat liver microsomes.

Table 7: Summary of metabolite identification in microsome samples following incubation with 100 µM dimethachlor sulfoxide (CGA048088)

Reference	RLM	RNM	HLM	HNM1	HMN2
CGA048088 *O*-dealkylated dimethachlor sulfoxide	✓	✓	✓	✓	✓
SYN551884 *p*OH dimethachlor sulfoxide	ND	✓	✓	ND	ND
SYN550717 *N*-dealkylated dimethachlor sulfoxide	✓	✓	✓	✓	✓
SYN551849 *p*OH *N*-dealkylated dimethachlor	ND	ND	ND	ND	ND
CGA048090 Dimethachlor sulfone	✓	✓	✓	✓	✓
SYN551848 *p*OH dimethachlor sulfone	ND	ND	ND	ND	ND
CSDL904931 *N*-dealkylated dimethachlor sulfone	✓	✓	✓	ND	ND
SYN551885 *p*OH *N*-dealkylated dimethachlor sulfone	ND	ND	ND	ND	ND

*ND = not detected

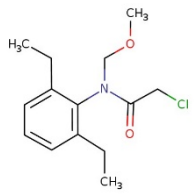
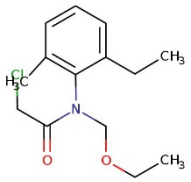
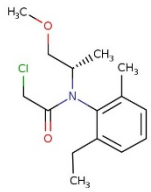
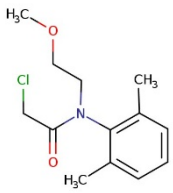
RLM = rat liver microsome, RNM = rat nasal microsomes, HLM = human liver microsomes, HNM = Human nasal microsomes

A similar study was performed again in 2021 (Anonymous et al., 2021). Similar results (see Table below) are reported, except that in this case para-hydroxylation was shown also in rat liver microsomes.

Summary of metabolite identification in microsome samples following incubation with 100 µM dimethachlor sulfoxide (CGA048088)						
Reference	**RT (min)**	**NC**	**HLM**	**HNM**	**RLM**	**RNM**
CGA048088 Dimethachlor sulfoxide	11.1	✓	✓	✓	✓	✓
SYN551884 *p*OH dimethachlor sulfoxide	4.7-5.3	ND	✓	ND	✓	✓
SYN550717 *N*-dealkylated dimethachlor sulfoxide	10.1	✓	✓	✓	✓	✓
SYN551849 *p*OH *N*-dealkylated dimethachlor sulfoxide	3.0	ND	ND	ND	ND	ND
CGA048090 Dimethachlor sulfone	14.8	✓	✓	✓	✓	✓
SYN551848 *p*OH dimethachlor sulfone	7.4	ND	ND	ND	ND	ND
CSDL904931 *N*-dealkylated dimethachlor sulfone	13.4	ND	✓	✓	✓	✓
SYN551885 *p*OH *N*-dealkylated dimethachlor sulfone	5.0	ND	ND	ND	ND	ND

NC = negative control, ND = not detected

With regards to the comparison with other chloroacetanilides, the Table from the CLH report is adapted, the substance S-metolachlor is added. The molecular structures of the substances and incidences of nasal tumours in male rats are provided.

	Alachlor	**Acetochlor**	**S-metolachlor**	**Dimethachlor**
Structure				
Dose	3000 ppm [a,b]	5000 ppm [a,c]	3000 ppm	4000 ppm
Adenoma	65/103 (63 %)	18/69 (26 %)	1/57 (1.8 %)	3/60 (5 %)
Carcinoma	7/103 (7 %)	2/69 (2.9 %)	2/69 (2.9 %) or 1/59 (1.7 %) [d]	0/60 (0 %)
classification	Carc. 2	Carc. 2	Carc. 2*	

[a] nasal tumours also in females;

[b] tumours observed down to 300 ppm;

[c] tumours observed down to 500 ppm;

[d] original report and re-evaluation.

*conclusion in RAC opinion (2022); not only based on nasal tumours

RAC evaluation of reproductive toxicity: Additional key elements

Some of the results from the 2-generation rat study (Anonymous, 1994) are provided in more detail in the Tables below. The first Table provides the data on body weight and food consumption in the two parental generation and the F1 generation, together with available data on the fertility. The highest dose has some effects on food consumption; however, this did not result in a statistically significant effect on body weights (not in the in P1, second P1 and F1).

Dose in ppm (~mg/kg bw/d)	**0**	**20 (1.33)**	**300 (20)**	**2000 (133)**	**4000 (267)**
P males					
Body weight (g), day 99, prior to first mating	546.9	532.4	529.1	535.4	512.2
Food consumption (g/animal/day), day 1-99	29.3	28.8	29.1	28.8	28.2
Body weight (9), day 29, prior to second mating	606.2	590.0	590.2	609.4	576.4
Food consumption (g/animal/day), day 1-29	26.5	26.5	28.6	28.1	27.9
P females					
Body weight (g), day 99, prior to first mating	314.8	318.4	314.2	312.2	296.7
Food consumption (g/animal/day), day 1-99	21.4	21.1	20.9	20.9	19.9
Body weight (g), day 20 of first pregnancy	444.0	444.0	460.8	455.0	420.0
Food consumption (g/animal/day), GD1-20	No differences				
Body weight (g), day 29, prior to second mating	343.0	343.1	339.6	334.9	324.6
Food consumption (g/animal/day), day 1-29	22.1	20.9	20.7	21.0	19.9
Body weight (g), day 20 of second pregnancy second mating	468.2	463.4	453.8	448.3	444.1
First mating					
Insemination index (%)	100	100	96	96	100
Females pregnant	24	22	22	20	24
Females delivering	24	22	22	20	23
Mean no. pups delivered	14.71	14.86	15.00	13.75	13.33
Live birth index (%)	100	99	98	99	95
Viability index (%)	97	97	92	95	92
Weaning index (%)	47	51	56	38	43
Second mating					
Insemination index (%)	96	100	95.8	100	100
Females pregnant	20	19	19	21	18
Females delivering	20	19	19	20	18
Mean no. pups delivered	12.51	11.05	11.74	9.75	11.17
Live birth index (%)	95	99	92	98	100
Viability index (%)	92	98	89	99	96
Weaning index (%)	99	97	85	98	94
Second mating					
Insemination index (%)	96	100	100	100	100
Females pregnant	21	24	21	21	25
Females delivering	21	23	21	21	25
Mean no. pups delivered	14.05	14.71	13.62	13.14	14.00
Live birth index (%)	96	94	96	99	98
Viability index (%)	92	95	92	87	97
Weaning index (%)	96	93	98	91	100
F1 males					
Bw change (g), day 1-99	362.1	3.59.7	350.6	315.5	322.2
Food consumption (g/animal/day), day 1-99	32.5	31.8	30.7	29.8	30.9
F1 females					
Bw change (g), day 1-99	158.7	166.7	162.0	151.2	142.3
Food consumption (g/animal/day), day 1-99	23.8	24.2	23.9	22.3* (6 %)	22.0* (8 %)

* Statistically significant difference from control group mean, $p < 0.05$

The second Table provides the pup body weights from the different generations in the 2-generation rat study (Anonymous, 1994).

Dose in ppm (~mg/kg bw/d)	0	20 (1.33)	300 (20)	2000 (133)	4000 (267)	0	20 (1.33)	300 (20)	2000 (133)	4000 (267)
Pup body weights	**MALE**					**FEMALE**				
F1a offspring										
PND1	6.92	6.89	6.79	6.77	6.79	6.51	6.44	6.41	6.44	6.36
PND4	8.84	8.75	8.44	8.66	8.79	8.39	8.30	8.22	8.22	8.34
PND7	12.04	12.71	12.03	11.52	12.48	11.97	12.40	11.56	11.41	12.33
PND14	31.84	30.73	28.09	26.44	29.65	30.61	30.73	28.63	29.51	28.02
PND21	53.73	55.51	50.43	44.53* (17 %)	46.79	51.29	52.64	47.17	49.22	45.10* (12 %)
F1b offspring										
PND1	7.52	7.47	7.40	7.29	7.39	7.06	7.04	6.84	6.72	6.74
PND4	10.48	10.33	9.99	9.99	10.43	9.92	9.79	9.43	9.21	9.41
PND7	15.92	16.28	15.25	14.73	15.90	15.00	15.53	14.99	13.76	14.66
PND14	32.13	32.72	31.84	29.28	29.03	30.90	31.76	30.34	28.08	28.11
PND21	53.95	55.58	54.42	48.13	46.37* (13 %)	52.20	54.47	51.29	46.53* (11 %)	44.01** (16 %)
F2 offspring										
PND1	6.77	7.06	6.95	6.75	6.95	6.38	6.54	6.43	6.40	6.53
PND4	9.70	9.70	9.66	9.03	9.52	9.19	9.17	9.19	8.56	9.06
PND7	15.66	15.41	15.58	14.68	14.79	14.61	14.04	14.91	13.60	13.85
PND14	31.83	32.02	31.96	29.64	28.53* (10 %)	29.95	30.29	30.74	28.31	27.16
PND21	54.06	53.67	53.72	47.3** (13 %)	43.8** (19 %)	50.83	50.50	51.70	45.29* (11 %)	41.81** (18 %)

* Statistically significant difference from control group mean, $p < 0.05$
** Statistically significant difference from control group mean, $p < 0.01$
PND4 data are pre-cull.
NB some percentages adapted after recalculation, different from values in the DRAR.

In the available repeated dose toxicity studies, no effects were reported with regards to changed weights of testes or ovaries. In the 28-day rat study (Anonymous, 1993), minimal to moderate reduction of spermatogenesis was reported in 3/10 males dosed with 750 mg/kg bw/d, these animals died.

With regards to the developmental toxicity studies, more detailed information is provided in the Tables below. The first Table presents the data of the rat developmental toxicity study (Anonymous, 1994), with exposure to dimethachlor from GD 6-16.

Dose level (mg/kg bw/d)		0	50	350	700
Body weight gain (g) GD6-16		66.8	71.3	63.3	55.2*(17 %)
Mean body weight (g) GD21 [1]		377.3	385.5	374.8	363.3
Food consumption (g/animal/day) GD0-6		20.1	21.5	21.9	21.6
Food consumption (g/animal/day) GD6-11		24.2	24.9	21.5**(11 %)	20.5
Food consumption (g/animal/day) GD11-16		26.3	27.5	24.8	23.9*(10 %)
Food consumption (g/animal/day) GD16-21		26.7	29.1	28.6	28.0
Animals pregnant		24	25	24	25
Animals pregnant, died/sacrificed moribund		0	0	0	5 [2]
Pregnant Animals with viable foetuses		24	25	24	19
Mean number corpora lutea		16.8	16.8	17.7	17.6
Mean number implantations		14.8	14.9	15.3	14.4
Mean pre-implantation loss (%)		11.2	11.8	13.5	17.8
Mean number foetuses		14.4	13.9	14.3	13.8
% Live foetuses		100	100	100	100
Mean number early resorptions		0.5	1.0	1.0	0.6
Mean number late resorptions		0	0	0	0
Mean post-implantation loss		3.4	6.6	7.4	4.0
Mean foetal body weight (g)		5.4	5.4	5.5	5.3
Number foetuses / litters evaluated		345/24	348/25	343/24	262/19
Total malformations	foetal incidence (%)	0	0	0	1 (0.4)
	litter incidence (%)	0	0	0	1 (5.3)

	% affected foetuses/litter	0	0	0	0.44
Total anomalies	foetal incidence (%)	8 (2.3)	11 (3.2)	15 (4.4)	21 (8.0)
	litter incidence (%)	7 (29.2)	10 (40.0)	12 (50.0)	11 (57.9)
	% affected foetuses/litter	2.47	3.39	4.58	8.11
Total variations	foetal incidence (%)	188 (54.5)	187 (53.7)	185 (53.9)	144 (55.0)
	litter incidence (%)	24 (100.0)	25 (100.0)	24 (100.0)	19 (100.0)
	% affected foetuses/litter	54.48	53.92	54.60	54.92

1. Body weights are not presented in the CLH report and the DRAR. These are derived from the study report.
2. One animal found dead on day 9, four animal sacrificed on day 9, 11, and 14.
*: $p < 0.05$; **$p < 0.01$ in d=ANOVA+Dunnett-test

From the DRAR (2022) a detailed overview of the skeletal findings in the rat developmental toxicity study (1993) is provided. Please note, at the highest dose, there is marked maternal toxicity (mortality 25 %).

Findings		**0**	**50**	**350**	**700**
Number foetuses / litters evaluated		177/24	181/25	177/24	137/19
Wide fontanel	foetal incidence (%)	0	0	2 (1.1)	3 (2.2)
	litter incidence (%)	0	0	1 (4.2)	3 (15.8)
	% affected foetuses/litter	0	0	1.4	2.4
Irregular ossification occipital bone	foetal incidence (%)	0	0	2 (1.1)	3 (2.2)
	litter incidence (%)	0	0	2 (8.3)	3 (15.8)
	% affected foetuses/litter	0	0	1.2	2.2
Bipartite occipital bone	foetal incidence (%)	0	0	0	2 (1.5)
	litter incidence (%)	0	0	0	1 (5.3)
	% affected foetuses/litter	0	0	0	1.2
Metatarsal 1 absent ossification	foetal incidence (%)	10 (5.6)	27** (14.9)	23* (13.0)	35** (25.5)
	litter incidence (%)	5 (20.8)	9 (36.0)	9 (37.5)	11 (57.9)
	% affected foetuses/litter	5.3	4.9	11.6	24.9
Anterior digit 2. Proximal phalanx absent ossification	foetal incidence (%)	2 (1.1)	8 (4.4)	0	16** (11.7)
	litter incidence (%)	2 (8.3)	2 (8.0)	0	5 (26.3)
	% affected foetuses/litter	1.2	5.2	0	10.4
Anterior digit 5. Proximal phalanx absent ossification	foetal incidence (%)	6 (3.4)	13 (7.2)	8 (4.5)	23** (16.8)
	litter incidence (%)	3 (12.5)	4 (16.0)	6 (25.0)	9 (47.4)
	% affected foetuses/litter	3.2	8.4	4.4	15.6
Anterior digit 5. Proximal phalanx poor ossification	foetal incidence (%)	4 (2.3)	3 (1.7)	8 (4.5)	12* (8.8)
	litter incidence (%)	2 (8.3)	3 (12.0)	6 (25.0)	6 (31.6)
	% affected foetuses/litter	2.1	1.5	4.2	9.5
Posterior digit 2. Proximal phalanx absent ossification	foetal incidence (%)	31 (17.5)	39 (21.5)	54** (30.5)	59** (43.1)
	litter incidence (%)	15 (62.5)	13 (52.0)	15 (62.5)	13 (68.4)
	% affected foetuses/litter	17.7	21.1	29.2	40.9
Posterior digit 3. Proximal phalanx absent ossification	foetal incidence (%)	24 (13.6)	30 (16.6)	36 (20.3)	48** (35.0)
	litter incidence (%)	12 (50.0)	11 (44.0)	12 (50.0)	11 (57.9)
	% affected foetuses/litter	13.8	16.6	19.3	33.6
Posterior digit 4. Proximal phalanx absent ossification	foetal incidence (%)	24 (13.6)	34 (18.8)	36 (20.3)	50** (36.5)
	litter incidence (%)	12 (50.0)	11 (44.0)	11 (45.8)	12 (63.2)
	% affected foetuses/litter	13.7	18.6	19.2	34.7
Posterior digit 4. Proximal	foetal incidence (%)	5 (2.8)	6 (3.3)	14 (7.9)	13* (9.5)
	litter incidence (%)	5 (20.8)	4 (16.0)	9 (37.5)	6 (31.6)

phalanx poor ossification	% affected foetuses/litter	3.0	3.0	7.3	8.3

* Statistically significant difference from control group mean, p < 0.05
** Statistically significant difference from control group mean, p < 0.01

In the Table below the results from the rabbit developmental toxicity study (Anonymous, 1993) are provided.

Dose level (mg/kg bw)	**0**	**10**	**100**	**350**
Body weight *gain* (g) GD6-19 (treatment days)	352.7	308/7	365.5	230.5
Body weight *gain* (g) GD0-28	828.8	817.0	902.1	791.4
Food consumption (g/animal/day) GD6-19	220.0	217.5	232.2	187.6* (15 %)
Food consumption (g/animal/day) GD0-28	202.7	201.9	216.8	211.0
Does found dead	0	0	1	2
Pregnant females	19	16	18	14
Does with total litter resorption	1	1	0	0
Does with abortion (killed)	0	1	0	1
Does with live foetuses	18	14	17	11
Corpora lutea/doe	12.2	11.7	11.8	11.4
Implants/doe	9.3	9.8	7.7	6.2
Pre-implantation loss (%)	23.5	16.1	35.0	43.3
Post-implantation loss (%)	9.7	4.7	16.3	10.4
Live foetuses/doe	8.4	9.4	6.4	5.5
Dead foetuses/doe	0.0	0.0	0.2	0.1
Resorptions/doe – Early	0.8	0.5	1.0	0.5
Resorptions/doe – Late	0.0	0.0	0.1	0.0
Sex ratio (% males)	61.6	53.3	42.8*	69.4
Mean foetal body weight (g)	39.0	36.3	40.9	40.2
Litter weight	319.7	333.4	257.7	209.4*
Number of litters examined	18	14	17	11
Malformation and variation data				
No. foetuses examined	152	131	109	61
No. foetuses with external/visceral malformations No. litters affected	4 3	1 1	6 4	1 1
No. foetuses with skeletal malformations No. of litters affected	6 5	4 3	10 6	5 2
Total number (%) malformed foetuses Total number (%) litters affected	9 (5.9) 5 (27.8)	5 (3.8) 4 (28.6)	13 (11.9) 7 (41.2)	5 (8.2) 2 (18.2)
No. foetuses with external/visceral variations No. of litters affected	1 1	0 0	0 0	0 0
No. foetuses with skeletal variations No. litters affected	152 18	130 14	108 17	60 11

* p < 0.05

ANNEXES:

Annex 1 The Background Document (BD) gives the detailed scientific grounds for the opinion. It is the combined Draft Renewal Assessment Report prepared according to Regulation (EC) N° 1107/2009 and Proposal for Harmonised Classification and Labelling (CLH Report) according to Regulation (EC) N° 1272/2008.

Annex 2 Comments received on the CLH report, response to comments provided by the Dossier Submitter and RAC (excluding confidential information).