Committee for Risk Assessment

RAC

Opinion

proposing harmonised classification and labelling
at EU level of

S-metolachlor (ISO);

2-chloro-*N*-(2-ethyl-6-methylphenyl)-*N*-[(2*S*)-1-methoxypropan-2-yl]acetamide; (R_aS_a)-2-chloro-*N*-(6-ethyl-*o*-tolyl)-*N*-[(1*S*)-2-methoxy-1-methylethyl]acetamide

[contains 80-100% 2-chloro-*N*-(2-ethyl-6-methylphenyl)-*N*-[(2*S*)-1-methoxypropan-2-yl]acetamide and 0-20% 2-chloro-*N*-(2-ethyl-6-methylphenyl)-*N*-[(2*R*)-1-methoxypropan-2-yl]acetamide]

EC Number: -
CAS Number: 87392-12-9

CLH-O-0000007145-77-01/F

Adopted

2 June 2022

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2 June 2022

CLH-O-0000007145-77-01/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 an opinion on the proposal for harmonised classification and labelling (CLH) of:

Chemical name: ***S*-metolachlor (ISO);**

2-chloro-*N*-(2-ethyl-6-methylphenyl)-*N*-[(2*S*)-1-methoxypropan-2-yl]acetamide; (R_aS_a)-2-chloro-*N*-(6-ethyl-*o*-tolyl)-*N*-[(1*S*)-2-methoxy-1-methylethyl]acetamide

[contains 80-100% 2-chloro-*N*-(2-ethyl-6-methylphenyl)-*N*-[(2*S*)-1-methoxypropan-2-yl]acetamide and 0-20% 2-chloro-*N*-(2-ethyl-6-methylphenyl)-*N*-[(2*R*)-1-methoxypropan-2-yl]acetamide]

EC Number: **-**

CAS Number: **87392-12-9**

The proposal was submitted by **Germany** and received by RAC on **25 May 2021.**

In this opinion, all classification and labelling elements are given in accordance with the CLP Regulation.

PROCESS FOR ADOPTION OF THE OPINION

Germany has submitted 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 **5 July 2021**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **3 September 2021**.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Nathalie Printemps**

Co-Rapporteur, appointed by RAC: **Laure Geoffroy**

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 RAC opinion on the proposed harmonised classification and labelling was adopted on **2 June 2022** by **consensus**.

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	607-432-00-4	S-metolachlor (ISO); 2-chloro-N-(2-ethyl-6-methylphenyl)-N-[(2S)-1-methoxypropan-2-yl]acetamide; (RaSa)-2-chloro-N-(6-ethyl-o-tolyl)-N-[(1S)-2-methoxy-1-methylethyl]acetamide	-	87392-12-9	Skin Sens. 1 Aquatic Acute 1 Aquatic Chronic 1	H317 H400 H410	GHS07 GHS09 Wng	H317 H410			
Dossier submitter's proposal	607-432-00-4	*S*-metolachlor (ISO); 2-chloro-*N*-(2-ethyl-6-methylphenyl)-*N*-[(2*S*)-1-methoxypropan-2-yl]acetamide; (R_aS_a)-2-chloro-*N*-(6-ethyl-*o*-tolyl)-*N*-[(1*S*)-2-methoxy-1-methylethyl]acetamide [contains 80-100% 2-chloro-*N*-(2-ethyl-6-methylphenyl)-*N*-[(2*S*)-1-methoxypropan-2-yl]acetamide and 0-20% 2-chloro-*N*-(2-ethyl-6-methylphenyl)-*N*-[(2*R*)-1-methoxypropan-2-yl]acetamide]	-	87392-12-9	**Retain** Aquatic Acute 1 Aquatic Acute 1 **Add** Carc. 2 Repr. 2 STOT RE 2	**Retain** H400 H410 **Add** H351 H361d H373 (skin)	**Retain** GHS09 Wng **Add** GHS08	**Retain** H410 **Add** H351 H361d H373 (skin)		**Add** M = 10 M = 10	
RAC opinion	607-432-00-4	*S*-metolachlor (ISO); 2-chloro-*N*-(2-ethyl-6-methylphenyl)-*N*-[(2*S*)-1-methoxypropan-2-yl]acetamide; (R_aS_a)-2-chloro-*N*-(6-ethyl-*o*-tolyl)-*N*-[(1*S*)-2-methoxy-1-methylethyl]acetamide [contains 80-100% 2-chloro-*N*-(2-ethyl-6-methylphenyl)-*N*-[(2*S*)-1-methoxypropan-2-yl]acetamide and 0-20% 2-chloro-*N*-(2-ethyl-6-methylphenyl)-*N*-[(2*R*)-1-methoxypropan-2-yl]acetamide]	-	87392-12-9	**Retain** Aquatic Acute 1 Aquatic Chronic 1 **Add** Carc. 2	**Retain** H400 H410 **Add** H351	**Retain** GHS07 GHS09 Wng **Add** GHS08	**Retain** H410 **Add** H351	**Add** EUH066	**Add** M = 10 M = 10	

Resulting Annex VI entry if agreed by COM	607-432-00-4	*S*-metolachlor (ISO); 2-chloro-*N*-(2-ethyl-6-methylphenyl)-*N*-[(2*S*)-1-methoxypropan-2-yl]acetamide; (R_aS_a)-2-chloro-*N*-(6-ethyl-*o*-tolyl)-*N*-[(1*S*)-2-methoxy-1-methylethyl]acetamide [contains 80-100% 2-chloro-*N*-(2-ethyl-6-methylphenyl)-*N*-[(2*S*)-1-methoxypropan-2-yl]acetamide and 0-20% 2-chloro-*N*-(2-ethyl-6-methylphenyl)-*N*-[(2*R*)-1-methoxypropan-2-yl]acetamide]	-	87392-12-9	Carc. 2 Skin Sens. 1 Aquatic Acute 1 Aquatic Chronic 1	H351 H317 H400 H410	GHS08 GHS07 GHS09 Wng	H351 H317 H410	EUH066	 M = 10 M = 10	

GROUNDS FOR ADOPTION OF THE OPINION

RAC general comment

S-metolachlor is a mixture of the 1S (80-100%) and 1R (20-0%) isomers, each of which is a racemic mixture of rotamers. Metolachlor is a mixture of the S and R stereoisomers, and it contains the two isomers in equal amount (1:1 ratio). The S-isomer, which is the main isomer of s-metolachlor is considered more active as a herbicide than the R-isomer. For carcinogenicity and adverse effects on sexual function and fertility, studies were only available with metolachlor in the CLH dossier.

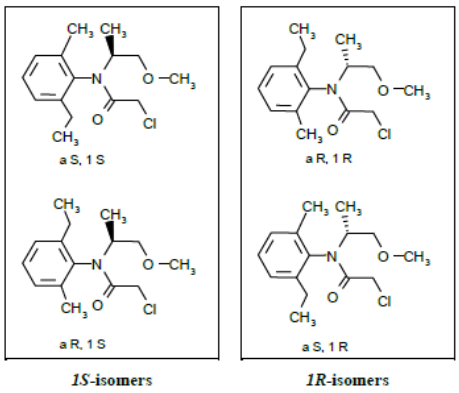

***Figure**: Structural isomers of s-metolachlor and metolachlor*

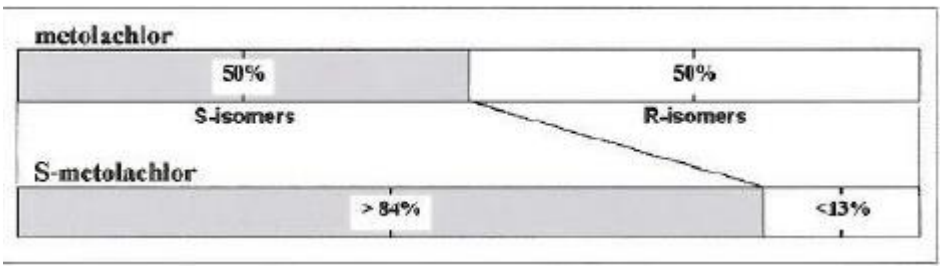

***Figure**: Isomer composition of metolachlor and s-metolachlor*

In the available toxicokinetic studies, a similar oral absorption and metabolic pathway was observed for the racemic mixtures and the S-enantiomer. In addition, some toxicological data were available for both metolachlor and s-metolachlor. Metolachlor, which had a similar acute toxicity profile. The substances were not irritant and were both skin sensitisers. The same target organs (liver, kidney) and similar NOAEL/LOAEL were observed in the short-term toxicity studies. A similar toxicological profile was also observed in the developmental toxicity studies, whose results were comparable between metolachlor and s-metolachlor.

Based on this, RAC agrees with the dossier submitter (DS) to consider the read across between the two compounds to be fully acceptable.

HUMAN HEALTH HAZARD EVALUATION

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

Summary of the Dossier Submitter's proposal

Oral rat repeated-dose toxicity studies were available with *S*-metolachlor or metolachlor. The DS described one 28-d (Anonymous (12), 1995) and two 90-d rat toxicity studies (Anonymous (4), 1995; Anonymous (13), 1999) with *S*-metolachlor, and one 28-d (Anonymous (12), 1995) and one 90-d (Anonymous (14), 1999) toxicity studies in rats with metolachlor. In addition, two 90-d oral toxicity studies in dogs were available with *S*-metolachlor (Anonymous (3), 1995; Anonymous (43), 1999) and a 6-month (Anonymous (44), 1980) and a 1-year oral dog toxicity study (Anonymous (20), 1989) were included with metolachlor. Moreover, a 21-d dermal rabbit toxicity study (Anonymous (30), 1987) was available with metolachlor.

Oral exposure

After oral administration, no effects of sufficient severity were reported in the available rat or dog studies to justify classification for STOT RE.

Dermal exposure

In the 21-d dermal repeated-dose toxicity study, similar to OECD TG 410, metolachlor was administered at dose levels of 0, 10, 100 and 1000 mg/kg bw/d in male and female rabbits. Significant local effects including dry skin, erythema and fissuring were observed in treated animal of all dose groups at ≥ 10 mg/kg bw/d. Microscopical examination revealed changes in the skin at all dose levels (minimal hyper- and parakeratosis, congestion, and subacute dermal inflammation of the dermis) in both sexes. Wrinkling of the skin was only noted at the top dose level.

The DS noted that the effects observed at 10 mg/kg bw/d occurred at a dose level relevant for STOT RE 1 (≤ 86 mg/kg bw/d for a 21-d dermal study). Nevertheless, as the effects were considered more as a sign of significant rather than severe toxicity, STOT RE 2 (H373) was proposed for skin effects.

Comments received during consultation

One Member State Competent Authority (MSCA) commented that the skin effects observed in the dermal toxicity study were not severe enough for classification. The MSCA noted that the congestion and subacute dermal inflammation may have been related to skin sensitisation for which the substance is already classified.

Assessment and comparison with the classification criteria

In the 21-d repeated-dose dermal toxicity study (Anonymous (30), 1987), rabbits were exposed to metolachlor by dermal application on skin for six hours per day at 10, 100 or 1000 mg/kg bw/d (five/sex/group). There was no treatment related mortality in the study. Kidney and liver weight changes were noted at the top dose. Bilirubin concentrations were significantly increased in females at the mid and high dose levels.

Dry skin and erythema were observed at the site of application in all dose groups. Erythema was graded as Draize score 1 except in one male in the mid dose group having a score of 2. Fissuring

was only noted in one female at 10 mg/kg bw/d and in both sexes at ≥ 100 mg/kg bw/d. Wrinkles of the skin was only noted at 1000 mg/kg bw/d. No other gross macroscopic pathologies were observed. Histopathological skin lesions included hyper- and parakeratosis at all dose-levels in both sexes and were reported to be of minimal grade by the DS. Additionally, congestion and subacute lymphocytic dermal inflammation of the dermis was observed in both sexes at ≥ 10 mg/kg bw/d.

Macroscopic findings were seen first around day 4-8 depending on the finding, with little differences between the dose groups.

***Table**: Dermal observations in the 21-d dermal rabbit toxicity study*

Dose (mg/kg bw/d)	**0**		**10**		**100**		**1000**	
	M	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Macroscopical pathology								
Erythema (grade 1)	0	0	5	5	5	5	5	5
Erythema (grade 2)	0	0	0	0	1	0	0	0
Dry skin	0	0	5	5	5	5	5	5
Fissuring	0	0	0	1	2	2	5	5
Wrinkles	0	0	0	0	0	0	5	5
Histopathology (dermis, back)								
Skin – back: hyperkeratosis	0	0	5*	3	5*	5*	5*	5*
Skin – back: parakeratosis	0	0	1	3	3	4*	2	5*
Skin - dermis back: focal subacute lymphocytic inflammation	0	0	0	3	3	3	5*	4*
Skin - dermis back: focal congestion	0	0	1	3	3	4	5*	5*
Skin - dermis back: focal haemorrhage	0	0	1	0	0	0	1	1
Skin - dermis back: focal oedema	0	0	0	0	0	3	1	2

Metolachlor up to 20000 mg/kg bw and *S*-metolachlor at 2000 mg/kg bw were not acutely toxic by the dermal route in rabbits and there is no classification for acute dermal toxicity. Slight to moderate dermal irritation appeared in these studies.

In the skin irritation studies available in the renewal assessment report (RAR), six rabbits were exposed to *S*-metolachlor for four hours. The test material produced very slightly to well-defined erythema (Score: 1-2) and very slight to slight oedema (Score: 1-2) within four to 96 hours. All irritations were cleared by day 7. No classification was warranted based on the CLP criteria. Based on this irritation study, single dermal exposure produced noticeable skin inflammation in rabbits, lasting at least for a few days, although their severity did not meet the classification criteria for Skin Irrit. 2. Therefore, it is possible that repeated, occlusive dermal exposure to *S*-metolachlor could lead to significant skin irritation over time.

Skin erythema noted in the 21-d dermal rabbit toxicity study were mainly graded 1. No increase in severity was reported over time. According to the DS, hyperkeratosis and parakeratosis were only graded minimal. Therefore, RAC concluded that the effects may not be severe enough for classification.

In addition, RAC notes that consideration of local skin effects under STOT RE for classification purposes is not straightforward. According to the CLP Regulation (section 3.9.1.1), the target organ toxicity (repeated exposure) does not include other specific toxic effects that are addressed in sections 3.1 to 3.8 and 3.10 of the CLP Regulation and this includes skin irritation.

When taking the lack of (acute) skin irritation/corrosion classification and the proposed Skin Sens. 1 (H317) classification into account, RAC considers that the skin effects observed in this study in this specific case **do not warrant classification for STOT RE**.

However, as in the repeated-dose toxicity study skin dryness was noted in all exposed animals and fissuring was seen in some animals, RAC concludes that an additional warning for the local skin effects is necessary and that *S*-metolachlor meets the CLP criteria for the **additional hazard phrase EUH066 "Repeated exposure may cause skin dryness or cracking"**.

RAC evaluation of germ cell mutagenicity

Summary of the Dossier Submitter's proposal

S-metolachlor was negative in three *in vitro* gene mutation assays in bacteria (Anonymous (23), 1995c; Sokolowski, 2014; Schulz, 2018) and in two gene mutation assays in mammalian cells (mouse lymphoma TK cells, Wollny, 2014) and Chinese hamster V79 cells (Anonymous (36), 2018). Equivocal results were observed *in vitro* in a chromosomal aberration assay (Anonymous (2), 2014) and in a micronucleus assay (Anonymous (32), 2019) with *S*-metolachlor.

Positive results were observed with metolachlor in a mouse lymphoma assay (Anonymous (1), 1984) and in a chromosomal aberration assay (Roloff, 1992). Polyploidy was increased in a mammalian chromosomal aberration assay in human lymphocyte (Anonymous (36), 1990). These studies were only considered as supplementary by the DS due to limitations.

In vivo, negative results were observed in two micronucleus assays (Anonymous (21), 1995a; Anonymous (9), 2014). The DS noted that the power of the studies may have been reduced due to the low number of cells analysed. In addition, the DS pointed out that no decrease in PCE/NCE ratio was noted in the studies. Nevertheless, proof of exposure was demonstrated as *S*-metolachlor was detected in mice plasma (one or four hours after exposure but not at the 24-hour time point) in a proof of exposure study (Anonymous (41), 2017). However, the DS pointed out that the small window of exposure might be an issue to doubt on aneugenicity.

Negative results were obtained in three *in vivo* unscheduled DNA synthesis (UDS) tests with *S*-metolachlor or metolachlor (Anonymous (6), 1988; Anonymous (18), 1994; Anonymous (22), 1995b).

Overall, based on the negative outcome obtained in the *in vivo* studies, the DS proposed no classification for germ cell mutagenicity.

Comments received during consultation

One MSCA agreed with no classification for germ cell mutagenicity but pointed out that the micronucleus assays had clear deficiencies and that the UDS test was only an indicator test and relatively insensitive. Therefore, the MSCA considered that the reason for no classification could be "data lacking" or "inconclusive".

Assessment and comparison with the classification criteria

In vitro data

Three negative bacterial gene mutation assays were available with *S*-metolachlor (Anonymous (23), 1995c; Sokolowski, 2014; Schulz, 2018). The studies were performed according to OECD TG 471 and were GLP-compliant.

Two negative *in vitro* gene mutation assays were available with *S*-metolachlor (Wollny, 2014; Anonymous (36), 2018). The studies were performed according to OECD test guidelines and were GLP-compliant. An equivocal gene mutation study (mouse lymphoma TK) was available with metolachlor (Anonymous (1), 1984). The study was only considered supplementary by the DS, was not GLP-compliant and no historical controls were available. The positive outcome obtained in this study is considered of lower weight than the negative results obtained in the two well-conducted studies with *S*-metolachlor.

A non-reproducible increase was observed in a chromosomal aberration assay (Anonymous (2), 2014), following four hours exposure of human lymphocytes with *S*-metolachlor, in the presence of metabolic activation. In addition, an increase in micronuclei was noted both with and without metabolic activation in a micronucleus assay (Anonymous (32), 2019) following four hours exposure of human lymphocytes to *S*-metolachlor. In the absence of metabolic activation, the increase was inside the historical control data (HCD) range. In the presence of metabolic activation, positive results were observed above historical control in one experiment, but the positive outcome was not reproduced in a second experiment.

Inconsistent results were obtained with metolachlor. The substance was not clastogenic in a chromosomal aberration test in Chinese Hamster Ovary cells (Anonymous (36), 1990) but an increase in polyploidy metaphases was detected at the highest concentration, without metabolic activation (three hours exposure). The study was only rated supplementary due to several deficiencies and polyploidy was not noted in other chromosomal aberration studies. A positive result was also observed in a non-guideline cytogenic study in human lymphocytes (Roloff, 1992) also showing deficiencies.

Overall, there are indications that *S*-metolachlor can be clastogenic *in vitro* in the presence of metabolic activation, even after a short exposure (four hours).

In vivo data

Negative results were obtained i*n vivo*, in two micronucleus tests performed in mice (Anonymous (21), 1995a; Anonymous (9), 2014). The studies were similar to OECD TG 474 and performed by oral gavage.

In Anonymous (21) (1995a), mice were exposed up to 2000 mg/kg bw. The main limitation in the study is the low number of cells scored for micronucleus induction (1000). In this study, it is stated that the maximum tolerable dose (MTD) was reached based on the observed clinical signs.

Mice were exposed at 800 mg/kg bw in the main study (Anonymous (9), 2014). The top dose of 800 mg/kg bw was determined as the MTD based on lethalities and severe toxicity. Only 2000 cells were analysed instead of 4000 recommended in the OECD TG.

With regards to bone marrow exposure, only weak direct evidence of exposure was noted:

- No shift in the ratio of polychromatic erythrocytes (PCE) to normochromatic erythrocytes (NCE) was noted in the studies.
- Clinical signs were noted at the time of administration at 2000 mg/kg bw: ataxia and tremor in males and females (Anonymous (21), 1995a).
- At 800 mg/kg bw, mice displayed hunched or abdominal posture, partially closed eyes, ruffled fur, slight reduction in spontaneous activity, Straub's phenomenon, trembling and tippy toe walk (Anonymous (9), 2014). The neurotoxic findings provide some evidence of systemic toxicity.

Nevertheless, there is some indirect evidence of systemic toxicity:

- A proof of exposure study was performed in mice exposed to 400 mg/kg bw *S*-metolachlor, *S*-metolachlor was detected in 2/3 animals at the one hour and 1/3 animals at the four-hour time point. *S*-metolachlor was not detected 24 hours after gavage (three mice). The substance was detected in variable amounts in the three animals. The data indicate a fast metabolisation of *S*-metolachlor.
- In the 'absorption, distribution, metabolism, and excretion' (ADME) studies, a very efficient oral absorption of *S*-metolachlor was observed in rats. Nevertheless, RAC notes that there is no information available regarding potential differences in mice.
- No short-term toxicity studies were performed in mice to indicate potential systemic toxicity. However, in the two-year mouse chronic study (Anonymous (38), 1982), a significant increase in liver and kidney weight and lower body weight at 571 mg/kg bw/d may also provide indication of systemic toxicity.

Overall, the negative *in vivo* micronucleus assays may be considered as an appropriate follow-up to the positive results observed in the *in vitro* cytogenicity studies. RAC considers that there was some evidence of bone marrow exposure in the *in vivo* studies but acknowledges the uncertainties raised by the DS on potential exposure levels and the issue of fast metabolisation to assess the aneugenicity endpoint.

There are two negative *in vivo* UDS assays (Anonymous (6), 1988; Anonymous (18), 1994) with metolachlor, and the one with *S*-metolachlor (Anonymous (22), 1995b) can also be considered as supportive data.

Comparison with the classification criteria

Based on the negative results observed in the *in vivo* micronucleus assays RAC agrees with the DS that according to the CLP criteria, **no classification is warranted for germ cell mutagenicity**.

RAC evaluation of carcinogenicity

Summary of the Dossier Submitter's proposal

The assessment of the DS is based on two long-term studies with metolachlor in rats and mice. There are no long-term studies available with *S*-metolachlor. In addition, several mechanistic studies were available to investigate the potential liver tumour mode of action (MoA) in rats.

No tumours were seen in the carcinogenicity study in mice (Anonymous (38), 1982). Nevertheless, the study in mice was not considered acceptable by the DS due to high mortality rate in mice (> 50%).

In the rat carcinogenicity study (Anonymous (39), 1983), the DS considered the following tumours for classification:

- Increased incidence in adenoma and carcinoma in the pituitary of females.
- Increased combined incidence of neoplastic nodules and carcinomas in liver of males and females.
- Increased nasal turbinates adenocarcinoma in male rats.
- Increased thyroid adenoma in females.

Eleven mechanistic studies[1]) were provided in the CLH dossier to investigate a potential CAR/PXR-mediated MoA for liver tumours. The arguments for human non-relevance were not accepted by the DS. The DS noted the lack of further experiments to exclude other mechanisms possibly responsible for tumour formation than CAR activation (no CAR knockout hepatocyte or humanised-CAR data, lack of PROD activity in human hepatocytes, missing positive control in some studies and effects not always comparable to the positive control). The DS concluded that the relevance or not to humans of different mechanisms for liver tumours is not sufficiently demonstrated.

In the many epidemiological studies reported[2], the DS noted that some associations were observed between metolachlor exposure and increased likelihood to develop certain tumours (lung cancer, colon cancer, liver cancer, follicular cell lymphoma). The DS pointed out that the positive exposure-response association between liver cancer and metolachlor use (Silver et al., 2015) identified in a prospective cohort through 2010/20, may be of particular concern as in the long-term rat study, liver tumours were observed. Nevertheless, although data were stratified for confounders, its participants were also exposed to other compounds. The DS concluded that no classification in Category 1A was warranted but that there is some limited evidence of carcinogenicity in humans that might support a need for classification.

Based on the weight of evidence considering both human and animal data, the DS proposed to classify *S*-metolachlor as Carc. 2; H351.

Comments received during consultation

One MSCA agreed with the DS's proposal, considering that Category 2 is more appropriate than Category 1B based on the available data. Another MSCA agreed to classify *S*-metolachlor at least in Category 2 based on the epidemiological findings in combination with the multiple tumours observed in rats (liver, pituitary, nasal turbinates and thyroid). The MSCA pointed out that the case may be borderline between Category 2 and 1B.

Industry representatives disagreed with the classification proposal and provided the following arguments in favour of no classification:

- The preneoplastic nodules in the liver have been demonstrated to be due to CAR activation, via a MoA not relevant to human.
- The findings in the pituitary and nasal passages were incidental and not treatment related.
- No findings were noted in the carcinogenicity study in mice, and they further noted that the study should be considered acceptable and similar to OECD TG.
- The epidemiological data do not provide conclusive evidence. Although there was an increased incidence in particular cancers, these cancers could not be attributed to metolachlor only. The industry representatives highlighted that the cohort study included pesticide applicators exposed to numerous plant protection products.

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[1] Anonymous (17), 1994; Anonymous (22), 1995b; Anonymous (35), 1995; Anonymous (10), 2014; Anonymous (27), 2006; Anonymous (34), 2014; Takeuchi, 2008; Kuelbeck, 2011; Kojima, 2011; Anonymous (11), 2014; Anonymous (5), 2019

[2] Rusiecki, 2006; Silver et al., 2015; Alavanja et al., 2004; Andreotti et al., 2009, 2010; Lee et al., 2007, 2005; Koutros et al., 2010; Berry et al., 2011; De Roos et al., 2003; Flower et al., 2004; Thorpe and Shirmohammadi, 2005; Metayer et al., 2013

Industry provided a review of the epidemiological data in humans, a discussion on the acceptability of the mouse carcinogenicity study and a justification for the non-relevance of the liver tumours in rat.

The re-analysis of the epidemiological data concluded that there is no clear link between *S*-metolachlor exposure and incidence of cancer in human. In addition, the latest publication from the AGRICAN cohort (Leon *et al*., 2019; Lerro et al*.,* 2018, 2019, 2020) were included as additional data to support the absence of a link between *S*-metolachlor exposure and increased tumour incidence.

Moreover, in order to support the acceptability of the mouse carcinogenicity study, additional detailed information on survival was provided (see table below). The industry representatives noted that the mouse study performed over 24 months instead of 18 months as recommended in the current OECD TG 453 (study performed prior to this OECD TG) was of unusual duration. They noted that following 18 months exposure, survival was acceptable and was not less than 73% in any group. Therefore, they considered that the study is valid for the assessment of the carcinogenic potential of the substance.

Sex	**Males**				**Females**			
Dose levels (ppm)	0	300	1000	3000	0	300	1000	3000
Number*	60	60	60	60	60	60	60	60
Number dying prior to week 79	11	10	9	15	8	15	12	20
% survival until week 79	81.7	83.3	85	75	86.7	75	80	66.7

* Excluding animals scheduled for interim sacrifice in week 52.

In the position paper, no new information was provided on the MoA of rat liver tumours.

In response, the DS noted that the human data investigated the association of alachlor with cancer and that no association of metolachlor and cancer can be drawn from this study (Lerro et al*.,* 2018). Similarly, as metolachlor was not specifically investigated in Lerro et al. (2019), this new study did not provide additional information on a potential association of *S*-metolachlor and cancer. The DS provided a critical assessment of the meta-analysis of Leon et al. (2019), suggesting the exposure to metolachlor may have been underestimated and also provided a more in-depth analysis of the results of Silver et al. (2015).

In addition, the DS provided the table of individual lifetime observations as reported in the original study report of the mice carcinogenicity study, which is reported in the section below.

Assessment and comparison with the classification criteria

Metolachlor has been studied for carcinogenicity potential in mice (Anonymous (38), 1982) and rats (Anonymous (39), 1983).

No treatment related carcinogenic effects were noted in the mouse study which was performed partly in compliance with OECD TG 451. RAC agrees with the DS that the acceptability of the study is questionable due to several limitations, including:

- Accidental water restriction during the first week of the study.
- Ethanol was used to prepare the diet (control as well as metolachlor diets) for the first 18 weeks. Small amounts of ethanol may have remained in the diet.
- Several investigations were conducted in too few animals (e.g., determination of feed intake in 10 animals/sex/dose only, determination of effects on haematological or clinical

chemistry parameters, at month 12 and 18, only a limited number of organs were weighed).

- Sendai virus infection affected the survival rate in all dose groups at the beginning of the study and more particularly in females of the high dose group. The significant increase in mortality rate in the high dose group in females at the end of the study may have been the result of these early deaths.
- There is no information if the tissues of animals that died between weeks 79 and 105 were of sufficient quality. However, RAC assumes that this was the case as no statement was available in the study.
- In the OECD TG 451, for mice, a duration of 18 months is considered more appropriate than 24 months.

With regards to the survival rates in the study, RAC notes that survival was above 50% at all dose levels including controls at week 79. Therefore, the effect on survival may not have affected the validity of the study. However, RAC notes that there is no information available if histopathological examination was influenced by the high mortality rate in females after two years.

***Table**: Relative survival in the mice study (Anonymous (38), 1982) according to individual lifetime observations as reported in the original study report (provided by DE-CA during targeted public consultation)*

Dose level (ppm)	Males		Females	
	Week 79	Week 105	Week 79	Week 105
N[1]	52	52	52	52
0	79%	38%	85%	52%
300	81%	48%	71%	38%
1000	83%	56%	77%	44%
3000	71%	54%	62%	33%*

* $p<0.05$; [1]The total number of animals does not include the eight animals per groups sacrified at 12 and 18 months.

In Anonymous (39) (1983), metolachlor was administered to Sprague-Dawley rats, for two years, in diet at 0, 20, 300 and 3000 ppm corresponding to about 0, 1.5, 15 and 150 mg/kg bw/d (60 rats/sex/group). In addition, five animals/sex/group were killed at week 53 for the toxicity study and at week 57 to investigate recovery. There were no treatment related effects on survival or clinical signs. During week 9, animals were affected with alodacryoadenitis virus (SDAV). Nevertheless, according to the authors, no histopathological findings would be attributed to this infection. At the top dose, a decreased in body weight gain was noted in males. In females, decreased body weight was noted during weeks 6-78 (by 5-10%). Based on total study duration, differences in body weight gain were about 13% in females and 7% in males. There was a slight trend in lower feed intake in females. Overall, RAC considers that there was no excessive toxicity in the study up to the highest dose.

Four types of tumours were discussed in the CLH dossier as potentially relevant for classification: liver, nasal turbinates, thyroid and pituitary tumours.

Liver tumours

Foci of cellular alteration (eosinophilic, clear cell and basophilic) were dose-dependently increased in both sexes. The increase was statistically significant in female rats. In addition, 'proliferative foci (neoplastic nodules)', was reported at the top dose in both male and female rats, positive in trend-test in both sexes. Although the terminology used is not standard, according to the study report terminology, 'proliferative foci (neoplastic nodules)' refers to primary benign neoplasms. As such, they were called 'adenoma' by the Co-RMS in the RAR.

The increase in hepatocellular carcinoma was statistically significant (one-sided trend-test) at the top dose in females. Total nodules and carcinoma were increased at the top dose in both males and females, with a positive trend-test and in a pairwise analysis in females in the original report. The increase in neoplastic nodules was above the provided HCD in males and females: maximum one incidence (2.1%) in females and two (4.4%) in males. The increase in adenocarcinoma was also outside historical controls for females. In males, the increase was also outside the HCD so as the negative controls. RAC notes that the HCD were very limited as only referring to one study from the same laboratory and strain with two controls (1982). Liver tumours were reassessed in 1984 following an EPA request and lead to a similar conclusion.

There was no evidence of reduced time latency as most of the tumours were observed at terminal sacrifice.

The table below presents the incidence of tumours in the rat carcinogenicity study available with metolachlor, re-evaluated in 1984. Similar results were observed in the re-evaluation.

***Table**: Liver tumour incidence in **female** rats treated with metolachlor for two years (terminal kill & moribund/died on test)*

Dose (mg/kg bw/d)[1]	**0**	**1.5**	**15**	**150**	**Control 1 /Control 2[2]**
Number examined	60	60	60	60	47/46
Eosinophilic foci Re-evaluation, 1984	4 5	7 6	5 9	23* 17*	
Total number of animals with foci (eosinophilic+clear +basophilic)	13	15	18	34*	
Proliferative foci (Neoplastic nodules) Re-evaluation, 1984	0 0/60	0 1/60	1 (1.7%) 2/60	4 (6.7%)# 6/60 (10%)*#	1 (2.1%)/ 0
Hepatocellular carcinomas Re-evaluation, 1984	0 0	0 0	0 0	2 (3.3%)# 1 (1.7%)#	0/1 (2.2%)
Total nodules and carcinomas Re-evaluation, 1984	0 0	0 1 (1.7%)	1 (1.7%) 2 (3.3%)	6 (10%)#* 7 (11.7%)*	

[1] Dose calculated using a default conversion factor of 20; [2]HCD available from two controls of the same study; # Cochrane-Armitage Trend-Test, one-sided; *: Fisher's exact test.

***Table**: Liver tumour incidence in **male** rats treated with metolachlor for two years*

Dose (mg/kg bw/d)[1]	**0**	**1.5**	**15**	**150**	**Control 1 /Control 2[2]**
Number examined (original/re-evaluation)	59/60	59/60	60/60	60/60	45/45
Eosinophilic foci Re-evaluation, 1984	10 12	15 13	14 19	21 22	
Total number of animals with foci (eosinophilic+clear+basophilic)	19 (32%)	24 (41%)	22 (37%)	29 (48%)	
Proliferative foci Neoplastic nodules (re-evaluation, 1984)	0 1	0 1	0 0	4 (6.7%)# 4 (6.7%)#	0/2 (4.4%)
Hepatocellular carcinoma Re-evaluation, 1984	2 (3.4%) 2 (3.4%)	1 (1.7%) 1 (1.7%)	3 (5%) 3 (5%)	2 (3.3%) 3 (5%)	0/1 (2.2%)
Total nodules and carcinoma Re-evaluation, 1984	2 (3.4%) 3 (5%)	1 (1.7%) 2 (3.3%)	3 (5%) 3 (5%)	6 (10%)# 7 (11.7%)	

[1] Calculated using default conversion factor of 20; [2]HCD available from two controls of the same study; #: Cochrane-Armitage trend-test, one-sided.

Overall, the significant increase in combined liver adenoma and carcinoma in females and males may be treatment related and considered relevant for classification.

Mode of action of liver tumours

A MoA data package consisting of 11 studies was provided in the CLH dossier to assess the human relevance of the rat liver tumours. The postulated MoA is that the activation of CAR and PXR nuclear receptors in rats results in the increase in hepatic cell proliferation leading to hepatocellular tumours.

The mechanistic studies available in the CLH dossier are described in the in-depth analysis by RAC section below.

There are two events that should be considered in case of CAR-mediated MoA in rodent: activation of CAR/PXR nuclear receptors and hepatocellular proliferation.

Activation of CAR and PXR nuclear receptors

In vitro, S-metolachlor was able to activate human CAR and human PXR (hPXR) but not human Arylhydrocarbon receptor (AhR) (Kuelbeck et al., 2011) in a C3A hepatoma cells reporter assay. Similarly, no activation was observed on human AhR (hAhR) in a screening assay for agonistic activity in Takeuchi et al. (2008). Metolachlor was an agonist of hPXR and mice PXR (mPXR) in Kojima et al. (2011). In a transactivation assay (Anonymous (34), 2014), *S*-metolachlor was shown to be an agonist of rat (57-fold), human (9-fold) and mouse (27-fold) CAR nuclear receptor.

In vivo, CAR activation was investigated *in vivo* in a 7-d and 28-d study in rats up to 426 mg/kg bw/d in males and 435 mg/kg bw/d in females (Anonymous (35), 1995).

PROD (marker of CYP2B, CAR) enzyme activities were statistically significantly increased (8x) in males at ≥ 242 mg/kg bw/d and in females (31x) at ≥ 257 mg/kg bw/d in response to *S*-metolachlor, corresponding approximately to the top dose level used in the carcinogenicity study. In addition, EROD (marker of CYP1A1 or CYP1A2) was increased dose-dependently and was statistically significant in both sexes (2.2x in males and 2.3x in females at 257 and 242 mg/kg bw/d, respectively). After 28 days of exposure, there was no increase in the total number of hepatocellular nuclei or labelling index. There was a moderate increase of smooth endoplasmic reticulum.

An increase in CAR-dependent enzymes was observed in female rats exposed to *S*-metolachlor at 3000 ppm in diet (235 mg/kg bw/d) for 14 or 60 days, similar to the dose level used in the carcinogenicity study (Anonymous (27), 2006). BROD (marker of CYP2B and CYP3A, CAR/PXR) and PROD (marker of CYP2B, CAR) enzyme activities were strongly increased at 14 and 60 days, respectively. MROD and EROD activities were also significantly increased at 60 days only. Hepatic CYP2B1, CYP3S and CYP1A2 protein levels were increased (statistical significance not assessed). In females treated for 3, 5, 7, 14, 28 and 60 days, no treatment related effects were observed in hepatocellular proliferation. There was no positive control in this study.

Associated events of CAR/PXR activation, such as altered gene expression, were not assessed in the mechanistic studies. Nevertheless, associated events such as increased liver weights and hepatocellular hypertrophy was observed in the 28-d rat toxicity studies (Anonymous (12), 1995). RAC noted the absence of liver hypertrophy in both the 90-d and carcinogenicity studies.

Overall, activation of CAR and PXR as well as an increase in CAR-dependent enzyme activity in response to *S*-metolachlor/metolachlor was observed. The liver induction profile of *S*-metolachlor can be considered consistent with CAR/PXR activation.

Increased hepatocellular proliferation

In vitro, proliferation of female rat hepatocyte was observed (Anonymous (14), 2014). Inconsistent results were observed *in vivo*. Hepatocellular proliferation as shown by BrDU labelling of hepatocytes was not increased after 7, 14, or 60-d exposure in the *in vivo* rat studies (Anonymous (35), 1995; Anonymous (27), 2006). In these studies, dose levels were similar to the dose used in the carcinogenicity study. Nevertheless, in a cell proliferation assay in SD rat, an increase in DNA synthesis was observed 72 hours after gavage at 500 mg/kg bw in males and at 1000 mg/kg bw metolachlor (but not at 500 or 100 mg/kg bw) in females (Anonymous (17), 1994).

Although hepatocellular proliferation was not investigated in longer term studies, an increase in a pre-neoplastic lesion (altered foci) was observed in both males and females at the top dose in the rat carcinogenicity study, which was consistent with hepatocellular proliferation.

Human non-relevance of the MoA

There were two *in vitro* studies in human hepatocytes (Anonymous (11), 2014; Anonymous (5), 2019) and one *in vitro* study in female rat hepatocytes (Anonymous (10), 2014).

***Table**: Comparative in vitro studies in human and rat primary hepatocytes*

	Human hepatocytes						**Female rat hepatocytes**	
	Female donor[1]		**Female donor[2]**		**Female donor[2]**			
Concentrations tested (µM)	*S*-metolachlor	PB	*S*-metolachlor	PB	*S*-metolachlor	PB	*S*-metolachlor	PB
Cell proliferation (by BrdU incorp.)	-	-	-	-	-	-	↑ (1.9x)	↑ (1.6x)
PROD activity (Cyp2b)	-	↑ (2.2x)	-	-	-	-	-	↑ (2.8x)
BROD activity (Cyp2b/Cyp3a)	↓ (0.19x)	↑ (3.1x)	-	↑ (2.0x)	-	↑ (2.1x)	↑ (1.3x)	↑ (4.7x)

[1] Anonymous (11), 2014; [2] Anonymous (5), 2019; PB: phenobarbital (100-1000µM); *S*-metolachlor: 5-75µM.

These studies showed that the increase in cell proliferation observed in rat hepatocytes was not observed in human donors. Epidermal growth factor (EGF) was used as a positive control and induced the expected cell proliferation. Overall, these studies showed that there were quantitative differences in the activation of CAR by *S*-metolachlor in rats and humans. RAC notes the lack of activation of BROD and/or PROD in human hepatocytes, as noted with the positive control, as an uncertainty of the proposed MoA.

Exclusion of alternative MoAs

There was no CARKO/PXRKO double knockout study or humanised CAR animals to show if the presence of CAR and/or PXR is essential in the initial hepatic proliferative response.

S-metolachlor is not genotoxic.

In the 90-d rat repeated-dose toxicity study, liver toxicity was observed but necrosis was not found. Therefore, cytotoxicity may not be the main MoA for rat liver tumours.

No evidence of activation of PPARγ was noted in the *in vivo* mechanistic study.

Treatment with *S*-metolachlor had no effect on the CYP3A1, CYP3A2, CYP4A1/A- and CYP4A3content. Therefore, peroxisomal proliferation can be ruled out. The substance was not an agonist *in vitro* of hAhR. Nevertheless, EROD was increased dose-dependently in the 28-d *in vivo*

study. In addition, CYP1A2 protein level was increase after 60 days exposure to *S*-metolachlor. Therefore, AhR activation *in vivo* cannot be fully ruled out.

There is no data in the CLH report suggesting that other MoA such as porphyria, statins/altered cholesterol synthesis, oestrogenic activity and immunosuppression would be likely for *S*-metolachlor.

Overall, RAC concludes that the proposed MoA is plausible in rats. However, the following uncertainties are noted:

- Inconsistency in the proliferative response in the *in vivo* studies at dose levels similar to the carcinogenicity study.
- Lack of activation of PROD and BROD in human hepatocytes.
- No *in vivo* CAR/PXR knock out animals or humanised-CAR animals were performed to confirm the *in vitro* results. This is especially needed as *S*-metolachlor is extensively metabolised, and no measures were taken to further stimulate the metabolism in the *in vitro* studies.
- Some of the alternative MoAs cannot be excluded.

Based on the above uncertainties, RAC agrees with the DS that the available data are not sufficient to conclude on human non-relevance.

Nasal turbinates

Treatment-related neoplastic findings (nasal turbinate tumours) have been observed with substances from the same chemical class (chloroacetanilide herbicides): alachlor, butachlor and acetochlor. The 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. Therefore, a re-analysis of the incidence of nasal turbinates was performed to exclude potential class effect. There is no explanation why a lower number of animals was used in the re-examination study. The DS proposed that some of the tissues may not have been suitable for re-examination due to the time elapse between the study and the re-examination.

An increase in nasal turbinate tumour was noted in males at the top dose. The incidence was 2/69 males in the original study report and 1/59 in the re-evaluation report. The increase was not statistically significant and was not observed in females.

The HCD provided are limited: they were from six studies performed between June 1975 and June 1987 in the same laboratory. The rat carcinogenicity study was dated 1985. In these studies, nasal turbinates were only investigated in case of macroscopic lesions in two out of 397 males and two out of 398 females. No neoplastic findings were noted. Although the HCD are limited, they support that this type of tumour is very rare. During the consultation, the industry representatives also provided HCD from the RITA database. In 54 studies between 1984 and 2013, the HCD range was 0-1 (0-1.7%) in male Wistar or Sprague-Dawley rats. RAC notes that these controls were performed in other laboratories and during a period larger than the ±5-year preferred range. Nevertheless, these HCD also support that it is a rare tumour type.

***Table**: Nasal turbinate tumour incidence in male and females in the original study report or after re-evaluation.*

Dose (mg/kg bw/d)[1]	0	1.5	15	150
Males				
Adenomatous polyps Re-evaluation	1/67[1] 1/57	0/59 0/59	0/53 0/53	0/69 1/59
Adenocarcinoma Re-evaluation	0/67 0/57	0/59 0/59	0/53 0/53	2/69# 1/59#
Fibroadenoma (original report)	0/67	0/59	0/53	1/69
Neurofibrosarcoma (re-evaluation)	0/57	0/59	0/53	1/59
Females				
Adenoma papilloma Re-evaluation	0/67 0/57	0/58 0/57	1/59 1/59	0/69 0/59
Squamous cell papilloma Re-evaluation	0/67 0/57	1/58 0/57	0/59 0/59	1/69 1/59
Odontoma Re-evaluation	1/67 1/57	0/58 0/57	0/59 0/59	0/69 0/59

[1] Including animal of interim sacrifice; # Cochrane-Armitage trend-test, one-sided.

Overall, although the incidences were low and the increase only in males, RAC considers that the increase in nasal turbinates tumours is of concern, as it is a rare tumour. Nevertheless, RAC acknowledges that the low incidence raises some uncertainties about the toxicological relevance of the observed effect.

Pituitary tumours

In the pituitary, a significant increase in adenoma and carcinoma was observed at the top dose in females. The increase in adenoma was positive in both pairwise and trend-test and the increase in carcinoma was positive in a trend-test only. No HCD were provided for this type of tumour, but no carcinoma was noted in the control group.

There were no preneoplastic findings such as hyperplasia in the pituitary gland and no tumours were observed at the 12-month time point. Nevertheless, adenomas were also significantly increased in female rats at the top dose level.

During the consultation, HCD from the RITA database were provided. In 41 rat carcinogenicity studies conducted between 1985 and 2014, 62 female animals showed pituitary carcinoma (2.8%; range 0-10%) and 67 females invasive brain carcinoma (2.9%; 0-12%). Although the HCD are limited (not in the ±5-year range, different laboratories), the increase in malignant carcinoma in pituitary gland is slightly above the HCD range.

***Table**: Pituitary tumour incidence in female rats*

Dose (mg/kg bw/d)*	0	1.5	15	150
Pituitary (terminal sacrifice) tumour in pituitary gland				
Benign adenoma	11	20	20	31#**
Malignant carcinoma	1/32	0/27	1/27 (3.2%)	5/39 (12.8%)#
Pituitary tumour in brain (terminal sacrifice)				
Invasive carcinoma	0/33	2/30	2/29	4/40 (10%)

Cochrane-Armitage trend-test, one-sided; ** p<0.001, Fisher exact test.

Therefore, RAC considers this type of tumour treatment related and that it should be taken into account for classification.

Thyroid

In addition, an increase in thyroid follicular cell adenoma was noted at the top dose in females (5%), above the HCD range from the study with two controls (maximum 1/45 in one of the

controls, or 2.2%) performed at the same time as the study. However, as commented for the liver tumours, these HCD are very limited. No progression to malignancy was observed and incidences were low. Therefore, RAC considers that this type of tumour could be incidental and provides insufficient evidence for classification.

***Table**: Thyroid tumour incidence in female rats*

Dose (mg/kg)*	**0**	**1.5**	**15**	**150**
Thyroid (total terminal kill and died on test and moribund)				
Clear cell adenoma	4	2	2	7
Clear cell carcinoma	2	0	1	1
Follicular cell adenoma	0	0	2 (3.5%)	3 (5%)#

Cochrane-Armitage trend-test, one-sided; ** p<0.001, Fisher exact test.

Human data

In humans, epidemiological studies presented in the CLH dossier showed some associations of metolachlor exposure in particular for certain tumours: liver cancer, follicular cell lymphoma, lung cancer, colon cancer.

Liver tumours

Silver et al. (2015) evaluated cancer incidence in the prospective cohort Agricultural Health Study (AHS) through 2010-2011 for 49616 applicators, 53% of whom reported ever using metolachlor. The cohort included licensed private and commercial pesticide applicators in Iowa and North Carolina recruited in 1993-1997. The authors used the Poisson regression to evaluate relations between two metrics of metolachlor use (lifetime days, intensity-weighted lifetime days) and cancer incidence (risk ratio and 95% confidence intervals (CI)). Intensity-weighted lifetime days take into account exposure modifying factors like use of personal protective equipment, methods of pesticide application, whether the applicator also repaired or cleaned pesticide application equipment and whether the applicator themself mixed pesticides. The intensity weighting factors were further adjusted against exposure monitoring data from consequent field studies and those (slightly) modified factors were used in this study. RAC notes that such an exposure metric may be more relevant than the exposure lifetime days metric. The authors categorised the metrics with quartiles based on the distribution among the cancer cases.

The authors also compared tumour incidence either with the low exposed group (1st quartile) or with unexposed applicators as reference groups. The authors noted, as in a previous study (Rusiecki et al., 2006), that the demographic characteristics for groups with high metolachlor use were more similar than those using less metolachlor than the unexposed applicators. In particular, applicators reporting use of metolachlor were more likely to have consumed alcohol in the past year and to have at least a high school education. In addition, according to Rusiecki et al. (2006), 80% of the metolachlor exposed applicators were from Iowa and 20% North Carolina whereas for unexposed metolachlor applicators about 60% were from Iowa and 20% from North Carolina. In addition, Silver et al., 2015 reported that the applicators in the highest usage group of metolachlor (4th quartile) were also most likely to have used one or more of the highly correlated pesticides compared to the 'no use' group. There were little differences with respect to age, smoking rate, family history of cancer.

***Table**: Selected demographic and lifestyle characteristics of applicators by cumulative metolachlor use in the AHS cohort, 1993-2011 (selected from table 1 of the published paper)*

Characteristics	No use (n=23111)	Quartile 1 (n=7866)	Quartile 4 (n=6803)
Alcohol consumption over past year (drinks/month)			
Never in past year	36.2%	28.8%	23%
< 1.875	14.5%	15.3%	13.5%
≥ 1.875 - < 14.5	25.5%	29.8%	31.4%
≥ 14.5	21.3%	24.2%	30.7%
Missing	2.4%	2.0%	1.3%
Education			
> High school	10.1%	7.1%	4.7%
High school graduate/GED	46.3%	48.6%	45.6%
> High school	41.2%	42.4%	47.6%
Missing	2.4%	1.8%	2.1%
Use of dicamba			
No	64.6%	47%	27%
Yes	32.3%	49.6%	69.8%
Missing	3.2%	3.4%	3.2%
Use of Alachlor			
No	64%	43%	25.5%
Yes	33.7%	54.5%	72%
Missing	2.2%	2.5%	2.5%
Use of atrazine			
No	47%	27.6%	7.5%
Yes	51%	71%	91.6%
Missing	1.9%	1.4%	0.9%
Use of trifluraline			
No	65.3%	43.5%	22.8%
Yes	31%	53.2%	74.2%
Missing	3.7%	3.3%	3.0%
State of residence			
Iowa	57.1%	72.8%	75.9%
North Carolina	42.9%	27.2%	20.6%

For liver cancer, in analyses restricted to exposed workers ('low-metolachlor use' category used as referent), no significant differences were noted. However, trends for both lifetime and intensity-weighted lifetime days of metolachlor use were positive and statistically significant with the 'no use' category used as referent.

***Table**: Rate ratios[a] for liver cancers with at least 20 exposed cases by quartiles of lifetime exposure days and intensity-weighted lifetime exposure days to metolachlor among AHS cohort applicators, with unexposed person-time as the referent, 5-year lag (Silver* et al.*, 2015)*

Cancer site	Lifetime days			Intensity-weighted lifetime days		
	N[b]	RR (95% CI)	p-trend	N	RR (95% CI)	p-trend
Liver						
Unexposed	17	1.00		15	1.00	
Q1[c]	2	0.97 (0.17–5.50)		3	1.65 (0.37–7.23)	
Q2	4	1.79 (0.54–5.93)		3	1.33 (0.35–4.99)	
Q3	**7**	**3.06 (1.05–8.90)**		**8**	**3.14 (1.11–8.88)**	
Q4	**10**	**3.99 (1.43–11.1)**	**< 0.01**	**9**	**3.18 (1.10–9.22)**	**0.03**

a Adjusted for age, smoking, alcohol, applicator status (private or commercial), family history of cancer (any site), state of residence and the pesticides most highly correlated with metolachlor (alachlor, atrazine, dicamba, imazethapyr, trifluralin). All cancers combined also adjusted for sex and race. Lung and prostate cancers also adjusted for race.

b Median number of cases over five imputations.

c For lifetime days analyses with a 5-year lag, unexposed = 0 days, Q1 > 0 – ≤ 15 days, Q2 > 15 – ≤ 38.75 days, Q3 > 38.75 – ≤ 108.5 days, Q4 > 108.5 days. For intensity-weighted lifetime days analyses, unexposed = 0 days, Q1 > 0 – ≤ 490, Q2 > 490 – ≤ 1403, Q3 > 1403 – ≤ 4103, Q4 > 4103 units.

CI = confidence interval; RR = rate ratio.

***Table**: Rate ratios[a] for liver cancers with at least 20 exposed cases by quartiles of lifetime exposure days and intensity-weighted lifetime exposure days to metolachlor among AHS cohort applicators, with person-time in the low-metolachlor exposure category as referent, 5-year lag (Silver* et al*., 2015)*

Cancer site	Lifetime days			Intensity-weighted lifetime days		
	N[b]	RR (95% CI)	p-trend	N	RR (95% CI)	p-trend
Q1	2	1.00		3	1.00	
Q2	4	1.86 (0.31-11.1)		3	0.85 (0.16-4.52)	
Q3	7	3.13 (0.56-17.4)		8	1.83 (0.42-8.02)	
Q4	10	4.01 (0.68-23.5)	0.1	9	1.71 (0.33-8.83)	0.44

a Adjusted for age, smoking, alcohol, applicator status (private or commercial), family history of cancer (any site), state of residence and the pesticides most highly correlated with metolachlor (alachlor, atrazine, dicamba, imazethapyr, trifluralin). All cancers combined also adjusted for sex and race. Lung and prostate cancers also adjusted for race.

b Median number of cases over five imputations.

c For lifetime days analyses with a 5-year lag, unexposed = 0 days, Q1 > 0 – ≤ 15 days, Q2 > 15 – ≤ 38.75 days, Q3 > 38.75 – ≤ 108.5 days, Q4 > 108.5 days. For intensity-weighted lifetime days analyses, unexposed = 0 days, Q1 > 0 – ≤ 490, Q2 > 490 – ≤ 1403, Q3 > 1403 – ≤ 4103, Q4 > 4103 units.

CI = confidence interval; RR = rate ratio.

No association between metolachlor use and incidence of all cancers combined (n = 5701) with a 5-year lag or most site-specific cancers were seen.

RAC notes that in this study, an association was noted between metolachlor exposure and liver tumours. The study of Silver et al. (2015) has the strength that it is based on a large sample size and several confounding factors were taken into account and adjusted for. The statistically significant increase in liver cancer was noted to be dose-related and an association was noted both considering lifetime exposure days and intensity-weighted lifetime exposure days. In addition, liver tumours were observed in the rat carcinogenicity study, supporting a biological plausibility. However, although a link was observed, the association may have been due to potential bias/chance or confounding factors that are discussed below.

- <u>Reference groups</u>

Evidence of a link between liver cancer and metolachlor exposure is only evident using the unexposed group as the referent. According to the authors, the group with higher metolachlor use (4th quartile) was more similar to those using less metolachlor than the unexposed applicators. There is no quantitative assessment or statistical analysis of the differences between the characteristics of the groups. On the one hand, RAC acknowledges that due to potential differences in some baseline parameters as potential confounding factors, the use of the most representative group (1st quartile group) as reference could be relevant. The absence of effects noted when the low-exposure group is used as referent may be due to several potential factors. Although rate ratios were adjusted for potential confounding factors such as alcohol use, full

adjustment may have been challenging. On the other hand, given the relatively small numbers of liver cancers in the low metolachlor exposure categories (1st quartile), considering the low exposure group as reference also leads to uncertainties and may explain the observed difference.

- Co-exposure with other pesticides

Applicators in the highest usage group of metolachlor (4th quartile) were more likely to have used one or more of the highly correlated pesticides. It is noted that one of the correlated exposures adjusted for was dicamba. More recently, in the same AHS cohort, Lerro et al. (2020) reported a statistically significant increasing trend of risk of cancer of the liver and bile ducts ($p < 0.001$) by increasing intensity-weighted lifetime exposure days of dicamba. Also, the relative risk in the highest exposure quartile was statistically significantly increased with dicamba (RR = 1.80, 95% CI: 1.26 – 2.56). Adjustment has been performed in Silver et al., 2015 for potential confounding effect of exposure to dicamba. However, due to the relatively small numbers of liver cancers in the metolachlor exposure categories (e.g., two and three cases in the 1st quartile), a full adjustment for the confounding effect of the potentially highly correlated exposure to dicamba may have been challenging. It may also be noted that dicamba is currently not classified for carcinogenicity in Annex VI of the CLP Regulation. In RAC 61, June 2022, the CLH proposal for dicamba was discussed and RAC concluded on no classification for carcinogenicity for dicamba due to inconclusive data.

- Alcohol use

In Lerro *et al*. (2019), a statistically significantly reduced risk of liver tumours was reported in the AHS cohort (SIR = 0.56, 95% CI: 0.45 – 0.70) based on 78 observed cases compared to the general population due to e.g., lower alcohol consumption. However, in this AHS follow-up study only cohort level risks were reported, without assessment of risk by different pesticide exposures or other risk factors. In Silver et al. (2015), alcohol consumption was only adjusted for alcohol use during the year before enrolment. Although a more detailed alcohol exposure characteristics would have been needed to control this potential factor, this could be a reasonable proxy of overall alcohol use.

Overall, RAC considers that this study may support the observed effect in the liver in the rat carcinogenicity study, acknowledging that it is not possible to fully exclude potential residual confounding factors such as co-exposure with other pesticides such as dicamba or other potential bias (e.g., alcohol exposure). RAC notes that Silver *et al*. (2015) considered that additional follow-up would facilitate assessment of whether the differences in the results reflect greater statistical power with a larger reference category or other exposure-related factors that they were unable to control. Further follow-up would permit better assessment of the role of latency in these associations, as well as evaluation of the role of metolachlor exposure in other health outcomes, particularly those for which cases are sparse or for which a longer lag period may be more biologically plausible. RAC agrees that further studies assessing the association between metolachlor and liver cancer would be needed.

Follicular cell lymphoma

Silver et al. (2015) also reported a significant increasing trend in the incidence of follicular cell lymphoma in the AHS cohort. No effects were noted on other lymphoma subtypes. The association was only observed when the unexposed group was used as referent.

***Table**: Rate ratios[a] for follicular cell lymphoma with at least 20 exposed cases by quartiles of lifetime exposure days and intensity-weighted lifetime exposure days to metolachlor among AHS cohort applicators, with person-time in the low-metolachlor exposure category or 'non-use' group as referent, 5-year lag (Silver* et al.*, 2015).*

Lifetime days			**Trend**	**Intensity-weighted lifetime days**		**Trend**
	N[b]	**RR (95% CI)**		**N[b]**	**RR (95% CI)**	
Follicular cell lymphoma: unexposed as referent						
Unexposed	24	1.00		24	1.00	
Q1[c]	4	0.93 (0.31–2.79)		6	1.37 (0.52–3.57)	
Q2	10	**2.43 (1.07–5.52)**		6	1.45 (0.56–3.78)	
Q3	7	1.76 (0.64–4.81)		10	**2.67 (1.10–6.49**)	
Q4	9	**2.89 (1.13–7.38)**	0.03	8	2.57 (0.95–6.95)	0.04
Follicular cell lymphoma: low exposure group as referent						
Q1	5	1.00		7	1.00	
Q2	10	2.48 (0.84-7.32)		6	1.08 (0.36-3.24)	
Q3	7	1.84 (0.53-6.34)		10	2.04 (0.71-5.88)	
Q4	9	3.24 (0.96-11)	0.14	8	2.08 (0.61-2.12)	0.21

a Adjusted for age, smoking, alcohol, applicator status (private or commercial), family history of cancer (any site), state of residence and the pesticides most highly correlated with metolachlor (alachlor, atrazine, dicamba, imazethapyr, trifluralin). All cancers combined also adjusted for sex and race. Lung and prostate cancers also adjusted for race.

b Median number of cases over five imputations.

c For lifetime-days analyses with a 5-year lag, unexposed = 0 days, Q1 > 0 – ≤ 15 days, Q2 > 15 – ≤ 38.75 days, Q3 > 38.75 – ≤ 108.5 days, Q4 > 108.5 days. For intensity-weighted lifetime-days analyses, unexposed = 0 days, Q1 > 0 – ≤ 490, Q2 > 490 – ≤ 1403, Q3 > 1403 – ≤ 4103, Q4 > 4103 units.

CI = confidence interval; RR = rate ratio.

Using intensity-weighed lifetime days as a metric, no dose-response was noted.

In a more recent study, Leon *et al*. (2019) investigated the relationship between the use of 14 selected pesticides, including metolachlor, and non-Hodgkin's lymphoid malignancies and major subtypes. In this study, the analysis combined two cohort studies: the French AGRICAN study, that enrolled 181747 men and women in 2005-2007 and followed-up until 2009 and the AHS cohort including linkage until 2010-2011. Leon et al. (2019) did not find a significant association with follicular lymphoma (43 cancer cases exposed to metolachlor, hazard ratio of 1.05 and 95% CI: 0.59 - 1.86) or other non-Hodgkin's lymphoid malignancies. However, as noted by the DS there were some limitations in this study. Leon et al. 2019 estimated the exposure to active substances for the AGRICAN cohort by country-specific crop-exposure matrices (CEM), which infers the exposure to a specific pesticide from the cultivated crop, pesticide sales and pesticide. Leon et al. (2019) considered the use of the CEM method as a limitation, as it might lead to lower specificity than self-reported pesticide use at the active ingredient level. The CEM likely lead to an overestimation of the exposure to metolachlor in the AGRICAN cohort. In addition, the DS noted that Leon et al. (2019) did not provide a separate analysis of the hazard ratio for the AHS and AGRICAN cohort.

Overall, RAC notes that the association between follicular cell lymphoma and metolachlor was weaker than for liver. In addition, considering the inconsistent results obtained for this type of tumour in human, the data are insufficient for classification.

Other tumour types

An increase in lung tumour was reported in two AHS cohort studies. In Alavanja et al. (2004), the authors evaluated cancer incidence through 2001. The odds ratio (OR) was 4.1 (95% CI: 1.1-9.22) using the no exposure group as referent and 5 (95% CI: 1.6-10.4) when the 'no use' was used as the referent group. In Rusiecki et al. (2006), the authors evaluated cancer incidence during a similar period, through 2002. Lung cancer showed a significant trend along the highest tertile of the lifetime exposure days (RR = 2.37, 95% CI: 0.97-5.82) using the low-metolachlor exposure group as referent. However, using the intensity-weighted lifetime days exposure, no association was found. As these earlier suggestions of increased lung cancer risk at high levels of metolachlor use in this cohort was not confirmed in the update published by Silver et al. (2015), including more comprehensive adjustment for potential confounding factors, the evidence is considered insufficient for classification.

In Andreotti et al. (2010), the authors evaluated cancer incidence through 2005 in the AHS cohort. A statistical increase in hazard ratio for colon cancer was published when body mass index (BMI) was ≥ 30, showing that BMI is an interaction factor. In a nested case-control study of the AHS cohort, Koutros et al., 2010 evaluated cancer incidence through 2002 and showed increased colon cancer in person with 8q24 variants genetic factor for prostate cancer in the high exposure group to metolachlor. Association between colon cancer and metolachlor was not reported in other AHS cohort studies (Silver et al., 2015; Rusiecki et al., 2006; Lee et al., 2007). Thus, no consistent evidence on colon cancer is available for metolachlor.

No association or decreased risk was observed between metolachlor and pancreatic cancer (Andreotti et al., 2009), prostate cancer (Barry et al., 2011; Rusiecki et al., 2006), or childhood cancer (Flower et al., 2004).

Two case-control studies were also reported in the CLH report. No association was observed with the use of metolachlor with non-Hodgkin's lymphoma (De Roos et al., 2003). An increase OR was noted for metolachlor and brain cancer but was not statistically significant (Lee et al., 2005).

Comparison with CLP criteria

There is limited evidence of carcinogenicity in humans for liver tumours and follicular cell lymphoma reported in one cohort study, including a high number of people in the US. Nevertheless, due to potential co-exposure, Category 1A is not considered justified.

Liver combined adenomas and carcinomas were significantly increased in female and male rats at the top dose only. Pre-neoplastic lesions and progression to malignancy has been noted. RAC considers that the incidence for carcinoma being low is an uncertainty. Based on the mechanistic data available in the dossier, a CAR/PXR mediated effect, which is not relevant to humans, is plausible although uncertainties have been noted. It was not possible to fully exclude potential other MoAs and some inconsistencies in the studies. Therefore, this type of tumour provides limited supportive evidence of carcinogenicity.

An increase in pituitary carcinoma in female rats was also noted. There were no relevant HCD available in the CLH dossier. Overall, RAC agrees with the DS that this type of tumour may have been treatment related.

With regard to the nasal turbinate malignant tumours, an increase incidence was noted in males. Although the incidence was very low (two males), this is a very rare tumour of concern.

Although the tumours were only recorded at the top dose, excessive toxicity was not observed in the rat carcinogenicity study and is thus not a potential confounding factor.

In addition, RAC notes that the mouse carcinogenicity study inadequately informs on liver, thyroid, pituitary and nasal turbinate due to the high mortality rate particularly in the high dose females.

In humans, Silver et al. (2015), reported an association between liver cancer and metolachlor exposure that may support the observed effect in rats. However, at present it is not possible to fully exclude potential confounding factors by co-exposure or alcohol use. Therefore, they only provide limited evidence of carcinogenicity.

Based on multiple tumours in rats in both sexes, classification of *S*-metolachlor as Carc. 1B could be warranted. However, RAC considers that there are several factors that justify downgrading the classification from Category 1B to Category 2:

- Tumours are observed in one species.
- A CAR-mediated MoA for liver tumours is plausible although some uncertainties remain.
- The incidence in nasal turbinate in rats was low, raising some uncertainties.
- *S*-metolachlor is not genotoxic.
- Human data do not clearly overlap except for liver tumours, and potential confounding factors cannot be excluded.

Therefore, RAC concluded that **classification as Carc. 2; H351 is warranted.**

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

Adverse effects on sexual function and fertility

The DS based its evaluation on a two-generation reproductive toxicity study in rats performed with metolachlor (Anonymous (33), 1981; GLP, similar to OECD TG 416). In this study, no effects on parameters investigating sexual function and fertility were observed. The main limitation pointed out by the DS was the absence of measurements of parameters such as oestrus cyclicity, ovarian follicles or developmental landmarks in offspring.

Overall, no classification was proposed by the DS for adverse effects on sexual function and fertility.

Adverse effects on development

The DS based its evaluation on five studies. Two teratogenicity studies in rats (Anonymous (24), 1995; Anonymous (26), 1985), two teratogenicity studies in rabbits (Anonymous (25), 1980; Anonymous (16), 1995) and the two-generation reproductive toxicity study (Anonymous (33), 1981).

The DS proposed to classify *S*-metolachlor as Repr. 2 (H361d) for developmental toxicity on the following basis:

- Hydrocephalus was observed in a small number of foetuses in two strains of rabbits in two independent studies.
- As the effects were observed in presence of overt maternal toxicity, Category 2 was considered more appropriate than Category 1B.

Adverse effects on or via lactation

No classification was proposed by the DS. The DS considered that no data were available to conclude whether there are specific effects on or via lactation.

Comments received during consultation

Comments were only received on developmental toxicity.

One MSCA agreed with the DS's proposal to classify *S*-metolachlor as Repr. 2; H361d. Another MSCA disagreed with the proposal and preferred no classification as hydrocephalus were likely a secondary consequence of maternal toxicity.

Industry representatives disagreed with the classification proposal. They proposed no classification since they considered that the two rabbit studies should be evaluated in isolation and the incidences of hydrocephaly should not be combined.

The DS responded that the hydrocephaly was not combined in the CLH report. The incidence of foetal hydrocephaly in both rabbit studies were within HCD range on a litter basis and observed at maternally toxic dose levels. The DS acknowledged that the HCD might raise doubt but show that hydrocephaly is a very rare malformation and the presence of these malformations in two studies with metolachlor and *S*-metolachlor raised concern.

The industry representatives highlighted that at the time of the study the two foetuses were reported to have "possible hydrocephaly". The technique may not have been sensitive enough and may produce possible artefactual alterations of the skull architecture and bone morphology.

The DS responded that either the results should be considered reliable, or a new rabbit study should have been performed, which was not the case.

Assessment and comparison with the classification criteria

Adverse effects on sexual function and fertility

No effects were seen on sexual function and fertility in the rat two-generation study. RAC notes that the top dose used in this study (Anonymous (33), 1981), around 55 mg/kg bw/d in males and 72 mg/kg bw/d in females, may have been insufficient to fulfil the requirements of OECD TG 416. There were no effects on body weight, clinical signs or mortality in parental animals. The effects on organ weights were of equivocal toxicological significance according to the DS. At the top dose in parental animals, there was only a slight but significant reduction in food intake in female of the F1 generation. In addition, RAC notes that several endpoints (e.g., oestrus cycle, sexual maturation) were not investigated as these endpoints were not recommended in the OECD TG available at the time of the study.

No relevant effects were noted in the repeated-dose toxicity studies.

In conclusion, RAC agrees with the DS's proposal that **no classification for adverse effects on sexual function and fertility is warranted**. Nevertheless, RAC considers the data inconclusive due to insufficient dose levels.

Adverse effects on development

In the two available prenatal developmental toxicity studies performed in rats with metolachlor or *S*-metolachlor (Anonymous (24), 1995; Anonymous (26), 1985), no effects relevant for classification were observed.

There are two rabbit developmental toxicity studies that raised concern on potential effects (Anonymous (25); 1980; Anonymous (16), 1995).

In the most recent study, dated 1995 (conducted in 1983), New Zealand White (NZW) rabbits (Har:PF/CF(NZW)BR) were exposed to *S*-metolachlor, by gavage at 0, 20, 100 or 500 mg/kg bw/d, during gestation days 7-19.

***Table**: Malformations observed in the rabbit study (Anonymous (16), 1995).*

Dose (mg/kg bw/d)	**0**	**20**	**100**	**500**
Pups/litter	161/19	107/15	129/16	143/18
Visceral malformations				
Cleft palate	0/0	0/0	0/0	4/1*
Hydrocephaly	0/0	0/0	0/0	2/2 (1*)
Thymus enlarged	1/1	0/0	0/0	0/0
Gonad malpositioned	0/0	1/1	0/0	0/0
Trachea reduced in size	0/0	0/0	0/0	1/1*
Tongue curled	0/0	0/0	0/0	3/1*
Skeletal malformations				
Zygomas/squamosals short	0/0	0/0	0/0	5/1*
Wavy ribs	0/0	0/0	0/0	4/1*
Short and bowed ulna radius	0/0	0/0	0/0	5/1*
External malformations				
Abnormal limb flexure	0/0	0/0	0/0	4/1*

* Observations from same litter; n.a.: not available

Most of the malformations occurred in only one high dose litter (BT14), including only five foetuses having all the multiple malformations. The dam of this litter had the lowest body weight in the high dose group between days 14 and 25. It also consumed very little food (reduced by about 50%).

The increase in malformations was primarily due to hydrocephaly. This severe malformation was observed in one out of five multi-malformed foetuses in one litter and in one foetus in a second litter (dam BS14). The incidence of two foetuses with hydrocephaly out of 143 foetuses in two litters is above the mean of HCD. Nevertheless, the incidence of hydrocephaly is within the HCD range from the same time period from the laboratory and same strain of rabbits, consisting of 12 studies. One out of 145 foetuses had hydrocephalus in one study and two foetuses in two separate litters out of 143 foetuses in another study. RAC notes that one foetus was from a litter that had a cluster of multi-malformed foetuses. This may reflect a total failure of foetal developmental in this dam. The occurrence of one hydrocephalus in a second litter, within HCD is insufficient for classification.

There were no other significant treatment related effects in the study except a statistically significant increase in fully formed ribs (variations) at the top dose.

At the top dose, maternal toxicity was observed. There was a dose-related increase in reduced or soft stool. A marked reduction in food consumption with concomitant body weight loss and reduced body weight gain was also seen. This was also reflected by reduced food efficiency during exposure. One death was also considered treatment related. Data on corrected body weight were not provided.

In the oldest study (Anonymous (25), 1980), female DLI:NZW rabbits were exposed to 0, 36, 120 or 360 mg/kg bw/d metolachlor on gestation days 6-18. The study was similar to OECD TG 414, but some deviations were noted (no distinction between malformation and variation in the study report, no precise data on food consumption, late terminal sacrifice on day 30 of gestation).

Two dead foetuses with hydrocephalus and small encephalocele were observed in the litter of one dam that died on day 29 of gestation. Although the study authors considered the death not related to treatment, it is not possible to exclude it. There was only one other death in the study

at the low dose, not related to treatment. Industry representatives argued that in the Anonymous (25) (1980) study, in three litters sired by the same buck, various malformations were observed, including the high dose litter with the two malformed foetuses with hydrocephaly. Although a genetic effect cannot be excluded, RAC agrees that with the DS that this remains speculative.

At the mid and top dose, miosis and vaginal bleeding was noted in the dams. Mean absolute body weight were significantly reduced during the treatment period.

There was no difference with regards to frequency of external, visceral and skeletal malformations but hydrocephalus was observed in two pups of the same litter of the high dose group. According to the study report, the HCD for hydrocephalus in the laboratory showed an incidence of 1:1000 litters. Ninety-nine studies were available in a 10-year range (1980-1990) in the same laboratory and rabbit strain. In 15 of the studies, one foetus was affected (number of foetuses affected/number of foetuses: 1/136, 1/94, 1/97, 1/132, 1/150, 1/138, 1/112, 1/87, 1/111, 1/98, 1/140, 1/40, while in the remaining study, using unusual dosing via inter-uterine device during gestation, three foetuses in two litters displayed hydrocephalus).

The two foetuses with hydrocephalus were from the same litter in a dam that died, although rarely occurring, this finding may have been secondary to the high maternal toxicity observed in the dam.

RAC agreed with the DS that the presence of four hydrocephalus in three litters from two different strain of NZW rabbits in two independent studies is of concern. Nevertheless, one hydrocephalus occurred in a multi-malformed litter in the first study and the two cases of hydrocephalus in the other study occurred in a dam that died probably due to treatment. In the remaining litter, the presence of one case of hydrocephalus in one study, within HCD, is not sufficient for classification.

Therefore, RAC concludes that **no classification for adverse effects on development is warranted**.

Adverse effects on or via lactation

The small decrease in foetal weight during lactation is not considered sufficient for classification. Therefore, RAC concludes that **no classification for adverse effects on or via lactation is warranted**.

ENVIRONMENTAL HAZARD EVALUATION

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier Submitter's proposal

The information presented by the DS is taken from the RAR (Rev. 1 – January 2018) and list of endpoints (January 2018) for *S*-metolachlor. Additional information on aqueous photolysis in natural water is taken from the RAR (Rev. 1 - 21 August 2020). Solely studies for *S*-metolachlor are considered for classification. Studies for metolachlor are listed by the DS for completeness.

Data are available for all three trophic levels. For acute toxicity, the primary producers, algae and aquatic plants are the most sensitive species with E_rC_{50} values of 0.056 mg/L (*P. subcapitata*) and 0.062 mg/L (*E. canadensis*). The lowest observed acute toxicities to fish and crustaceans are located between 1 and 10 mg/L (most sensitive species for fish and crustaceans are *O. mykiss* and *M. bahia* with LC_{50} of 1.23 and 1.4 mg/L, respectively).

For chronic toxicity, the most sensitive species for fish is *P. promelas* with a NOEC of 0.03 mg/L, the most sensitive species for crustaceans is *M. bahia* with an EC_{10} of 0.182 mg/L and most sensitive species for algae and aquatic plants is *L. gibba* with a NOEC of 0.0021 mg/L.

Based on Grade (1996), *S*-metolachlor is not readily degradable, where the mineralization of *S*-metolachlor under the test conditions was 0% in 29 d, based on an OECD TG 301B study.

Based on the experimentally determined BCF in fish of 255, *S*-metolachlor is not considered to have a potential to bioconcentrate for classification purposes.

The DS concluded that *S*-metolachlor can be classified as Aquatic Acute 1 with an M-factor of 10 (0.01 mg/L < $L(E)C_{50}$ ≤ 0.1 mg/L) based on the acute toxicity to algae and as Aquatic Chronic 1 with a M-factor of 10 (0.001 mg/L < NOEC ≤ 0.01 mg/L) based on the long-term toxicity to aquatic plants and the not rapidly degradable property.

Comments received during consultation

During the consultation, one MSCA supported the DS classification proposal and a National Authority agreed on the DS proposal and asked for clarifications about:

- The OECD TG 239 validity criteria, regarding the mean coefficient of variation for yield based on measurements of shoot fresh weight in the control not exceeding 35% between replicates in Teixeira (2006a), is met. The DS confirmed that the coefficient of variation for yield based on shoot wet weight was 19.91% and then, the validity criteria given in OECD TG 239 (< 35%) was met.
- To compare the robustness of E_rC10 and E_rC20 endpoints with the coefficient of variations which should not exceed the level effect value and to determine if the NOEC should be preferred in Teixeira (2006a) study. The DS agreed that according to OECD TG 239 the 7-d E_rC_{10} of 0.0049 mg/L (mean measured: mm) is not reliable, as the Coefficient of Variance is 19.91% (> 10%). However, it is noted that (i) OECD TG 239 for *Myriophyllum spicatum* is only used as surrogate guideline for the study with *Elodea canadensis*, (ii) the width of the confidence interval of the E_rC_{10} (0.0013 - 0.011 mg/L) is acceptable and below the E_rC_{20} (0.029 mg/L) and (iii) the 7-d E_rC_{10} of 0.0049 mg/L is not a relevant endpoint for the classification of *S*-metolachlor. Therefore, a change to the NOE_rC as relevant long-term endpoint is not considered by the DS sufficiently justified and they would retain the current classification.
- A need to report the EC_{10} and EC_{20} values in *Lemna gibba* study (Eckenstein, 2014) as they are preferred over NOEC values for the classification purposes. As fronds were removed from test vessels for use in the recovery phase before the final dry weight determination, the National Authority emphasised these uncertainties and considers that dry weight endpoints should not be used for classification and that the endpoints based on frond number are more relevant. The DS presented the dose-response curve and considered that as all replicates of the second and third lowest treatment level are above the value predicted by the model, the uncertainty in the model itself and the derivation of an E_rC_{10} is considered to be high. As the NOEC can unambiguously be set at 0.0021 mg/L, the DS considered that this endpoint is the most reliable endpoint relevant for classification purposes. The DS considered that endpoints related to dry weight are reliable and can be used for classification purposes. Twelve fronds were taken at the end of the 7-d exposure phase for a subsequent study of the recovery. In the treatments with expected low frond numbers (100 – 1000 µg a.s./L) three additional treatments were available to conduct the recovery study. In the lower treatments (control, 2.1, 9.8 and 22 µg a.s./L), the amount of fronds observed in the replicates were between 87 and 167. The dry weights were corrected for the missing 12 fronds. Due to the high amount of

fronds in the affected treatments, the DS considered that the missing 12 fronds randomly taken from each replicate were not expected to modify the overall results.

Assessment and comparison with the classification criteria

Degradation

The table below summarised the relevant information on rapid degradability.

***Table**: Relevant information on degradation*

Method	Results	Reference
OECD TG 301B	Ready biodegradability CO_2 formation 0% in 29 d *S*-metolachlor is not readily degradable Deviation: one scrubber used Reliability = 1	Grade, 1996
OECD TG 111	Hydrolytic degradation of the active substance and metabolites > 10% pH 5 at 25°C: no degradation within 30 d pH 7 at 25°C: no degradation within 30 d pH 9 at 25°C: no degradation within 30 d	Keller, 1996
OECD TG 309	Aerobic mineralisation in surface water *S*-metolachlor DT_{50} values are normalised to 20°C DT_{50} whole system = 74 d [at 10 µg/L] DT_{50} whole system = 97 d [at 95 µg/L] Metabolite CGA40172: Max in total system 9.1% after 58 days. DT_{50}-values were not applicable Mineralisation: Fresh water plus suspended sediment [10 µg/L]: 4.5% after 58 d Fresh water plus suspended sediment [95 µg/L]: 3.9% after 58 d Reliability 1	Crabtree, 2014
BBA Guideline Part IV; 5-1	Degradation in water/sediment system: DT_{50} between 33.6 and 54.8 days at 20°C Mineralisation 2% max after 180 days Not readily biodegradable Reliability = 1	Mamouni, 1997

Under a pH range (1-9), *S*-metolachlor is found to be hydrolytically stable in a valid OECD TG 111 assay, with a degradation half-life far above 200 days.

The ready biodegradability of *S*-metolachlor is measured by CO_2 production in a valid OECD TG 301B assay during 29 days at 21 ± 2°C (Grade, 1996). No inhibition of the test reference (sodium benzoate) was observed with *S*-metolachlor.

The mineralisation rate and route of degradation of ^{14}C-*S*-metolachlor was investigated in natural water with a valid OECD TG 309 assay. The systems were incubated under aerobic conditions and maintained under a diffuse non-UV light/dark cycle (16 hours/8 hours) at 20°C for up to 58 days. The mean mass balances for all incubation groups were 94.0% to 96.6%. For the non-sterilised, viable test systems, the mean levels of parent compound decreased to between 54.0 and 62.2% at the end of the incubation period (58 days), with resultant $DegT_{50}$ values ranging from 74 to 97 days. For the sterilised samples, *S*-metolachlor was found to be stable with 92.4% applied radioactivity (AR, mean) remaining at 58 DAT. CGA40172 was the only metabolite found

at ≥ 5%, reaching a maximum level of 9.1% AR at 58-d. Less than 5% of *S*-metolachlor was mineralised to carbon dioxide.

The mineralisation rate and route of degradation of ^{14}C-*S*-metolachlor was investigated in river and pond in water/sediment system in a valid BBA guideline assay. For river and pond, under aerobic and anaerobic incubation conditions the same range of DT_{50} values of between 42 and 53 days at 20°C were determined. At temperatures below 10°C, the degradation half-life was by a factor of three longer. Two main metabolites were detected.

The DS presented in the CLH report studies regarding degradation of *S*-metolachlor in soil with a metabolic pathway proposal. This information is not used for classification purpose.

RAC concludes that *S*-metolachlor was found not to be readily biodegradable in the OECD TG 301B and limited mineralisation was observed in the water surface simulation and water/sediment simulation studies. Thus, RAC concurs with the DS conclusion and considers ***S*-metolachlor as not rapidly degradable**.

Bioaccumulation

Bioconcentration factors of *S*-metolachlor were measured and calculated in bluegill sunfish (*Lepomis macrochirus*) assay (Anonymous, 2001). Bioconcentration factors (measured and calculated) were based on analyses of water and fish tissues for total radioactive residues. The study was conducted with nominal concentrations of 0.03 and 0.003 mg/L *S*-metolachlor. The study is considered valid as temperature variations were less than ± 1°C, the dissolved oxygen remained above 60% air saturation value (ASV), test item concentrations were maintained within ± 20% of the mean measured values during the accumulation phase, mortality of the batch of fish used was less than 5% during the 7 days preceding the test and were low (1 fish) during the accumulation phase.

Nevertheless, the study was not performed according to the newest guideline OECD TG 305 of October 2nd, 2012. It is stated in OECD TG 305 that "*the increase in fish mass during the test will result in a decrease of test substance concentration in growing fish (so-called growth dilution), and thus the kinetic BCF will be underestimated if not corrected for growth*"; this was not done in the study. In the study report and the summary provided by the applicant it is not clear if BCF was based on the S-isomer or total radioactivity. Lipid content for whole fish at day 28 was not reported but needed to express the BCF based on 5% lipid content as laid out in OECD TG 305. Lipid normalisation will therefore be based on initial lipid content. Feeding was relatively high in the study (2% of wet body weight per day). This may have led to a relatively high increase of the lipid content and a dilution of *S*-metolachlor in fat.

To derive a BCF for the assessment of bioaccumulation, the worst-case BCF value of 112 (whole fish, low dose) is normalised to 5% lipid using the lipid content of 2.2 measured at the first day of exposure as a reference. This yields a BCF_{ss} of 255.

Overall from this study RAC and the DS concluded that *S*-metolachlor BCF in fish is 255, which is below the CLP criteria of 500 that indicates **a low bioconcentration potential for classification purposes**.

Acute aquatic toxicity

The DS presented metolachlor and *S*-metolachlor data. RAC considers that for classification purpose of *S*-metolachlor and as the dataset is complete, only data on this substance is taken into account and data on metolachlor is considered as additional data.

The table below presents a summary of relevant valid information on acute aquatic toxicity of *S*-metolachlor.

***Table**: Relevant information on acute aquatic toxicity*

Method	Species	Test material	Results	Remarks	Reference
Fish					
EPA-660/3-75009; 1975	***Oncorhynchus mykiss (Salmo gairdneri)***	**CGA 77102 (*S*-metolachlor)**	**LC_{50} (96 h) = 1.23 mg a.s./L (initial Measured, im)**	**Key study Minor deviation from validity Reliability 2**	**Anonymous, 1983a**
EPA-660/3-75009; 1975	*Lepomis macrochirus*	CGA 77102 (*S*-metolachlor)	LC_{50} (96 h) = 3.16 mg a.s./L (im)	Minor deviation from validity Reliability 2	Anonymous, 1983b
FIFRA Guideline 72-1	*Oncorhynchus mykiss*	CGA 77102 (*S*-metolachlor)	LC_{50} (96 h) = 12 mg a.s./L	Minor deviation from validity Reliability 2	Anonymous, 1995a
OECD TG 203	*Cyprinus carpio*	CGA 77102 (*S*-metolachlor)	LC_{50} (96 h) = 20 mg a.s./L (mm)	Reliability 1	Anonymous, 2006
OPPTS 850.1075	*Cyprinodon variegates*	CGA 77102 (*S*-metolachlor)	LC_{50} (96 h) = 17 mg a.s./L (mm)	Reliability 1	Anonymous, 2004
Invertebrates					
ASTM 1981; EPA660/3-75-009	*Daphnia magna*	CGA 77102 (*S*-metolachlor)	EC_{50} (48 h) = 11.24 mg/L (im)	No analytical verification of test concentrations at the end of the test. Reliability 2	Spare, 1983c
EPA 850.1035, 723	***Mysidopsis bahia***	**CGA 77102 (*S*-metolachlor)**	**LC_{50} (96 h) = 1.4 mg/L (mm)**	**Key study Reliability 1**	**Spare, 1983d**
FIFRA Guideline Number 72-2(a)	*Daphnia magna*	CGA 77102 (S-metolachlor)	LC_{50} (48 h) = 26 mg/L (mm)	Exceedance of the allowed solvent concentration. Reliability 2	Collins, 1995b
OPPTS Number 850.1025	*Crassostrea virginica*	CGA 77102 (*S*-metolachlor)	EC_{50} (96 h) = 4 mg/L (mm)	Reliability 1	Palmer et al., 2004b
Algae and aquatic plants					
OECD TG 201	*Skeletonema costatum*	CGA 77102 (*S*-metolachlor)	E_rC_{50} (72 h) = 0.340 mg/L E_rC_{10} (72 h) = 0.013 mg/L (mm)	Minor deviation from validity criteria Reliability 2	Hoberg, 1995b
OECD TG 201	***Pseudokirchneriella subcapitata***	**CGA 77102 (*S*-metolachlor)**	**E_rC_{50} (72 h) = 0.056 mg/L**	**Key study Reliability 1**	**Memmert, 2006**

			NOEC (growth, 72 h) = 0.012 mg/L (mm)		
OECD TG 201	*Navicula pelliculosa*	CGA 77102 (S-metolachlor)	E_rC_{50} (72 h) = 31 mg/L NOEC (growth, 72 h) = 9.7 mg/L (mm)	Reliability 1	Desjardins et al., 2003
OPPTS 850.4450	***Elodea canadensis***	**CGA 77102 (*S*-metolachlor)**	**E_rC_{50} (7 d) = 0.062 mg/L E_rC_{10} (7 d) = 0.0049 mg/L (mm)**	**Key study** **Reliability 2**	**Teixeira, 2006a**
OPPTS 850.4450	*Myriophyllum heterophyllum*	CGA 77102 (*S*-metolachlor)	E_rC_{50} (7 d) = 0.065 mg/L NOEC (growth, 7 d) = 0.01 mg/L (mm)	Supplemental information	Teixeira, 2006b
OECD TG 221	*Lemna gibba*	CGA 77102 (*S*-metolachlor)	E_rC_{50} (7 d) = 0.133 mg/L NOEC (growth, 7 d) = 0.0021 mg/L (mm)	Reliability 1	Eckenstein, 2014
OECD TG 221	*Lemna gibba*	CGA 77102 (*S*-metolachlor)	E_rC_{50} (7 d) = 0.149 mg/L NOEC = 0.00384 mg/L (mm)	Reliability 1	Kümmric, 2019

Valid results for the three trophic levels (fish, invertebrates and primary producers) are available. For fish and invertebrates, the $L(E)C_{50}$ are above 1 mg/L and the most sensitive fish species is the *O. Mykiss* (rainbow trout, LC_{50} = 1.23 mg/L) and *M. Bahia* (EC_{50} = 1.4 mg/L) is the most sensitive invertebrates. As expected for an herbicide, primary producers are the most sensitive and the reference values are below 1 mg/L for *P. subcapitata* and *E. canadensis*, E_rC_{50} = 0.056 mg/L and 0.062 mg/L respectively.

RAC concurs with the DS proposal that *S*-metolachlor fulfils the classification criteria for Aquatic Acute 1 with an M-factor of 10 as 0.01 mg/L < $L(E)C_{50}$ ≤ 0.1 mg/L based on the acute toxicity to algae.

Chronic aquatic toxicity

The DS presented metolachlor and S-metolachlor data. RAC considers that for classification purpose of *S*-metolachlor and as the dataset is complete only data on this substance is taken into account and data on metolachlor is considered as additional data.

The table below presents a summary of relevant valid information on chronic aquatic toxicity of *S*-metolachlor.

Method	Species	Test material	Results	Remarks	Reference
Fish					
FIFRA Guideline 72-4	***Pimephales promelas***	**CGA 77102 (*S*-metolachlor)**	**NOEC (35 d) = 0.03 mg/L (mm)**	**Key study Reliability 1**	**Anonumous , 1999**
FIFRA Guideline Reference No. 72-4	*Cyprinodon variegatus*	CGA 77102 (*S*-metolachlor)	NOEC (34 d) = 1.3 mg/L (mm)	Reliability 1	Anonymous, 2000
Invertebrates					
OECD TG 211	*Daphnia magna*	CGA 77102 (*S*-metolachlor)	NOEC (21 d) = 5.2 mg/L EC_{10} (21 d) = 1.29 mg/L (mm)	Reliability 1	Palmer et al., 2004
EPA 850.1300, 72-4	**Mysidopsis bahia**	**CGA 77102 (*S*-metolachlor)**	**NOEC (28 d) = 0.15 mg/L EC_{10} (28 d) = 0.182 mg/L (nominal)**	**Key study Reliability 1**	**Lima, 1999**
Guideline Proposal 1995	*Chironomus riparius*	CGA 77102 (*S*-metolachlor)	NOEC (28 d) = 8 mg/L (nominal)	Reliability 1	Grade, 1998
Algae and aquatic plants					
OECD TG 201	*Skeletonema costatum*	CGA 77102 (*S*-metolachlor)	ErC50 (72 h) = 0.340 mg/L E_rC_{10} (72 h) = 0.013 mg/L (mm)	Reliability 1	Hoberg, 1995b
OECD TG 201	*Pseudokirchneriella subcapitata*	CGA 77102 (*S*-metolachlor)	E_rC_{50} (72 h) = 0.056 mg/L NOEC (growth, 72 h) = 0.012 mg/L (mm)	Reliability 1	Memmert, 2006
OECD TG 201	*Navicula pelliculosa*	CGA 77102 (*S*-metolachlor)	E_rC_{50} (72 h) = 31 mg/L NOEC (growth, 72 h) = 9.7 mg/L (mm)	Reliability 1	Desjardins et al., 2003
OPPTS 850.4450	*Elodea canadensis*	CGA 77102 (*S*-metolachlor)	E_rC_{50} (7 d) = 0.062 mg/L E_rC_{10} (7 d) = 0.0049 mg/L (mm)	Reliability 2	Teixeira, 2006a
OPPTS 850.4450	*Myriophyllum heterophyllum*	CGA 77102 (*S*-metolachlor)	E_rC_{50} (7 d) = 0.065 mg/L NOEC (growth, 7 d) = 0.01 mg/L (mm)	Supplemental information	Teixeira, 2006b
FIFRA Guideline number 122-2 and 123-2	*Lemna gibba*	CGA 77102 (*S*-metolachlor)	E_rC_{50} (14 d) = 0.039 mg/L NOEC (growth, 14 d) = 0.0076 mg/L (mean measured)	Reliability 1	Hoberg, 1995d
OECD TG 221	***Lemna gibba***	**CGA 77102 (*S*-metolachlor)**	**E_rC_{50} (7 d) = 0.133 mg/L NOEC (growth, 7 d) = 0.0021 mg/L (mm)**	**Key study Reliability 1**	**Eckenstein, 2014**

Valid results for the three trophic levels (fish, invertebrates and primary producers) are available. *P. promelas* (NOEC = 0.03 mg/L) and *M. Bahia* (EC_{10} = 0.182 mg/L) are the most sensitive species for fish and invertebrates respectively. Primary producers are the most sensitive and the reference values are below 0.01 mg/L for *L. gibba* and *E. canadensis*.

As *S*-metolachlor is considered as not rapidly degradable, RAC concurs with the DS proposal that *S*-metolachlor fulfils the classification criteria for Aquatic Chronic 1 with a M-factor of 10 as 0.001 mg/L < NOEC, EC_{10} ≤ 0.01 mg/L based on the acute toxicity to algae

Conclusion on classification

RAC concluded that a classification for **Aquatic Acute 1 with an M-factor of 10** as 0.01 mg/L < L(E)C_{50} ≤ 0.1 mg/L and **Aquatic Chronic 1 with an M-factor of 10** as 0.001 mg/L < NOEC, EC_{10} ≤ 0.01 mg/L is warranted for *S*-metolachlor.

Additional references

Leon et al., 2019. Pesticide use and risk of non-Hodgkin lymphoid malignancies in agricultural cohorts from France, Norway and the USA: a pooled analysis from the AGRICOH consortium. Int J Epidemiol. 2019;48:1519-1535

Lerro et al., 2018. Use and Cancer Incidence in the Agricultural Health Study: An Updated Analysis. J Natl Cancer Inst. 2018;110:950-958

Lerro et al., 2019. Cancer incidence in the Agricultural Health Study after 20 years of follow-up. Cancer Causes Control. 2019;30:311-322

Lerro et al., 2020. Dicamba use and cancer incidence in the agricultural health study: an updated analysis. Int J Epidemiol. 2020;49:1326-1337

ANNEXES:

Annex 1 The Background Document (BD) gives the detailed scientific grounds for the opinion. The BD is based on the CLH report prepared by the Dossier Submitter; the evaluation performed by RAC is contained in 'RAC boxes'.

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