CLH report

Proposal for Harmonised Classification and Labelling

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

International Chemical Identification:

S-metolachlor (ISO);

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

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

EC Number:	**-**
CAS Number:	**87392-12-9**
Index Number:	**607-432-00-4**

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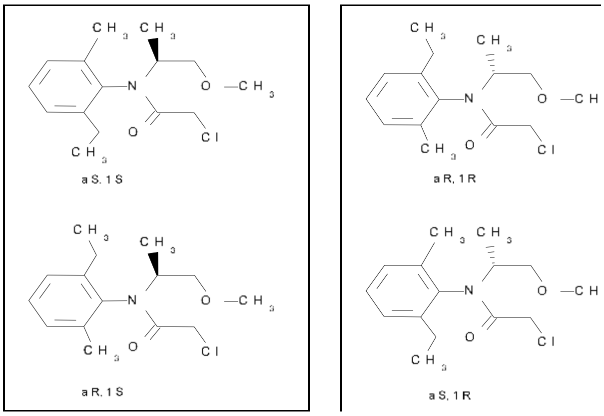
CONTENTS

1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

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

Name(s) in the IUPAC nomenclature or other international chemical name(s)	*Mixture of (aRS, 1 S)-2-chloro-N-(6-ethyl-o-tolyl)-N-(2-methoxy-1-methylethyl) acetamide (80-100 %) and* *(aRS, 1 R)-2-chloro-N-(6-ethyl-o-tolyl)-N-(2-methoxy-1-methylethyl) acetamide (20-0 %)*
Other names (usual name, trade name, abbreviation)	S-metolachlor
ISO common name (if available and appropriate)	S-metolachlor
EC number (if available and appropriate)	-
EC name (if available and appropriate)	-
CAS number (if available)	87392-12-9 (S-isomer), 178961-20-1 (R-isomer)
Other identity code (if available)	CIPAC number: 607
Molecular formula	$C_{15}H_{22}ClNO_2$
Structural formula	S-metolachlor is a mixture of the 1S and 1 R isomers each of which is a racemic mixture of rotamers as demonstrated by the structural formulas:  *1S* -isomers *1R* -isomers
Molecular weight or molecular weight range	283.8 g/mol
Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	S-metolachlor is a mixture of the 1 S (80 – 100 %) and 1 R (20 – 0 %) isomers each of which is a racemic mixture of rotamers
Degree of purity (%) (if relevant for the entry in Annex VI)	96 % or 960 g/kg Technical S-metolachlor consists of two isomers, CGA 77102 and CGA 77101. The content of the two isomers in the technical substance meets the following specification limits: minimum 840 g/kg of S-isomer (CGA 77102) maximum 130 g/kg of R-isomer (CGA 77101)

1.2 Composition of the substance

Table 2: Constituents (non-confidential information)

Constituent (Name and numerical identifier)	Concentration range (% w/w minimum and maximum in multi-constituent substances)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)
See table 1			

Table 3: Impurities (non-confidential information) if relevant for the classification of the substance

Impurity (Name and numerical identifier)	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)	The impurity contributes to the classification and labelling
There are no relevant impurities in the technical material.				

Table 4: Additives (non-confidential information) if relevant for the classification of the substance

Additive (Name and numerical identifier)	Function	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)	The additive contributes to the classification and labelling
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Table 5: Test substances (non-confidential information) (this table is optional)

Identification of test substance	Purity	Impurities and additives (identity, %, classification if available)	Other information	The study(ies) in which the test substance is used
-				

2 PROPOSED HARMONISED CLASSIFICATION AND LABELLING

2.1 Proposed harmonised classification and labelling according to the CLP criteria

Table 6:

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors	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 [contains 80-100 % 2-chloro-N-(2-ethyl-6-methylphenyl)-N-[(2S)-1-methoxypropan-2-yl]acetamide and 0-20 % 2-chloro-N-(2-ethyl-6-methylphenyl)-N-[(2R)-1-methoxypropan-2-yl]acetamide]	-	87392-12-9	Skin Sens. 1 Aquatic Acute 1 Aquatic Chronic 1	H317 H400 H410	GHS09 GHS07 Wng	H317 H410			
Dossier submitters proposal					**Add** STOT RE 2 Repr. 2 Carc. 2 **Retain** Skin Sens. 1 Aquatic Acute 1 Aquatic Chronic 1	**Add** H373 (skin) H361d H351 **Retain** H317 H400 H410	**Retain** GHS09 GHS07 Wng	**Add** H373 (skin) H361d H351 **Retain** H317 H410		**Add** M = 10 M = 10	
Resulting Annex VI entry if agreed by RAC and COM					Skin Sens. 1 STOT RE 2 Repr. 2 Carc. 2 Aquatic Acute 1 Aquatic Chronic 1	H317 H373 (skin) H361d H351 H400 H410	GHS09 GHS07 Wng	H317 H373 (skin) H361d H351 H400 H410		M = 10 M = 10	

Table 7: Reason for not proposing harmonised classification and status under public consultation

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

3 HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING

In the CLP-Regulation (EC) No 1272/2008 S-metolachlor was introduced as Skin Sens.1, H371, Aquatic Acute 1, H400 and Aquatic Chronic, H410, on proposal by Belgium. Reproductive toxicity was addressed, but no classification was proposed. No further details are known.

4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

[A.] There is no requirement for justification that action is needed at Community level.

S-metolachlor is an active substance in the meaning of Directive 91/414/EEC (repealed by the Regulation EC 1107/2009).

5 IDENTIFIED USES

S-metolachlor is an herbicide in maize and sunflower.

6 DATA SOURCES

Main data source for this CLH dossier are Volumes 1 and 3 of the Renewal Assessment Report (RAR) which was prepared for the pesticides procedure. Volume 3 is attached to the CLH dossier as a confidential annex. All toxicological studies included in this dossier were evaluated and assessed by the dossier submitter.

7 PHYSICOCHEMICAL PROPERTIES

Table 8: Summary of physicochemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
Physical state at 20 °C and 101,3 kPa	at 25 °C : clear extremely pale-yellow liquid	Das (1995)	Visual assessment
Melting/freezing point	freezing temp. (glass transition temp) = - 61.1 °C	Geoffroy (1995)	Measured EC A 1 (DSC)
Boiling point	boiling temp. = approx. 334 °C (could not be properly determined due to thermal decomposition at a temperature lower than that of the boiling point)	Das (1995)	measured EC A 2 (Siwoloboff-method with photocell detection)
Relative density	density at 20 °C = 1117 kg/m^3	Das (1995)	Measured EC A 3 (oscillating density meter)
Vapour pressure	vapour pressure at 25 °C = 3.7 x 10^{-3} Pa (extrapolated) Measurement between 40 °C am 90 °C	Widmer (1995)	Measured EC A 4 (automized gas saturation method with online GC-detection)
Surface tension	54.3 mN/m - 54.5 mN/m (90 % saturated aqueous solution; 22 °C) The substance is considered surface active.	O’Connor (2013)	Measured OECD 115 EC A 5
Water solubility	solubility at 25 °C in water (pH 7.3) = 480 mg/L	Stulz (1995)	Measured EC A 6 (flask method + HPLC-analysis)

Property	Value	Reference	Comment (e.g. measured or estimated)
	The a.s. has no dissociation constant in an accessible pH range (see also B.2.8), which means the pH has no effect on the water solubility of the compound in the pH range 4 - 10.		
Partition coefficient n-octanol/water	at 25 °C : log P_{ow} = 3.05 ± 0.02 (pH of aqueous phase = 7)	Stulz (1995)	Measured EC A 8 (shake-flask method + HPLC analysis)
Flash point	flash point (1013 mbar) = 190 °C	Schürch (1995)	Measured EC A 9 DIN 51758
	Statement on study for flash point (Schürch (1995)) with respect to data requirements of Reg. 1272/2008: EC Test A.9 does not define a method for flash point measurement, but merely lists acceptable national and international standards (e.g. ASTM, BS, DIN, ISO, NM). This is also the case in Section 32 of the UN Manual of Tests and Criteria, which covers the testing of flammable liquids as required for UN transport and UN GHS classification. For S-metolachlor, the flash point was originally determined according to the German DIN 51758 standard for closed-cup Pensky-Martens flash point testing. The original German standard has since been withdrawn but now exists in the form of DIN ISO 2719, which is the same as ISO 2719, the international standard for Pensky-Martens closed-cup testing. ISO 2719 is listed as an acceptable method for flash point in both EC Test A.9 and the UN MoTC. Therefore the original flash point is still valid and meets Reg (EU) 1272/2008 requirements.	Document M (2017)	Statement
Flammability	Not applicable (a.s. is a liquid with flash point > 55 °C)	DAR	Statement
Explosive properties	- no thermal sensitivity (effect of a flame) - no mechanical sensitivity (shock) friction testing method is not applicable for liquids => S-metolachlor is not considered an explosive	Schürch (1995)	Measured EC A 14
	An examination of the	Document M	Statement

Property	Value	Reference	Comment (e.g. measured or estimated)
	structures of S-metolachlor indicates that there are no bond groupings associated with explosive properties. Conclusions: (i) Based on this assessment, the substance is not an explosive. (ii) An experimental determination of the explosive properties, in accordance with UN Test Series 2, is therefore considered unnecessary and has not been carried out on this substance.	(2017)	
Self-ignition temperature	auto-ignition temperature = 430 °C	Schürch (1995)	Measured EC A 15 DIN 51794
	Statement on study for self-heating (Schürch (1995)) with respect to data requirements of Reg. 1272/2008: EC Test A.15 does not define a method for AIT measurement, but merely lists acceptable national and international standards (e.g. BS, DIN, IEC, NM). For S-metolachlor, the AIT was originally determined according to the DIN 51794 standard, which is still a valid national standard today. The apparatus defined in DIN 51794 is also covered by IEC 60079-20-1 Section 7, “Method of Test for Auto-Ignition Temperature”, which is a currently accepted international standard for AIT measurement. Therefore, the original AIT measurement is still valid. (Note: neither the UN transport recommendations nor the UN GHS address auto-ignition temperatures).	Document M (2017)	Statement
Oxidising properties	S-metolachlor technical is not an oxidising substance.	Jackson (2013)	Measured EC A 21
	Statement on study for oxidising properties (Jackson (2013)) with respect to data requirements of Reg. 1272/2008: The original test for oxidizing properties was carried out in accordance with EC Test A.21, which is identical to UN Test O.2 for substances testing negative, as was the case here. The result reported in the	Document M (2017)	Statement

Property	Value	Reference	Comment (e.g. measured or estimated)
	study is therefore considered to be still valid for use when classifying the material for UN transport or in accordance with the UN GHS, and therefore the requirements of Reg (EU) 1272/2008.		
Stability in organic solvents and identity of relevant degradation products	solubility at 25 °C in n-hexane : completely miscible toluene : completely miscible dichloromethane : completely miscible methanol : completely miscible n-octanol : completely miscible acetone : completely miscible ethyl acetate: completely miscible tested in the range from 5 % to 95 % (v/v)	Stulz (1995)	Measured SOP 209/5 (essentially an adaptation of CIPAC MT 157.3)
Dissociation constant	*consideration of structural formula :* no dissociation expected within pH-range 2-12 *experimental confirmation :* UV/VIS-absorption spectra (210-400 nm) recorded in neutral, acidic and basic solution are identical => no dissociation constant (pKa) in an accessible pH-range	Stulz (1995)	Measured OECD 112 (UV/VIS-absorption spectra)

8 EVALUATION OF PHYSICAL HAZARDS

This endpoint is not addressed in this CLH report and is outside the scope of the public consultation.

9 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

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

Summary of the relevance of the toxicokinetic studies for the classification proposal:

This summary is taken from Volume 1 (chapter 2.6.1) of the RAR, which was prepared for the renewal of the approval of the active substance. In case more detailed information on the reported effects is needed, it is referred to Volume 3, chapter B.6 of the RAR.

The oral absorption of metolachlor and S-metolachlor was very efficient, and amounted to respectively 92 % and 85 %. Total absorption seemed independent of sex, dose level, administration route or pre-treatment.
After absorption, the compound was found strongly associated to red blood cells (RBC) in the rat, and high levels were maintained up to 11 days. The calculated depletion halftime was about 26.5 days.
Apart from RBC, the compound was distributed in well-perfused organs like heart, lung, spleen, kidney and liver, and was found in highest concentrations 8 h post-dose. Pre-treatment at low dose did not influence tissue residue levels at d7, and decreased slightly (1.6-fold) the residues in RBC, when compared to single low-dose administration. In contrast, pre-treatment at high dose lowered consistently and significantly (about 50 %) residue concentrations in tissues and RBC. This reflected a partial saturation of RBC binding sites. High-dose acute administration (200-fold the low-dose level) resulted in approximately 150-300-fold increase of residues in both RBC and tissues.
Whole-body autoradiography at d8 revealed labelling in GI-tract, kidney, liver and lung, and to some extent in bone marrow. In the absence of high radioactivity measurements in the sum of all tissues at that sampling time (about 1.6-3.5 % of administered dose), it was concluded that no accumulation occurred.
For both metolachlor and S-metolachlor, the metabolite pattern and amount was independent of sex, pre-treatment or dose level. Among the 32 identified metabolites of the racemic mixture, 8 were considered as major (occurrence of > 5 % in any fraction). The three identified environmental metabolites (recovered amounts in soil/water 5-10 % of dose) accounted for maximally 0.3 % in the rat excreta. Not more than 3 % of the unchanged compound was recovered in the excreta at d7.
From the analysis of metabolite patterns in a bridging study, it was concluded that metabolic pathways of the racemic mixture and the S-enantiomer were similar. The proposed metabolic pathway for metolachlor is shown in Figure 1.
Excretion occurred moderately rapid and was completed by 72 h post-dose. The major excretion route was biliary (about 80 % at d2), and ultimately fecal, although the renal excretion seemed relatively more important in females when compared to males. Pre-treatment or dose level were without influence on the recovered % of administered doses in the excreta. The amounts of compound-related radioactivity in expired air were low.
A comparative in vitro metabolism study was performed using microsomes from rats and humans. S-metolachlor was extensively metabolized in the hepatic microsomes of both species. The quantity of metabolites was comparable, even though minor differences occurred. The metabolite M4 was evident in human microsomes only. It was not possible to conclude on its relevance as no information on its molecular structure or toxicological properties are given. Also, no information about the identity and toxicological profile of the metabolite M9, which was the major metabolite in humans but not in rats, is available. No data to compare metabolism in other key species (e.g. mice, rabbits and dogs) is available.
It is noted that the submitted study reports usually describe separation of metabolites with thin layer chromatography (TLC), which can be a quite insensitive method for detection. Additionally, in several studies, the metabolites were only separated but not identified. In none of the studies, chiral separation methods were used; hence, no firm conclusions can be drawn on possible enantiomeric preference of ADME.

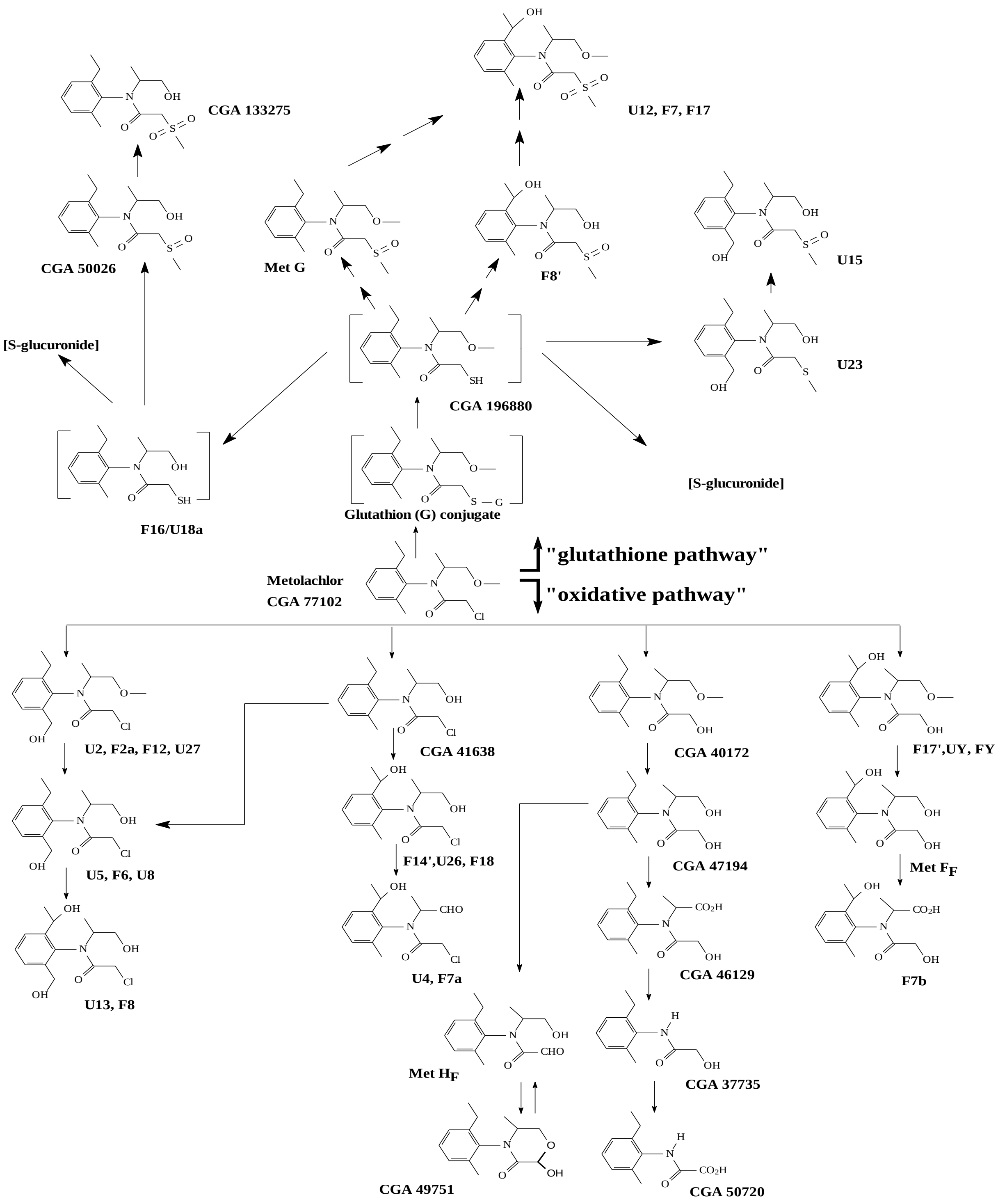
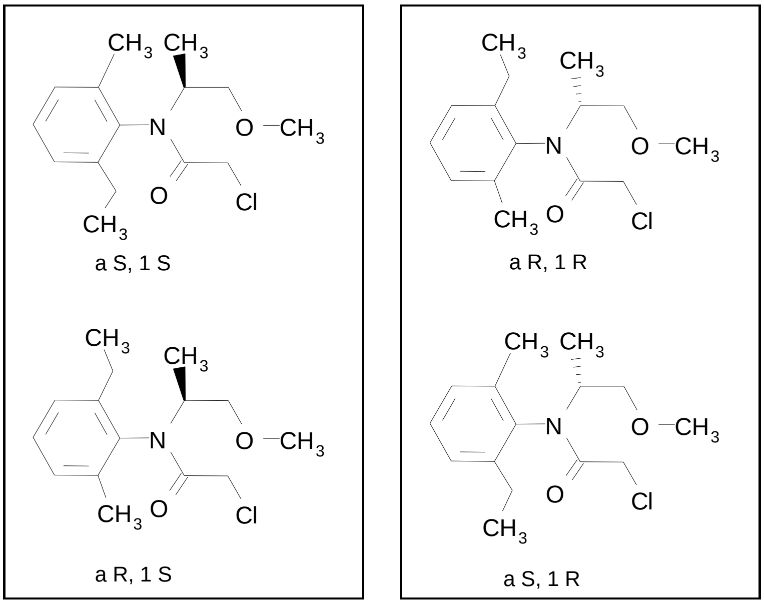


Figure 1: Proposed metabolic pathways of Metolachlor in rats

10 EVALUATION OF HEALTH HAZARDS

S-metolachlor is a mixture of the 1S and 1R isomers each of which is a racemic mixture of rotamers as demonstrated in the structural formulas in Figures 2 and 3.



***1S*-isomers** ***1R*-isomers**

Figure 2: Structural isomers of S-metolachlor and metolachlor

The isomers in the technical substance S-metolachlor meet the following specification limits: minimum 840 g/kg of S-isomer (CGA 77102), maximum 130 g/kg of R-isomer (CGA 77101).
Metolachlor is also a mixture of the S- and R- stereoisomers; it contains the two isomers in equal amounts, i.e. in a 50:50 ratio.

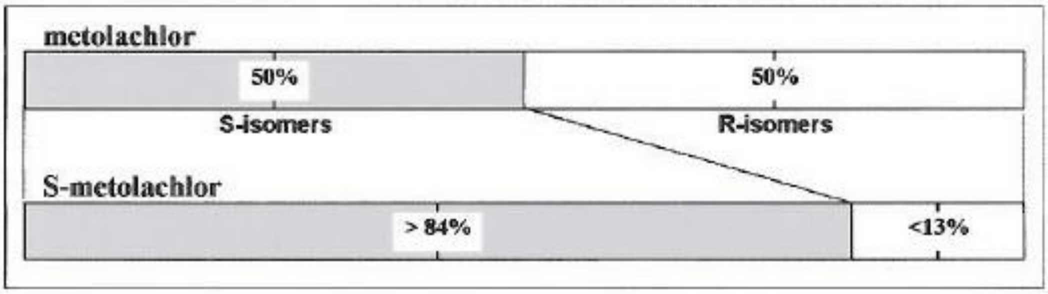


Figure 3: Isomer composition of metolachlor and S-metolachlor

Toxikokinetic bridging studies revealed similar oral absorption and similar metabolic pathways for the racemic mixtures and the S-enantiomer. S-metolachlor as well as metolachlor is of low acute toxicity and the LD_{50} is greater than 2000 mg/kg bw for oral and dermal exposure and above the maximal applied concentrations of 1.75 mg/L and 2.91 mg/L for inhalative exposure. Both substances are non-irritant and both show skin sensitizing properties. Observed no adverse effect levels in short-term studies (28- and 90-day) in rats were similar for S-metolachlor and metolachlor and liver was the target organ (increase in weight and hypertrophy) as well as the kidney, where increased weight was observed. For dogs, a 90-day study with S-metolachlor was submitted, along with a 6-month and 1-year study with metolachlor.

Regarding genotoxicity S-metolachlor showed overall negative results regarding all regular end points for genetic damage. Metolachlor showed in vitro inconsistent results (MLA: equivocal, two assays on CA: negative and positive results). In vivo metolachlor was only tested for the endpoint DNA damage and repair and showed negative results. Bone marrow exposure was demonstrated for S-metolachlor in mice, but S-metolachlor could only be detected for a maximum time period of four hours in the plasma of only two (one hour) and one (four hours) out of three tested animals.

Long-term studies were conducted with metolachlor only and a systemic and carcinogenic NOAEL at 15 mg/kg bw/d was derived.

Reproductive toxicity in terms of a multi-generation study was analysed in rats using metolachlor only. Developmental toxicity was analysed in rats and rabbits using S-metolachlor as well as metolachlor. Maternal and developmental NOAELs were similar. Fetal malformations (hydrocephalus) were observed in rabbits upon treatment with S-metolachlor as well as with metolachlor.

Overall, it can be concluded that the toxicological properties S-metolachlor and metolachlor as demonstrated in acute toxicity studies, 28- and 90-day repeated dose studies, genotoxicity studies and developmental toxicity studies are similar and a bridging between S-metolachlor and metolachlor is possible.

This section contains short summaries taken from Vol. 1 (chapter 2.6) of the RAR, which was prepared for the renewal of the approval of the active substance. All studies included in this dossier were evaluated and assessed by the dossier submitter. In case more detailed information on the reported effects is needed, it is referred to Volume 3, chapter B.6 of the RAR.

Acute toxicity

10.1 Acute toxicity - oral route

This endpoint is not addressed in this CLH report and is outside the scope of the public consultation.

10.2 Acute toxicity - dermal route

This endpoint is not addressed in this CLH report and is outside the scope of the public consultation.

10.3 Acute toxicity - inhalation route

This endpoint is not addressed in this CLH report and is outside the scope of the public consultation.

10.4 Skin corrosion/irritation

This endpoint is not addressed in this CLH report and is outside the scope of the public consultation.

10.5 Serious eye damage/eye irritation

This endpoint is not addressed in this CLH report and is outside the scope of the public consultation.

10.6 Respiratory sensitisation

This endpoint is not addressed in this CLH report and is outside the scope of the public consultation.

10.7 Skin sensitisation

This endpoint is not addressed in this CLH report and is outside the scope of the public consultation.

10.8 Genotoxicity / Germ cell mutagenicity

The genotoxicity of S-metolachlor was assessed in *in vitro* studies in bacteria and mammalian cells as well as in *in vivo* studies in somatic cells. The valid *in vitro* mutagenicity/genotoxicity studies are compiled in Table 9 and the valid *in vivo* mutagenicity/genotoxicity studies are given in Table 10. In both tables also results from genotoxicity testing using metolachlor were included.

Table 9: Summary table of mutagenicity/genotoxicity tests in vitro

Method, guideline, deviations if any	**Test substance**	**Relevant information about the study including rationale for dose selection (as applicable)**	**Observations**	**Reference**
S-metolachlor				
Reverse mutation assay OECD TG 471/1983 GLP	S-metolachlor Batch number: V4673/7 Purity: 95.6 % (S-enantiomeric content: 84 %)	*Salmonella typhimurium* (TA100, TA1535, TA102, TA 98, TA1537) and *E.coli* (WP2uvrA) ± S9 (Aroclor-induced rat liver S9-mix) Solvent: DMSO Study design: plate incorporation Concentrations: 312.5, 625, 1250, 2500, 5000 µg/plate (original experiment, all strains); 78.13, 156.25, 312.50, 625.00, 1250.00 µg/plate (confirmatory experiment, strains TA100, TA1535, TA1537, TA102); 312.5, 625, 1250, 2500, 5000 µg/plate (confirmatory experiment, strains WP2 uvrA, TA98) Preliminary range-finding test (20.6 - 5000 µg/plate; TA100 and E. coli WP2 uvrA), original experiment (312.5 – 5000 µg/plate), confirmatory experiment (78.13 – 5000 µg/plate)	+S9: negative -S9: negative Positive controls gave strong increases in revertants Cytotoxicity was seen ≥1250 µg/plate in strains TA100, TA1535, TA1537, TA102 w/o metabolic activation and at 5000µg/plate (TA98), 1250µg/plate (TA102), 2500µg/plate (TA100, TA1535) and 5000µg/plate (TA1537 and WP2 uvra) Non-mutagenic in tested *S. typhimurium* and *E. coli* strains	Anonymous (23), 1995c acceptable
Reverse mutation assay OECD TG 471/1997 GLP	S-metolachlor Batch number: SMU3BL13001 Purity: 97.1 %	*Salmonella typhimurium* (TA100, TA1535, TA1537, TA 98) and *E.coli* (WP2uvrApKM101, WP2pKM101) ± S9 (phenobarbitol and β-naphthoflavone-induced rat liver S9-mix) Solvent: DMSO Study design: plate incorporation (experiment I), pre-incubation (experiment II) Concentrations: 3, 10, 33, 100,	+S9: negative -S9: negative Positive controls gave strong increases in revertants Precipitation was seen in the overlay agar in the presence of metabolic activation in the test tubes from 2500 µg to 5000 µg/plate Cytotoxicity occurred in all strains except TA1535 Non-mutagenic in tested *S. typhimurium* and *E. coli* strains	Sokolowski, 2014 acceptable

Method, guideline, deviations if any	**Test substance**	**Relevant information about the study including rationale for dose selection (as applicable)**	**Observations**	**Reference**
		333, 1000, 2500, 5000 µg/plate		
Reverse mutation assay OECD TG 471/1997 GLP	S-metolachlor Batch number: CAB7C17042_ FORTIFIED Purity: 96.4 %	*Salmonella typhimurium* (TA100, TA1535, TA1537, TA 98) and *E.coli* (WP2uvrApKM101, WP2pKM101) ± S9 (phenobarbitol and β-naphthoflavone-induced rat liver S9-mix) Solvent: DMSO Study design: plate incorporation (experiment I), pre-incubation (experiment II) Concentrations: 3, 10, 33, 100, 333, 1000, 2500, 5000 µg/plate	+S9: negative -S9: negative Positive controls gave strong increases in revertants Precipitation was seen in the overlay agar in the test tubes from 2500 to 5000 µg/plate, precipitation on the incubated agar plates was observed at 5000 µg/plate Cytotoxicity occurred in strains TA 98, TA 100, WP2 pKM101, and WP2 uvrA pKM101 Non-mutagenic in tested *S. typhimurium* and *E. coli* strains	Schulz, 2018 acceptable
In vitro Mammalian Cell Gene Mutation Test OECD TG 476/1997 GLP	S-metolachlor Batch number: SMU3BL13001 Purity: 97.1 % (S-enantiomeric content: 86.3 %)	Mouse lymphoma L5178Y/TK$^{+/-}$ 3.7.2c ± S9 (phenobarbitol and β-naphthoflavone-induced rat liver S9-mix) Solvent: DMSO Concentrations: experiment I/-S9: 22.5; 45.0; 90.0; 135.0; and 180.0 µg/mL; experiment I/+S9: 22.5; 45.0; 90.0; 180.0; and 270.0 µg/mL; experiment II/-S9: 45.0; 90.0; 135.0; 160.0; and 180.0 µg/mL; experiment II/+S9: 45.0; 90.0; 160.0; 180.0; and 270.0 µg/mL	+S9: negative -S9: negative Positive control substances led to increases in revertant number, however, positive control responses differed largely between the experiments. No precipitation occurred up to the maximum concentration. Cytotoxicity occurred at concentrations ≥180 µg/mL (experiment I) or ≥160 µg/mL (experiment II)	Wollny, 2014 acceptable
In Vitro Mammalian Cell Gene Mutation Tests using the Hprt and xprt genes OECD TG 476/2016 GLP	S-metolachlor Batch number: CAB7C17042_ FORTIFIED Purity: 96.4 %	V79 cells (Chinese hamster lung fibroblasts) ± S9 (phenobarbitol and β-naphthoflavone-induced rat liver S9-mix) Solvent: DMSO Concentrations: -S9: 10.00; 150.0; 170.0; 190.0; and 210.0 µg/mL + S9: 50.0; 100.0; 150.0, 200.0; and 220.0 µg/mL	+S9: negative -S9: negative Positive controls gave responses (300 µg/mL EMS: 276.4; 1.1 µg/mL DMBA: 109.5 mutant colonies per 10^6 cells) which were in the lower range of the HCD (150 µg/mL and 300 µg/mL EMS: 53.9-872.3; 1.1 µg/mL and 2.3 µg/mL DMBA: 56.7-739.9 mutant colonies per 10^6 cells); HCD ranges are not given for the concentrations used, but as summarized data of different	Anonymous (36) 2018 acceptable

Method, guideline, deviations if any	Test substance	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
			concentrations Phase separation occurred at 210.0 µg/mL and above Severe cytotoxicity was observed at ≥230 µg/mL in the absence of metabolic activation and at ≥240 µg/mL in the presence of metabolic activation Non-mutagenic in V79 cells	
In Vitro Mammalian Chromosomal Aberration Test OECD TG 473/1997 GLP 200 cells were counted instead of 300	S-metolachlor Batch number: SMU3BL13001 Purity: 97.1 %	Human lymphocytes ± S9 Solvent: DMSO Concentrations: -S9: Experiment 1A: 173, 302.8, 529.9, 927.3 µg/mL; Experiment 1B: 150.0, 300.0, 600.0 µg/mL; Experiment 2: 52.2, 91.4, 159.9 µg/mL + S9: Experiment 1A: 173.0, 302.8, 529.9, 927.3 µg/mL; Experiment 2: 100.0, 200.0, 400.0 µg/mL	Statistically significant increase in number of mutant colonies in experiment 1B (4-hour incubation time, in the presence of metabolic activation) at 400 µg/mL, dose-response, value exceeded historical control data range Positive result was not reproducible Positive controls gave expected responses High variation in cytotoxicity between the experiments Phase separation was observed at 200 µg/mL and above Clastogenic potential in human lymphocytes is not clear	Anonymous (2), 2014 supplementary
In Vitro Mammalian Cell Micronucleus Test OECD TG 487/2016 GLP	S-metolachlor Batch number: CAB7C17042_FORTIFIED Purity: 96.4 %	Human lymphocytes ± S9 Solvent: DMSO Concentrations: -S9: Experiment I: 69.6, 122, 213, 373 µg/mL; Experiment IID: 38.9, 119, 138, 152 µg/mL + S9: Experiment I: 122, 213, 373, 653 µg/mL; Experiment IIB: 126, 152, 182, 319 µg/mL	Equivocal Non-reproducible statistically significant increases of micronucleated cells in the absence and presence of metabolic activation were observed, one value exceeded the HCD range Positive controls gave expected responses Phase separation was observed at 373 µg/mL and above Clastogenic/aneugenic potential in human lymphocytes is not clear	Anonymous (32), 2019 acceptable
Metolachlor				
In vitro Mammalian	Metolachlor	Mouse lymphoma cells	equivocal	Anonymous

Method, guideline, deviations if any	Test substance	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
Cell Gene Mutation Test Similar to OECD TG 490 No GLP Missing standard deviations and historical control data	Batch number: OP303010 Purity: 95.9 %	(L5178Y) ± S9 Solvent: DMSO Concentrations: -S9: 0; 9.5; 19; 38; 76; 114; 152; 190 nL/mL + S9: Experiment I: 0; 10.5; 21; 42; 84; 126; 168; 210 nL/mL; Experiment II: 0; 56; 112; 168; 196; 224; 252; 280 nL/mL		(1), 1984 supplementary
In Vitro Mammalian Chromosomal Aberration Test OECD TG 473/1983 GLP	Metolachlor Batch number: P802006 Purity: 97.4 %	CHO cells (CCL61) ± S9 Solvent: DMSO Concentrations: -S9, 3h: 0, 62.5, 125, 250 µg/mL -S9, 24h: 0, 15.63, 31.25, 62.5, 125, 250 µg/mL + S9, 3h: 0, 31.25, 62.5, 125, 250 µg/mL	+S9: negative -S9: negative At 250 µg/ml an increase in polyploid metaphases (endoreduplication figures) was detected, thus, an aneugenic effect of S-metolachlor seems possible. On the other hand, since this polyploidy was accompanied by suppression of mitotic activity by 56.4%, it could also result from cytotoxicity or cell cycle perturbation	Anonymous (36), 1990 supplementary
In Vitro Mammalian Chromosomal Aberration Test No GLP	Metolachlor Batch number: n.a. Purity: n.a.	Human lymphocytes ± S9 Solvent: isooctane Concentrations: -S9, 72h: 0, 0.01, 0.1, 1 µg/mL	Positive ± S9?	Roloff, 1992 supplementary

Table 10: Summary table of mutagenicity/genotoxicity tests in vivo in mammalian somatic or germ cells

Method, guideline, deviations if any	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
S-metolachlor				
Mammalian Erythrocyte Micronucleus Test OECD TG 474/1983 GLP	S-metolachlor Batch number: V4673/7 Purity: 95.6 %	Tif:MAGf mice Oral (gavage) Solvent: arachis oil Concentrations: 500; 1000; 2000 mg/kg 5 mice/sex/dose/time point	Negative No increases in micronuclei frequency in bone marrow cells of mice due to the test material in doses up to 2000 mg/kg bw. No indications of toxicity in bone marrow in terms of alterations of the ratio PCE/NCE were observed.	Anonymous (21), 1995a acceptable

Method, guideline, deviations if any	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
1000 cells were scored instead of 4000			(Additional proof of exposure study available).	
Mammalian Erythrocyte Micronucleus Test OECD TG 474/1997 GLP 2000 cells were scored instead of 4000	S-metolachlor Batch number: SMU3BL13001 Purity: 97.1 %	NMRI mice Oral Solvent: aqueous CMC containing Tween 80 Concentrations: 200, 400, 800 mg/kg bw 7 male mice/dose group, 5 male mice per control group	Negative No increases in micronuclei frequency in bone marrow cells of mice due to the test material in doses up to 800 mg/kg bw. No indications of toxicity in bone marrow in terms of alterations of the ratio PCE/NCE were observed. (Additional proof of exposure study available).	Anonymous (9), 2014 acceptable
Unscheduled DNA Synthesis (UDS) Test with Mammalian Liver Cells in vivo GLP	S-metolachlor Batch number: V4673/7 Purity: 95.6 % (S-enantiomeric content: 84 %)	Sprague Dawley Tif:RAIf rats 3 rats/sex/dose/time point Oral (gavage) Solvent: arachis oil Concentrations: 500, 1500, F:3200, M:5000 mg/kg,	Negative No increase in unscheduled DNA synthesis was reported in hepatocytes of treated rats.	Anonymous (22), 1995b acceptable
Metolachlor				
Unscheduled DNA Synthesis (UDS) Test with Mammalian Liver Cells in vivo GLP	Metolachlor Batch number: FL930326 Purity: 97.3 %	Sprague Dawley, CRL, CD7BR 3 rats/sex/dose/time point Oral (gavage) Solvent: corn oil Concentrations: 500; 1250; 2500; 4000 mg/kg bw (males); 0; 500; 1000 and 1500 mg/kg b.w. (females)	Negative No increase in unscheduled DNA synthesis was reported in hepatocytes of treated rats	Anonymous (18) 1994 acceptable
Unscheduled DNA Synthesis (UDS) Test with Mammalian Liver Cells in vivo GLP	Metolachlor Batch number: 841697 Purity: 96.4 %	Sprague Dawley rats 3 rats/sex/dose/time point Oral (gavage) Solvent: PEG Concentrations: 2.9, 31.9, 301, 450 mg/kg	Negative No increase in unscheduled DNA synthesis was reported in hepatocytes of treated rats	Anonymous (6), 1988 acceptable

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

The potential genotoxicity of S-metolachlor was investigated in a series of both in vitro and in vivo studies. All regular end points for genetic damage (point mutations, chromosome damage, DNA damage and repair) were assessed. For metolachlor only the endpoint DNA damage and repair was tested in vivo. In vitro data regarding mutagenicity and chromosomal aberrations are also available for metolachlor.
S-metolachlor was tested negative for genotoxicity in vitro in bacterial (Anonymous (23), 1995c, Sokolowski, 2014, Schulz, 2018) and mammalian (Wollny, 2014, Anonymous (36), 2018) cell mutagenicity studies. However, chromosome aberration in vitro showed a positive, but not reproducible result (Anonymous (2), 2014). A micronucleus test (Anonymous (32), 2019) gave equivocal results.
In vivo negative results for S-metolachlor were reported in two micronucleus assays (Anonymous (21), 1995a; Anonymous (9), 2014), but both assays showed deviations as too few cells were scored and therefore the experimental power of the assays was reduced. Bone marrow exposure was not sufficiently demonstrated in the studies (i.e. decrease in the PCE/NCE ratio), but in the study by Anonymous (21), (1995) neurological signs (ataxia, tremor) were observed in 3 out of 5 males and females, which might point to exposure of the bone marrow. In the study by Anonymous (9), 2014 observed neurological signs were rather mild and occurred only in the 0-1 hour post-treatment interval in few animals. ADME data suggest that the bone marrow was reached. As mentioned in Vol. 3, B.6.1.1:”In one study (Momose, 1988) tissue distribution was assessed by whole body autoradiography at 8h post-dose (1.5 mg/kg b.w.). Apart from G.I.-tract membranes, labelling was restricted to liver, kidney and lung, and also slightly to bone marrow.” Nevertheless, it was also discussed by the authors that the slight blackening of the bone marrow was possibly “possibly owing to blood”. Furthermore, radioactivitiy concentrations in tissues and organs were given in the study report and F-values are in the range of 9 – 16.73 % 24 hours after administration in male and female animals. In a proof of exposure study Anonymous (41), 2017) S-metolachlor was only one hour (in 2 out of 3 animals) and four hours (in 1/3 animals) after exposure and not after 24 hours detectable in plasma of male mice. However, according to the EFSA Scientific Opinion from 2017 (EFSA Journal 2017;15(12):5113) bone marrow exposure is sufficiently shown by the detection of the test substance in plasma. Even though the short abundance of S-metolachlor in the plasma, perhaps due to fast metabolization, might cause some uncertainty. On the pesticides peer review expert meeting (TC 27) the proof of exposure study was discussed and if, based on the results, bone marrow exposure for the in vivo micronucleus studies by Anonymous (21), 1995a and Anonymous (9), 2014 can be assumed. The already identified uncertainty in terms of a very small window (1 hr Cmax) of detection of S-metolachlor and fast metabolisation in mice as a possible reason were confirmed. It was noted that there is no data to compare the different metabolism between the species (rats vs. mice) as only humans were included in the in vitro comparative metabolism study. The small window of detection might be an issue to assess the aneugenicity endpoint (1 hour might not be sufficient). However, as S-metolachlor was detected at variable amounts in different animals at 1 and 4 hours, it was agreed that there is enough evidence to support bone marrow exposure.

No effect of S-metolachlor was seen in an unscheduled DNA synthesis (UDS) test (Anonymous (22), 1995b). Results for metolachlor from two acceptable unscheduled DNA synthesis (UDS) tests showed no genotoxic potential regarding the endpoint DNA damage and repair. In a mouse lymphoma assay (Anonymous 1, 1984) without S9 mix no mutagenic effect of metolachlor was observed, but contradictory results were seen with S9 mix. This study was of only supplementary informative value as several deviations were obvious (absence of information on the number of treated cells, no historical control data, to high doses to evaluate dose-response relation). Conflicting results were observed with regard to chromosome aberration in vitro as one test gave a negative result (Anonymous (36), 1990) and a further test showed a positive result (Roloff, 1992).

Overall, it was concluded that S-metolachlor is unlikely to have a genotoxic potential.

10.8.2 Comparison with the CLP criteria

Following criteria for classification for germ cell mutagens are given in CLP regulation:

Table 11: CLP criteria for classification for germ cell mutagens

CLP criteria
The classification in **Category 1A** is based on positive evidence from human epidemiological studies. Substances to be regarded as if they induce heritable mutations in the germ cells of humans.
The classification in **Category 1B** is based on: - positive result(s) from in vivo heritable germ cell mutagenicity tests in mammals; or - positive result(s) from in vivo somatic cell mutagenicity tests in mammals, in combination with some evidence that the substance has potential to cause mutations to germ cells. It is possible to derive this supporting evidence from mutagenicity/genotoxicity tests in germ cells in vivo, or by demonstrating the ability of the substance or its metabolite(s) to interact with the genetic material of germ cells; or - positive results from tests showing mutagenic effects in the germ cells of humans, without demonstration of transmission to progeny; for example, an increase in the frequency of aneuploidy in sperm cells of exposed people.
The classification in **Category 2** is based on: - positive evidence obtained from experiments in mammals and/or in some cases from in vitro experiments, obtained from: - somatic cell mutagenicity tests in vivo, in mammals; or - other in vivo somatic cell genotoxicity tests which are supported by positive results from in vitro mutagenicity assays.
Note: Substances which are positive in in vitro mammalian mutagenicity assays, and which also show chemical structure activity relationship to known germ cell mutagens, shall be considered for classification as Category 2 mutagens.

No human data are available for S-metolachlor, hence a classification in category 1A is not possible. *In vitro* studies (mutagenicity, clastogenicity) and/or the respective *in vivo* studies showed overall a negative outcome, hence a classification in category 2 is currently considered not warranted for S-metolachlor.

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

No classification for genotoxicity is proposed.

10.9 Carcinogenicity

Two studies on chronic toxicity and carcinogenicity of metolachlor in rats and mice are available, however, the study in mice was considered not acceptable due to high mortality (> 50 % in control and treatment groups). Results from rat and mice studies are summarised in Table 12.
Epidemiological studies with metolachlor are available. Most of them are based on data from the Agricultural Health Study (AHS). Results are summarised in Table 13.
Several mechanistic studies concerning a possible mode or mechanism of action for the observed tumour-formation in response to metolachlor were conducted. Results are summarised in Table 14.

Table 12: Summary table of animal studies on carcinogenicity

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
2-year chronic oral toxicity and oncogenicity study Oral (dietary) Rat, Sprague Dawley (Crl:CD(SD)BR) 60 animals/sex/dose (5/sex from control and high-dose group for interim kill) 10 rats/sex for haematology prestudy partly in compliance to B.33 of directive 92/69/EEC with deviations (e.g. weekly feed consumptions recorded on 10 animals/sex/dose instead of all animals, except at week 40, 52, 66, 78, 92 and 104; haematology and urinalysis on 8 animals/sex/dose, but no blood smears, animals were infected with sialodacryoadenitis virus (SDAV) GLP	Metolachlor (95.5 % purity, enantiomeric content: 47.7 %:47.7 % R/S enantiomer 0, 30, 300 and 3000 ppm, equivalent to 1.5, 15, and 150 mg/kg bw/d (calculated using default conversion factor of 20)	NOAELsystemic & carcinogenicity = 15 mg/kg bw/d Body weight (wk 8 – wk 78) ↓ (10 %) in females, Statistically significant increase in liver neoplastic nodules and combined incidence of both nodules and carcinoma in males and females exceeding historical control data (c. f. Table 15, Table 16) Statistically significant increase in adenoma and carcinoma of the pituitary in females (c. f. Table 15) Statistically significant increase in thyroid follicular cell adenoma in females (c. f. Table 15) exceeding historical control data Statistically significant increase in nasal turbinate adenocarcinoma in males (c. f. Table 17) exceeding historical control data	Anonymous (39), 1983 (including Amendment 1 + 2) Anonymous (31), 1988 Anonymous (19),1984 supplementary
Carcinogenicity study Oral (dietary) Mice (Crl:CD-1 (ICR)BR) 68 animals/sex/dose (8/sex for interim kill) 10 mice/sex for haematology prestudy partly in compliance to B.32 of directive 92/69/EEC and OECD 451 with deviations (e.g. Mortality > 50 % in control and other dose groups; food consumption recorded in 10 animals/dose/sex instead of all animals, accordingly haematology at 12 and 18 month in 8 animals/dose/sex only, accidental drinking water restriction in week 1 of the study, Sendai virus infections at early stages of the study) GLP	Metolachlor (95.3% purity, enantiomeric content: 47.7%:47.7% R/S enantiomer 0, 300, 1000 and 3000 ppm, equivalent to 0, 50, 171, 571 mg/kg bw/d in males and 0, 65, 228 and 733 mg/kg bw/d in females	NOAEL for carcinogenicity = not derived NOAEL for systemic toxicity = 171 mg/kg bw/d, LOAEL = 571 mg/kg bw/d Mortality > 50% in control and treatment groups No increase in tumour incidences Lower mean bodyweights in males at 571 mg/kg bw/d from week 2 on (↓ 5-10 %) Increased relative liver (+27 %) and kidney (+21 % left kidney, +13 % right kidney) weight at 571 mg/kg bw/d in males	Anonymous (38), 1982 not acceptable

Table 13: Summary table of human data on carcinogenicity

<table>
<tr><th>Type of data/report</th><th>Test substance</th><th>Relevant information about the study (as applicable)</th><th>Observations</th><th>Reference</th></tr>
<tr><td>Agricul-tural health study (AHS)</td><td>Metolachlor</td><td>Prospective study of pesticide applicators in Iowa and North Carolina AHS cohort, 1993 – 2002</td><td>
<p>Increased risk for lung cancer (lifetime days exposure, highest category of use)</p>
<p>Decreased risk for prostate cancer (lifetime days exposure, highest category & intensity-weighted lifetime days exposure, second highest category)</p>
<p>Rate ratios[1] from poisson regressions for selected cancers[2] by tertiles[3] of lifetime exposure-days and intensity-weighted lifetime exposure-days to metolachlor[4] among Agricultural Health Study cohort applicators with low-metolachlor exposed applicators as the referent:</p>
<table>
<tr><th rowspan="2">Cancer site</th><th colspan="4">Lifetime days[6]</th><th colspan="4">Intensity weighted lifetime days[7]</th></tr>
<tr><th><i>n</i>[5]</th><th>RR</th><th>95 % CI</th><th><i>p</i>-trend</th><th><i>n</i>[5]</th><th>RR</th><th>95 % CI</th><th><i>p</i>-trend</th></tr>
<tr><td colspan="9">All cancers</td></tr>
<tr><td>T1</td><td>225</td><td>1.00</td><td></td><td></td><td>229</td><td>1.00</td><td></td><td></td></tr>
<tr><td>T2</td><td>221</td><td>1.00</td><td>(0.83–1.21)</td><td></td><td>214</td><td>0.95</td><td>(0.78–1.15)</td><td></td></tr>
<tr><td>T_{3L}</td><td>117</td><td>1.05</td><td>(0.83–1.32)</td><td></td><td>113</td><td>0.83</td><td>(0.65–1.07)</td><td></td></tr>
<tr><td>T_{3U}</td><td>117</td><td>1.01</td><td>(0.78–1.30)</td><td>0.98</td><td>124</td><td>0.93</td><td>(0.72–1.21)</td><td>0.72</td></tr>
<tr><td></td><td>680</td><td></td><td></td><td></td><td>680</td><td></td><td></td><td></td></tr>
<tr><td colspan="9">Lung</td></tr>
<tr><td>T1</td><td>13</td><td>1.00</td><td></td><td></td><td>12</td><td>1.00</td><td></td><td></td></tr>
<tr><td>T2</td><td>11</td><td>1.02</td><td>(0.45–2.30)</td><td></td><td>16</td><td>1.44</td><td>(0.67–3.11)</td><td></td></tr>
<tr><td>T_{3L}</td><td>10</td><td>1.89</td><td>(0.79–4.48)</td><td></td><td>8</td><td>1.38</td><td>(0.51–3.72)</td><td></td></tr>
<tr><td>T_{3U}</td><td>12</td><td>2.37</td><td>(0.97–5.82)</td><td>0.03</td><td>10</td><td>1.65</td><td>(0.61–4.47)</td><td>0.65</td></tr>
<tr><td></td><td>46</td><td></td><td></td><td></td><td>46</td><td></td><td></td><td></td></tr>
</table>
<p>…</p>
<p>…</p>
</td><td>Rusiecki, J. A., et al., 2006,</td></tr>
</table>

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

	Lifetime days[6]				Intensity weighted lifetime days[7]			
	n[5]	RR	95 % CI	*p*-trend	*n*[5]	RR	95 % CI	*p*-trend
Prostate								
T1	115	1.00			108	1.00		
T2	99	0.84	(0.63–1.10)		101	0.91	(0.69–1.21)	
T_{3L}	47	0.79	(0.55–1.13)		46	0.66	(0.45–0.97)	
T_{3U}	38	0.59	(0.39–0.89)	0.21	44	0.67	(0.44–1.01)	0.38
	299				299			

1 Adjusted for age, sex, race, smoking, alcohol, applicator status (private or commercial) family history of cancer, state of residence, and the most highly correlated pesticides with metolachlor.
2 Cancers for which there were at least 20 exposed cases and 5 exposed cases in each category after accounting for missing covariate data.
3 Top tertile split for all cancers combined, colon, lung, prostate, and all lymphohematopoetic cancers.
4 Total number exposed to metolachlor: 22,781
5 Numbers of cancer-specific cases entered into the final models in each tertile of metolachlor exposure.
6 Tertiles for lifetime days: 2.5–20, 21–56, >56; when top tertile split, T_{3L}: >56–116, T_{3U}: >116.
7 Tertiles for intensity weighted lifetime days: 0.5–103, >103–362, >362; when top tertile split, T_{3L}: >362–924, T_{3U}: >924.

<table>
<tr><th>Type of data/report</th><th>Test substance</th><th>Relevant information about the study (as applicable)</th><th>Observations</th><th>Reference</th></tr>
<tr><td>Agricultural health study (AHS)</td><td>Metolachlor</td><td>Prospective study of pesticide applicators in Iowa and North Carolina

AHS cohort, 1993 – 2010 (North Carolina) / 2011(Iowa)</td><td>Increased risk for liver cancer (both metrics, two highest categories of use)

Increased risk for follicular cell lymphoma (both metrics) With person-time in the low metolachlor use category as referent: decreased risk for developing melanoma (no exposure-response) and increased risk for oral cavity cancer (no exposure-responseRate ratios[a] for cancers with at least 20 exposed cases by quartiles of lifetime exposure days and intensity-weighted lifetime exposure days to metolachlor among Agricultural Health Study cohort applicators (with unexposed person-time as the referent), 5-year lag:
<table>
<tr><th></th><th colspan="3">Lifetime days</th><th colspan="3">Intensity-weighted lifetime days</th></tr>
<tr><td>Cancer site</td><td>N[b]</td><td>RR (95 % CI)</td><td>p-Trend</td><td>N</td><td>RR (95 % CI)</td><td>p-Trend</td></tr>
<tr><td colspan="7">All cancers</td></tr>
<tr><td>Unexposed</td><td>3,248</td><td>1.00 (referent)</td><td></td><td>3,248</td><td>1.00 (referent)</td><td></td></tr>
<tr><td>Q1c</td><td>619</td><td>0.95 (0.86–1.04)</td><td></td><td>619</td><td>0.98 (0.89–1.08)</td><td></td></tr>
<tr><td>Q2</td><td>626</td><td>0.96 (0.88–1.06)</td><td></td><td>604</td><td>0.95 (0.86–1.05)</td><td></td></tr>
<tr><td>Q3</td><td>611</td><td>0.97 (0.88–1.06)</td><td></td><td>610</td><td>0.96 (0.87–1.07)</td><td></td></tr>
<tr><td>Q4</td><td>589</td><td>0.94 (0.85–1.04)</td><td>0.30</td><td>613</td><td>0.92 (0.83–1.02)</td><td>0.14</td></tr>
<tr><td colspan="7">Liver</td></tr>
<tr><td>Unexposed</td><td>17</td><td>1.00</td><td></td><td>15</td><td>1.00</td><td></td></tr>
<tr><td>Q1</td><td>2</td><td>0.97 (0.17–5.50)</td><td></td><td>3</td><td>1.65 (0.37–7.23)</td><td></td></tr>
<tr><td>Q2</td><td>4</td><td>1.79 (0.54–5.93)</td><td></td><td>3</td><td>1.33 (0.35–4.99)</td><td></td></tr>
<tr><td>Q3</td><td>7</td><td>3.06 (1.05–8.90)</td><td></td><td>8</td><td>3.14 (1.11–8.88)</td><td></td></tr>
<tr><td>Q4</td><td>10</td><td>3.99 (1.43–11.1)</td><td><0.01</td><td>9</td><td>3.18 (1.10–9.22)</td><td>0.03</td></tr>
</table>
…

…</td><td>Silver, S. R., et al., 2015,</td></tr>
</table>

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

	Lifetime days			Intensity-weighted lifetime days		
Cancer site	N[b]	RR (95 % CI)	p-Trend	N	RR (95 % CI)	p-Trend
Follicular cell lymphoma						
Unexposed	24	1.00		24	1.00	
Q1	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

[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–≤1,403, Q3 >1,403–≤4,103, Q4 > 4,103 units.

Rate ratios[a] for all cancers with 20 or more exposed cases by quartiles of lifetime days and intensity-weighted lifetime days of metolachlor use among Agricultural Health Study applicators (n = 26,505) who ever used metolachlor (with person-time in the low-metolachlor use category as referent), 5-year lag:

	Lifetime days			Intensity-weighted lifetime days		
Cancer site	N[b]	RR (95 % CI)	p-Trend	N	RR (95 % CI)	p-Trend
All cancers						
Q1[c]	699	1.00 reference		694	1.00 reference	
Q2	626	1.00 (0.90–1.13)		604	0.96 (0.86–1.08)	
Q3	611	1.00 (0.89–1.13)		610	0.97 (0.86–1.10)	
Q4	589	0.97 (0.86–1.11)	0.64	613	0.92 (0.80–1.05)	0.27

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

	Lifetime days			Intensity-weighted lifetime days		
Cancer site	N[b]	RR (95 % CI)	p-Trend	N	RR (95 % CI)	p-Trend
Liver						
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)	0.10	9	1.71 (0.33–8.83)	0.44
Follicular cell lymphoma						
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)	0.14	8	2.08 (0.61–7.10)	0.21
Melanoma						
Q1	29	1.00		38	1.00	
Q2	27	1.10 (0.63–1.91)		17	0.54 (0.30–0.97)	
Q3	29	1.20 (0.68–2.10)		27	0.91 (0.52–1.60)	
Q4	27	1.19 (0.65–2.18)	0.60	30	1.03 (0.55–1.93)	0.43
Oral cavity						
Q1	10	1.00		14	1.00	
Q2	21	2.34 (1.06–5.16)		12	1.06 (0.48–2.36)	
Q3	16	1.88 (0.82–4.31)		19	1.69 (0.79–3.61)	
Q4	14	1.78 (0.72–4.39)	0.63	16	1.66 (0.70–3.96)	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 (imazethapyr, alachlor, atrazine, dicamba,

<table>
<tr><th>Type of data/report</th><th>Test substance</th><th>Relevant information about the study (as applicable)</th><th>Observations</th><th>Reference</th></tr>
<tr><td></td><td></td><td></td><td>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, 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, Q1 >0–≤490, Q2 >490–≤1,403, Q3 >1,403–≤4,103, Q4 > 4,103 units.
d Subtypes for non-Hodgkin lymphoma as defined by Morton et al.</td><td></td></tr>
<tr><td>Agricultural health study (AHS)</td><td>Metolachlor</td><td>Prospectives tudy of pesticide applicators in Iowa and North Carolina
AHS cohort, 1993 -2001</td><td>Increased lung cancer risk for highest lifetime exposure days (> 457) independent of used referent groupLung cancer risk among applicators by lifetime exposure days of indicated pesticide, using two referent groups, Agricultural Health Study, 1993–2001:
<table>
<tr><th>Pesticide by lifetime exposure days</th><th>No. of exposed cases</th><th>Odds ratio*</th><th>95 % confidence interval</th><th>Odds ratio*</th><th>95 % confidence interval</th></tr>
<tr><td>No exposure</td><td>96</td><td>1.0</td><td>Referent</td><td></td><td></td></tr>
<tr><td><38.8</td><td>20</td><td>0.6</td><td>0.4, 1.0</td><td>1.0</td><td>Referent</td></tr>
<tr><td>38.8–116</td><td>20</td><td>1.0</td><td>0.6, 1.6</td><td>1.6</td><td>0.8, 3.0</td></tr>
<tr><td>116.1–457.0</td><td>8</td><td>0.9</td><td>0.4, 1.8</td><td>1.2</td><td>0.5, 2.9</td></tr>
<tr><td>>457.0</td><td>6</td><td>4.1</td><td>1.6, 10.4</td><td>5.0</td><td>1.7, 14.9</td></tr>
<tr><td>p-trend</td><td></td><td>0.015</td><td></td><td></td><td>0.0002</td></tr>
</table>
* Odds ratios adjusted for smoking (pack-years among current and pack-years among former smokers), age, gender, and total days of any pesticide application.</td><td>Alavanja, M. C. R., et al. (2004)</td></tr>
</table>

<table>
<tr><td>Agricultural health study (AHS)</td><td>Metolachlor</td><td>Prospective study of pesticide applicators in Iowa and North Carolina
AHS cohort,</td><td>No association between metolachlor and pancreatic cancer
Odds ratios and 95 % confidence intervals for pancreatic cancer in relation to ever/never pesticide exposure[1] among applicators and spouses in the agricultural health study, 1993–2004:
<table>
<tr><th>Pesticides</th><th colspan="2">Controls</th><th colspan="2">Pancreatic cancer cases</th><th>OR[2]</th><th>95 % CI[2]</th></tr>
<tr><td></td><td>Never</td><td>Ever</td><td>Never</td><td>Ever</td><td></td><td></td></tr>
<tr><td>Metolachlor</td><td>52,711</td><td>23,097</td><td>61</td><td>23</td><td>1.0</td><td>0.6–1.7</td></tr>
</table></td><td>Andreotti, G., et al. (2009)</td></tr>
</table>

<table>
<tr>
<td></td>
<td></td>
<td>1993 – 2004</td>
<td>1 Pesticides with at least 10 exposed cases, or for organochlorines at least 5 exposed cases, are reported.
2 Adjusted for age group, cigarette smoking (never, past and current), diabetes, and applicator type.
Odds ratios and 95 % confidence intervals for pancreatic cancer in relation to intensity-weighted pesticide exposure[1] among applicators in the agricultural health study, 1993–2004:
<table>
<tr><th>Pesticides</th><th>Intensity-weighted pesticide exposure[2]</th><th>Controls</th><th>Pancreatic Cancer Cases</th><th>OR[3]</th><th>95% CI[3]</th></tr>
<tr><td>Metolachlor</td><td>Never</td><td>25,658</td><td>34</td><td>1.0</td><td>–</td></tr>
<tr><td></td><td>≤224</td><td>10,727</td><td>14</td><td>1.2</td><td>0.7–2.3</td></tr>
<tr><td></td><td>≥225</td><td>10,732</td><td>6</td><td>0.6</td><td>0.2–1.4</td></tr>
<tr><td></td><td>p-trend</td><td></td><td></td><td></td><td>0.34</td></tr>
</table>
[1] Pesticides having at least 10 exposed cases and 5 cases per exposure group are shown.
[2] Intensity-weighted lifetime exposure days [(exposure days)x(intensity score)]; cutoffs based on median level among controls.
[3] Adjusted for age group, cigarette smoking (never, past, current), diabetes</td>
<td></td>
</tr>
<tr>
<td>Agricultural health study (AHS)</td>
<td>Metolachlor</td>
<td>Prospective study of pesticide applicators in Iowa and North Carolina
AHS cohort, 1993 – 2005</td>
<td>Positive association between body mass index (BMI) and colon cancer: increased risk for metolachlor users when BMI >=30 Hazard Rate Ratio (HR) and 95 % Confidence Intervals (CI) for Colon Cancer in relation to BMI at enrolment by ever/never use of pesticides among men:
<table>
<tr><th>BMI (kg/m²)</th><th>N</th><th>HR[1]</th><th>95 %CI[1]</th><th>p-interaction[2]</th><th>N</th><th>HR[1]</th><th>95 %CI[1]</th><th></th><th>p-interaction[2]</th></tr>
<tr><td></td><td colspan="4">No Metolachlor</td><td colspan="4">Metolachor</td><td></td></tr>
<tr><td><25</td><td>28</td><td>1.00</td><td>Ref</td><td></td><td>12</td><td>1.00</td><td>Ref</td><td></td><td></td></tr>
<tr><td>25–29.9</td><td>57</td><td>1.09</td><td>0.67–1.77</td><td></td><td>36</td><td>1.39</td><td>0.68–2.83</td><td></td><td></td></tr>
<tr><td>>=30</td><td>31</td><td>1.29</td><td>0.74–2.25</td><td></td><td>35</td><td>2.91</td><td>1.42–5.96</td><td></td><td></td></tr>
<tr><td>trend[1]</td><td></td><td>1.01</td><td>0.96–1.06</td><td>0.70</td><td></td><td>1.09</td><td>1.04–1.15</td><td>0.001</td><td>0.02</td></tr>
</table>
[1] Based on Cox regression using age as time-dependent variable; test of trend calculated using continuous BMI; models adjusted for race, education, family history colon cancer.
[2] Interaction calculated using never/ever pesticide use and continuous BMI</td>
<td>Andreotti, G., et al. (2010)</td>
</tr>
<tr>
<td>Agricultural health study</td>
<td>Metolachlor</td>
<td>Prospective study of pesticide applicators in Iowa and</td>
<td>No association between metolachlor and colorectal cancer.
Colorectal cancer risk among pesticide applicators by ever/never exposed to metolachlor in the Agricultural Health Study, 1993–2002:</td>
<td>Lee, W. J., et al. (2007)</td>
</tr>
</table>

<table>
<tr>
<td>(AHS)</td>
<td></td>
<td>North Carolina
AHS cohort, 1993 – 2002</td>
<td>
<table>
<tr><td>Pesticides[1]</td><td colspan="4">Colorectal (n = 305)</td><td colspan="4">Colon (n = 212)</td><td colspan="4">Rectum (n = 93)</td></tr>
<tr><td></td><td colspan="2">Observed cases[2]</td><td rowspan="2">OR[3]</td><td rowspan="2">95 % CI[3]</td><td colspan="2">Observed cases</td><td rowspan="2">OR</td><td rowspan="2">95 % CI</td><td colspan="2">Observed cases</td><td rowspan="2">OR</td><td rowspan="2">95 % CI</td></tr>
<tr><td></td><td>Exp.</td><td>Non-exp.</td><td>Exp.</td><td>Non-exp.</td><td>Exp.</td><td>Non-exp.</td></tr>
<tr><td>Metolachlor</td><td>107</td><td>146</td><td>1.0</td><td>0.8, 1.3</td><td>73</td><td>104</td><td>1.0</td><td>0.7, 1.4</td><td>34</td><td>42</td><td>1.0</td><td>0.6, 1.7</td></tr>
</table>
1 The information for ever/never exposed to 50 pesticides comes from the enrollment questionnaire.
2 Missing data for some questions are responsible for difference in total cancer cases.
3 OR, Odds ratio; CI, confidence interval. Odds ratio adjusted for age, smoking, state, total days of pesticide application among all enrolment applicators. The reference category was applicators who were not exposed to each pesticide.
</td>
<td></td>
</tr>
<tr>
<td>Agricultural health study (AHS)</td>
<td>Metolachlor</td>
<td>Prospective study of pesticide applicators in Iowa and North Carolina
AHS cohort, 1993 – 2003</td>
<td>Analysis of interaction of genetic risk factor for prostate cancer (8q24 variants), metolachlor use and prostate cancer. Exposure dependent increase of OR.

Metolachlor-8q24 SNP (rs12547643) interactions with increased trends across strata of lifetime exposure days and risk of prostate cancer in the AHS:
<table>
<tr><td colspan="2">Nonexposed</td><td colspan="2">Low exposed</td><td colspan="2">High exposed</td><td>p-interaction</td></tr>
<tr><td>Cases/ Controls</td><td>OR* (95 % CI)</td><td>Cases/ Controls</td><td>OR* (95 % CI)</td><td>Cases/ Controls</td><td>OR* (95 % CI)</td><td></td></tr>
<tr><td>369/711</td><td>1.05 (0.87–1.27)</td><td>179/302</td><td>1.15 (0.87–1.53)</td><td>133/302</td><td>1.47 (1.08–2.00)</td><td>0.05</td></tr>
</table>
*OR per risk allele assuming a log-additive model. Adjusted for age and state of residence.
</td>
<td>Koutros, S., et al. (2010).</td>
</tr>
<tr>
<td>Agricultural health study (AHS)</td>
<td>Metolachlor</td>
<td>Prospective study of pesticide applicators in Iowa and North Carolina
AHS cohort, 1993 – 2004</td>
<td>Negative association of high metolachlor exposure and prostate cancer.
<table>
<tr><td colspan="7">Associations between pesticide intensity-weighted lifetime days and prostate cancer:</td></tr>
<tr><td></td><td colspan="6">Pesticide exposure</td></tr>
<tr><td></td><td>None[a]</td><td colspan="2">Low</td><td colspan="2">High</td><td></td></tr>
<tr><td>Pesticide</td><td>Ca/Co</td><td>Ca/Co</td><td>OR (95 % CI)[b]</td><td>Ca/Co</td><td>OR (95 % CI)[b]</td><td>ptrend[c]</td></tr>
<tr><td>Metolachlor</td><td>369/712</td><td>190/304</td><td>1.21 (0.97, 1.52)</td><td>119/298</td><td>0.77 (0.60, 0.99)</td><td>0.02</td></tr>
</table>
Ca, cases; CI, confidence interval; Co, controls. [a] Referent group for estimated effects of low and high pesticide use.
[b] Adjusted for age and state. [c] p-Value for pesticide trend, adjusted for age and state.
</td>
<td>Barry, K. H., et al. (2011)</td>
</tr>
</table>

<table>
<tr><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>Case-control study</td><td>Metolachlor</td><td>Pooled data from three case-control studies conducted by the National Cancer Institute during the 1980s in the Midwestern United States of America.
47 pesticides</td><td>No association of metolachlor use and non-Hodgkin lymphoma (NHL)
Effect estimates for use of metolachlor and NHL incidence, adjusting for use of other pesticides (the estimate is adjusted for use of 46 other pesticides, age, and study site):
<table>
<tr><td colspan="2">Exposed [n (%)]</td><td>Logistic regression</td><td>Hierarchical regression</td></tr>
<tr><td>Cases (N=650)</td><td>Controls (N=1933)</td><td>OR (95 % CL)†</td><td>OR (95 % CL)</td></tr>
<tr><td>13 (2.0 %)</td><td>37 (1.9 %)</td><td>0.7 (0.3 to 1.6)</td><td>0.7 (0.4 to 1.5)</td></tr>
</table>
</td><td>De Roos, A. J., et al. (2003)</td></tr>
<tr><td>Case-Control study</td><td>Metolachlor</td><td>Population based on case-control study, telephone interviews with men and women diagnosed with gliomas (n=251) between 1988 and 1993 and controls (n=498)
Eastern Nebraska</td><td>Metolachlor was part of an herbicide group, an acetanilide group and a nitrosatable pesticides group. Non-significantly increased ORs (~ two-fold) in the herbicide group for glioblastoma and for glioblastoma and astrocytoma in the acetanilide group as well as in the nistrosatable pesticides group.
Non-significantly increased OR for metolachlor and brain-cancer among proxy-responders, not among self-responders.
Odds ratios (ORs) and 95 % confidence intervals (CIs) for brain cancer by ever-use of pesticide classes by histological types among adult male farmers:
<table>
<tr><td></td><td colspan="4">Glioblastoma multiforme</td><td colspan="3">Astrocytoma</td><td colspan="3">Other glioma</td></tr>
<tr><td></td><td></td><td colspan="3">Cases</td><td colspan="3">Cases</td><td colspan="3">Cases</td></tr>
<tr><td></td><td>Controls</td><td>n</td><td>OR*</td><td>95 % CI</td><td>n</td><td>OR*</td><td>95 % CI</td><td>n</td><td>OR*</td><td>95 % CI</td></tr>
<tr><td>Non-farmers</td><td>112</td><td>25</td><td>1.0</td><td>Ref‡</td><td>15</td><td>1.0</td><td>Ref‡</td><td>9</td><td>1.0</td><td>Ref‡</td></tr>
<tr><td>Herbicides</td><td>70</td><td>21</td><td>1.9</td><td>0.9-3.8</td><td>12</td><td>1.6</td><td>0.7-3.9</td><td>5</td><td>1.1</td><td>0.3-3.7</td></tr>
<tr><td>Acetanilide</td><td>34</td><td>10</td><td>1.9</td><td>0.8-4.7</td><td>9</td><td>2.1</td><td>0.8-5.5</td><td>3</td><td>0.9</td><td>0.2-3.8</td></tr>
<tr><td>Nitrosatable pesticides</td><td>61</td><td>18</td><td>2.0</td><td>0.9-4.2</td><td>14</td><td>2.2</td><td>0.9-5.1</td><td>4</td><td>0.9</td><td>0.3-3.5</td></tr>
</table>
</td><td>Lee, W. J., et al. (2005)</td></tr>
</table>

use§										

* Odds ratio adjusted for age (≤49, 50–59, 60–69, ≥70) and respondent type.
‡ Reference category: non-farmers.
§ Individual pesticides were grouped into herbicides (2,4,5-T, 2,4-D, alachlor, atrazine, bentazon, butylate, chloramben, cyanazine, dicamba, EPTC, glyphosate, metolachlor, metribuzin, paraquat, pendimethalin, propachlor, trifluralin) or chemical family: acetanilide herbicides (alachor, metolachlor, propachlor), nitrosatable pesticides (11 herbicides: 2,4,5-T, 2,4-D, atrazine, butylate, cyanazine, dicamba, EPTC, glyphosate, metolachlor, propachlor, trifluralin & 5 insecticides: bufencarb, carbaryl, carbofuran, famphur, nicotine)

Odds ratios (ORs) and 95 % confidence intervals (CIs) for brain cancer by ever-use of individual pesticides among adult male farmers:

	Overall				Self				Proxy			
		Cases				Cases				Cases		
	Controls	n	OR*	95 % CI	Controls	n	OR†	95 % CI	Controls	n	OR†	95 % CI
Non-farmers	112	49	1.0	Ref‡	40	20	1.0	Ref‡	72	29	1.0	Ref‡
Herbicides §	70	38	1.7	1.0–3.0	28	9	0.6	0.2–1.7	42	29	2.8	1.4–5.9
Acetanilide §	34	22	1.8	0.9–3.6	17	7	0.7	0.2–2.1	17	15	3.3	1.3–8.2
Nitrosatable pesticides use§	61	36	1.9	1.1-3.4	27	9	0.7	0.2-1.8	34	27	3.4	1.6-3.7
Metolachlor	14	6	1.2	0.4–3.6	8	2	0.4	0.1–2.3	6	4	2.6	0.6–11.3

* Odds ratio adjusted for age (≤49, 50–59, 60–69, ≥70) and respondent type.
† Odds ratio adjusted for age (≤49, 50–59, 60–69, ≥70).
‡ Reference category: non-farmers.
§ Individual pesticides were grouped into herbicides (2,4,5-T, 2,4-D, alachlor, atrazine, bentazon, butylate, chloramben, cyanazine, dicamba, EPTC, glyphosate, metolachlor, metribuzin, paraquat, pendimethalin, propachlor, trifluralin) or chemical family: acetanilide herbicides (alachor, metolachlor, propachlor)

<table>
<tr><td></td><td></td><td></td><td></td><td></td></tr>
<tr>
<td>Agricul-tural health study (AHS)</td>
<td>Metolachlor</td>
<td>Prospective study of pesticides applicators in Iowa and North Carolina
AHS cohort, but only private pesticides applicators were analysed
Cancer cases among the children of the cohort were both retrospectively and prospectively identified after parental enrolment
17,280 children in total included</td>
<td>No association between metolachlor and childhood cancer
Paternal[a] use of metolachlor and subsequent childhood cancer risk among 17,280 children of Iowa participants in the Agricultural Health Study
<table>
<tr><th>No. exposed (%)</th><th>No. exposed cases</th><th>OR[b] (95 % CI)</th></tr>
<tr><td>3,032 (18)</td><td>5</td><td>0.69 (0.26-1.84)</td></tr>
</table>
[a] Use of chemical by father before child’s birth. bAdjusted for child’s age at enrollment.</td>
<td>Flower, K. B., et al. (2004)</td>
</tr>
</table>

<table>
<tr>
<td>Hypothesis-generating study</td>
<td>Metolachlor, atrazine, simazine, alachlor, nitrates</td>
<td>Potential correlations of spatial patterns of four types of childhood cancer and the distribution of nitrates and herbicides (atrazine, simazine, alachlor and metolachlor) in groundwater were explored
Data from the Maryland Cancer Registry for bone and brain cancer, leukemia and lymphomas, for ages 0 – 17 years, during the years 1992-1998</td>
<td>Children potentially exposed to metolachlor had an increased risk for developing the analysed cancer types (Crude OR=1.54, 95 % CI, 1.1.4-2.07). The risk increased, when mixtures of the analysed herbicides (nitrazine, atrazine, simazine, alachlor) were considered.
Non-significant positive associations for metolachlor and bone cancer (Crude OR=2.26, 95 % CI, 0.97-5.24) / leukemia (Crude OR=1.48, 95 % CI,0.93-2.36) were observed.
Crude odds ratios (OR) for selected childhood cancers in Maryland (ages 0–17 years old) listed by potential exposure to all detectable concentrations of selected herbicides and nitrates. Range of OR for 95% confidence interval (CI) listed in parentheses:
<table>
<tr><th>Potential exposure</th><th>Crude odds ratio
(Range of 95 % CI)</th><th>P-value</th></tr>
<tr><td>Metolachlor</td><td>1.54 (1.14–2.07)</td><td>0.0061</td></tr>
<tr><td>Nitrate/Atrazine/Metolachlor</td><td>7.56(4.16–13.73)</td><td>< 0.0001</td></tr>
<tr><td>Nitrate/Metolachlor/Simazine/Alachlor</td><td>5.31 (2.84–9.93)</td><td>< 0.0001</td></tr>
</table>
Crude odds ratios (OR) for selected childhood cancers in Maryland (ages 0–17 years old) listed by potential exposure to all detectable concentrations of metolachlor; categorized by cancer type. Range of OR for 95 % confidence interval (CI) listed in parentheses:
<table>
<tr><th>Potential exposure</th><th>Crude odds ratio
(Range of 95 % CI)</th><th>P-value</th></tr>
<tr><td>Bone/metolachlor</td><td>2.26 (0.97–5.24)</td><td>0.0995</td></tr>
<tr><td>Leukemia/metolachlor</td><td>1.48 (0.93–2.36)</td><td>0.1256</td></tr>
</table>
</td>
<td>Thorpe, N. and A. Shirmohammadi (2005)</td>
</tr>
<tr>
<td>Case-control study</td>
<td>17 herbicides including metolachlor</td>
<td>Population based on case-control</td>
<td>No detection of metolachlor in house-dust of childhood ALL cases
Summary of house-dust metolachlor detection and concentrations for cases and controls: The Northern California Childhood Leukemia Study, 2001–2007:</td>
<td>Metayer, C., et al. (2013)</td>
</tr>
</table>

study in California (US).

Subset of Northern California Childhood Leukemia Study (NCCLS): 2001-2007, dust samples were collected in families with children <8years of age at the time of diagnosis

Analyte	Detection limit	Childhood ALL cases (n=252)				Controls (n=306)				
	(ng/g)	Detected (%)	Not detected (%)	Missing (%)[a]	Arithmetic mean[b] (SD) (ng/g)	Detected (%)	Not detected (%)	Missing(%)	Arithmetic mean[b] (SD)	P-value[d]
Metolachlor[c]	67.6	0 (0)	251 (100)	1 (<1)	NA	2 (<1)	304 (99)	0 (0)	0.5 (5.6)	0.2

[a] Missing because of insufficient dust or interferences in the chemical analyses.

[b] Analyte concentration in ng/g of dust.

[c] Hexane–acetone extraction method.

[d] P-value derived from Fisher's exact test comparing % detected between cases and controls.

Table 14: Summary table of other studies relevant for carcinogenicity

<table>
<tr><th>Type of study/data</th><th>Test substance, purity</th><th>Relevant information about the study (as applicable)</th><th>Observations</th><th>Reference</th></tr>
<tr>
<td>Cell proliferation assay in rat liver cells (in vivo)

No GLP</td>
<td>Metolachlor 97.3 %, batch number: P111072, S-enantiomeric content not reported</td>
<td>5 rats/sex/dose (Sprague Dawley, CRL, CD^{7}BR)

Oral (gavage)

Dose levels 0; 150; 500 and 1000 mg/kg bw

Positive control: dimethylnitrosamine (DMN; 10 mg/kg bw)

After dosing (0.3 – 2.3 h) animals were administered bromodeoxyuridine (BUdR) and sacrificed 72 h later.</td>
<td>500 mg/kg bw: DNA synthesis ↑: ~4-fold in males.

1000 mg/kg bw: liver weight ↑(+19 % in females, +9 % in males). DNA synthesis ↑: ~3-fold in females

Response to positive control in female animals markedly stronger

<table>
<tr><td>Dose (mg/kg b.w.)</td><td colspan="2">0</td><td colspan="2">150</td><td colspan="2">500</td><td colspan="2">1000</td><td colspan="2">DMN
15 mg/kg</td></tr>
<tr><td></td><td>M</td><td>F</td><td>M</td><td>F</td><td>M</td><td>F</td><td>M</td><td>F</td><td>M</td><td>F</td></tr>
<tr><td>liver weight (g) efficiency(%)</td><td>15.3</td><td>10.2</td><td>15.9</td><td>10.7</td><td>15.2</td><td>11.3</td><td>16.7 **
(↑9 %)</td><td>12.1**
(↑19 %)</td><td>14.1</td><td>10.6</td></tr>
<tr><td>L.I. (%)</td><td>3.1</td><td>3.7</td><td>3.0</td><td>n.a.</td><td>13.2 **
(↑425 %)</td><td>3.1</td><td>n.a.</td><td>10.6**
(↑286 %)</td><td>18**
(486 %)</td><td>33.5 **
(1080 %)</td></tr>
<tr><td colspan="11">histopathology</td></tr>
<tr><td>increase mitoses</td><td>0/5</td><td>1/5</td><td>0/5</td><td>0/5</td><td>2/5</td><td>0/5</td><td>0/5</td><td>0/5</td><td>5/5</td><td>4/4</td></tr>
<tr><td>glycogen storage</td><td>1/5</td><td>3/5</td><td>3/5</td><td>0/5</td><td>5/5</td><td>3/5</td><td>0/5</td><td>3/5</td><td>4/5</td><td>1/4</td></tr>
</table>
L.I: labelling index, n.a.: not analysed, ** significant at p≤0.01, c: four animals per group</td>
<td>Anonymous (17) 1994</td>
</tr>
</table>

<table>
<tr><th>Type of study/data</th><th>Test substance, purity</th><th>Relevant information about the study (as applicable)</th><th>Observations</th><th>Reference</th></tr>
<tr><td></td><td></td><td></td><td></td><td></td></tr>
<tr>
<td>In vitro/in vivo unscheduled DNA synthesis in rat hepatocytes

GLP</td>
<td>S-metolachlor, 95.6 %, B.n.:V4673/7; S-enantiomeric content: 84 %</td>
<td>3 rats/sex/dose/time point (Sprague Dawley,Tif:RAIf

Oral (gavage)

Dose levels 0; 500; 1500 and 5000 mg/kg b.w.(males) and 0; 500; 1500 and 3200 mg/kg b.w. (females)

Positive control: dimethylnitrosamine (DMNA, 15 mg/kg, 2h) or 4-acetylaminofluorene (4-AAF, 1000 mg/kg, 38h)

Animals were killed 2, 15 and 38 hours after dosing</td>
<td>Increase in DNA replication in male hepatocytes after 38 hours at 1500 mg/kg bw

Increase in DNA replication in female hepatocytes after 15 and 38 hours at 500 and 1500 mg/kg bw

No increase in unscheduled DNA synthesis
<table>
<tr><th colspan="10">Males</th></tr>
<tr><td>Dose (mg/kg b.w.)</td><td colspan="3">0</td><td colspan="3">500</td><td colspan="3">1500</td></tr>
<tr><td>treatment (h)</td><td>2</td><td>15</td><td>38</td><td>2</td><td>15</td><td>38</td><td>2</td><td>15</td><td>38</td></tr>
<tr><td>viability (%)</td><td>82</td><td>84</td><td>82</td><td>84</td><td>78</td><td>80</td><td>67</td><td>67</td><td>83</td></tr>
<tr><td>S-phase (%)</td><td>-</td><td>0.07</td><td>0.57</td><td>-</td><td>8</td><td>51</td><td>-</td><td>7</td><td>301</td></tr>
<tr><td>NGC (total)</td><td>0</td><td>-8</td><td>-</td><td>-7</td><td>-20</td><td>-</td><td>-3</td><td>-13</td><td>-</td></tr>
<tr><td>NGC (cells in repair)</td><td>27</td><td>32</td><td>-</td><td>33</td><td>44</td><td>-</td><td>32</td><td>33</td><td>-</td></tr>
<tr><td>% cells in repair</td><td>137</td><td>73</td><td>-</td><td>150</td><td>60</td><td>-</td><td>137</td><td>93</td><td>-</td></tr>
<tr><td colspan="10">Females</td></tr>
<tr><td>viability (%)</td><td>79</td><td>80</td><td>71</td><td>92</td><td>86</td><td>83</td><td>87</td><td>84</td><td>87</td></tr>
<tr><td>S-phase (%)</td><td>-</td><td>0.18</td><td>0.37</td><td>-</td><td>24</td><td>36</td><td>-</td><td>440</td><td>452</td></tr>
<tr><td>NGC (total)</td><td>0</td><td>0</td><td>-</td><td>10</td><td>-4</td><td>-</td><td>-3</td><td>2</td><td>-</td></tr>
<tr><td>NGC (cells in repair)</td><td>29</td><td>32</td><td>-</td><td>36</td><td>27</td><td>-</td><td>31</td><td>2</td><td>-</td></tr>
<tr><td>% cells in repair</td><td>140</td><td>83</td><td>-</td><td>290</td><td>137</td><td>-</td><td>190</td><td>130</td><td>-</td></tr>
</table>
NCG: mean (net) nuclear grain count, NGC>2: fraction of cells in repair

In the concomitant positive control at 38h, S-phase fraction increase was 27-fold (males) and 10-fold (females)</td>
<td>Anonymous (22), 1995b</td>
</tr>
</table>

Type of study/data	**Test substance, purity**	**Relevant information about the study (as applicable)**	**Observations**	**Reference**
28-day study. Replicative liver DNA synthesis assay, ultramorphological changes, biochemistry parameters No GLP	S-metolachlor, 95.6 %, batch number: V4673/7, S-enantiomeric content: 84 % & Metolachlor, 97.7 %; batch number: P111072	3 or 5 rats/sex/dose (Tif Ralf (SPF) Oral (dietary) Treatment for 28 days S-metolachlor: Dose levels of 0; 2.65; 24.5; 242; 426 mg/kg bw (males) and: 0; 2.73; 26.4; 257; 435 mg/kg bw (females) Metolachlor: Dose levels: 0; 265; 447 mg/kg b.w. (males) and: 0; 264; 433 mg/kg b.w. (females)	After 28 days: no increase of total number of hepatocellular nuclei or labelling index Moderate increase of smooth endoplasmatic reticulum for metolachlor and S-metolachlor CYP2B induction, and to a lesser extent CYP1A1 induction by metolachlor and S-metolachlor No positive control included Results in males (CGA77102 = S-metolachlor, CGA24705 = metolachlor):	Anonymous (35), 1995

Dose	P450 [nmol/g liver]	EROD [nmol/min/g liver]	PROD [nmol/min/g liver]	UDPGT [nmol/min/g liver]	GST [nmol/min/g liver]
Males					
Treatment Groups					
0 ppm	9.55 (0.61)	1.83 (0.39)	0.50 (0.19)	879 (85)	138 (15)
30 ppm CGA 77102	9.03 (2.83)	1.83 (1.00)	0.51 (0.29)	810 (277)	121 (29)
300 ppm CGA 77102	10.29 (1.18)	2.78 (0.47)	1.05 (0.35)	1095 (218)	119 (46)
3000 ppm CGA 77102	13.89* (3.36)	4.06* (1.54)	4.20*** (2.56)	1653** (428)	183 (77)
5000 ppm CGA 77102	12.64 (2.18)	4.83 ** (1.87)	4.91*** (2.04)	1729** (424)	179 (69)
3000 ppm CGA 24705	12.91 (2.62)	4.90** (2.11)	3.14*** (2.31)	1693** (520)	144 (36)
5000 ppm CGA 24705	14.56* (2.21)	5.33*** (0.64)	4.98*** (0.86)	2245*** (320)	180 (68)
Treatment/Recovery Groups					
0/0 ppm	8.92 (1.03)	2.51 (0.66)	0.46 (0.22)	1052 (232)	124 (11)
5000/0 ppm CGA 77102	8.97 (2.17)	2.52 (0.70)	0.62 (0.17)	956 (150)	111 (42)

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

5000/0 ppm CGA 24705	8.34 (1.44)	1.63 (0.28)	0.40 (0.14)	735 (151)	143 (12)

Results in females (CGA77102 = S-metolachlor, CGA24705 = metolachlor):

Dose	P450 [nmol/g liver]	EROD [nmol/min/g liver]	PROD [nmol/min/g liver]	UDPGT [nmol/min/g liver]	GST [nmol/min/g liver]
Females					
Treatment Groups					
0 ppm	7.14 (1.43)	1.32 (0.64)	0.07 (0.04)	625 (94)	122 (21)
30 ppm CGA77102	6.47 (1.37)	1.39 (0.55)	0.04 (0.01)	636 (103)	126 (26)
300 ppm CGA 77102	7.88 (0.78)	1.61 (0.26)	0.11 (0.04)	741 (60)	128 (26)
3000 ppm CGA 77102	9.02 (0.91)	2.97** (0.87)	2.15*** (0.41)	867** (78)	177* (20)
5000 ppm CGA 77102	9.68 (2.56)	3.27** (1.38)	4.34*** (2.16)	1027*** (236)	208** (34)
3000 ppm CGA 24705	9.20 (1.45)	3.09** (1.02)	1.71*** (0.64)	857** (115)	141 (36)
5000 ppm CGA 24705	11.29*** (0.88)	2.91* (0.96)	3.13*** (1.06)	1067*** (158)	188**(21
Treatment/Recovery Groups					
0/0 ppm	6.63 (1.04)	1.58 (0.85)	0.12 (0.01)	686 (67)	125 (35)
5000/0 ppm CGA 77102	5.79 (1.04)	1.09 (0.20)	0.07** (0.02)	622 (77)	113 (14)
5000/0 ppm CGA 24705	7.27 (0.90)	1.41 (0.36)	0.09 (0.03)	603 (93)	128 (25)
*: $p < 0.05$, **: $p < 0.01$; ****: $p < 0.001$ (two-sided Dunnett's test) (): standard deviation					

<table>
<tr><th>Type of study/data</th><th>Test substance, purity</th><th>Relevant information about the study (as applicable)</th><th>Observations</th><th>Reference</th></tr>
<tr><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>Assessment of hepatic cell proliferation, apoptosis and liver enzyme induction

No GLP</td><td>Metolachlor, 97.7 %, batch number: P.11072</td><td>15 female CD-Crl: CD (SD) rats

Oral (dietary)

Treatment for up to 3, 5, 7, 14, 28 and 60 days</td><td>PROD and BROD activities ↑ after 14 and 60 days , EROD and MROD ↑ after 60 days (to a lesser extent in comparison to PROD and BROD)

Protein levels of CYP2B and CYP3S ↑ after 14 and 60 days, CYP1A2 ↑ after 60 days

No positive control included
<table>
<tr><td>Dietary administration of 3000 ppm metolachlor</td><td>14 days</td><td>60 days</td></tr>
<tr><td>Mean PROD activity (% of control)</td><td>902***</td><td>1590***</td></tr>
<tr><td>Mean BROD activity (% of control)</td><td>1336***</td><td>1918***</td></tr>
<tr><td>Mean EROD activity (% of control)</td><td>100</td><td>193*</td></tr>
<tr><td>Mean MROD activity (% of control)</td><td>114</td><td>162*</td></tr>
</table>
* and ***: Statistically significantly different from control with p≤0.05 and p≤0.001, respectively.
<table>
<tr><td rowspan="2"></td><td colspan="2">14 Days</td><td colspan="2">60 Days</td></tr>
<tr><td>0 ppm</td><td>3000 ppm</td><td>0 ppm</td><td>3000 ppm</td></tr>
<tr><td>CYP1A1</td><td>n.d.</td><td>n.d.</td><td>n.d.</td><td>n.d.</td></tr>
<tr><td>CYP1A2</td><td>11143</td><td>11002</td><td>9572</td><td>17340</td></tr>
<tr><td>CYP2B</td><td>n.d.</td><td>20122</td><td>n.d.</td><td>26831</td></tr>
<tr><td>CYP3S</td><td>3391</td><td>14216</td><td>5758</td><td>19975</td></tr>
</table>
Units are relative area units derived from western blot band intensities. n.d.: Not detected. These data were not analysed for statistical significance. Data are group means.</td><td>Anonymous (27), 2006</td></tr>
</table>

<table>
<tr><th>Type of study/data</th><th>Test substance, purity</th><th>Relevant information about the study (as applicable)</th><th>Observations</th><th>Reference</th></tr>
<tr><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>CAR3 Transactivation assay

No GLP</td><td>S-metolachlor, 98.8 % w/w, batch number: CAB2H12058, 87.4 % S-enantiomeric content</td><td>CAR3 reporter constructs from humans, mice and rats
Positive control: CITCO, TCPOBOP and Clotrimazole</td><td>Activation of CAR3 from rat: 57-fold, mouse: 27-fold and human 9-fold

Activation of CAR3 by S-metolachlor in comparison to the used ‘positive controls’, direct-acting model substances (Clotrimazole, TCPOBOP, CITCO), was between 59 and 83 % (rat: 60 %, mouse: 59 %, human: 83 %).

Results for hCAR3:
<table>
<tr><th>Construct</th><th>Treatment</th><th>Normalised Luciferase activity</th><th>SD</th><th>Fold Change</th><th>SD</th><th>Statistical significance (p<0.01)</th></tr>
<tr><td>Empty Vector</td><td>DMSO</td><td>0.011601867</td><td>0.001689407</td><td>1.0000</td><td>0.1456</td><td></td></tr>
<tr><td></td><td>S-metolachlor 30µM</td><td>0.008282301</td><td>0.004001144</td><td>0.7139</td><td>0.3449</td><td>No</td></tr>
<tr><td>hCAR3</td><td>DMSO</td><td>0.006283901</td><td>0.002030466</td><td>1.0000</td><td>0.3231</td><td></td></tr>
<tr><td></td><td>PB 1mM</td><td>0.005458657</td><td>0.003216095</td><td>0.8687</td><td>0.5118</td><td>No</td></tr>
<tr><td></td><td>CITCO 5µM</td><td>0.064987884</td><td>0.025951624</td><td>10.3420</td><td>4.1299</td><td>Yes</td></tr>
<tr><td></td><td>S-metolachlor 1 µM</td><td>0.010589304</td><td>0.000771497</td><td>1.6851</td><td>0.1228</td><td>No</td></tr>
<tr><td></td><td>S-metolachlor 3 µM</td><td>0.017636257</td><td>0.000630748</td><td>2.8066</td><td>0.1004</td><td>Yes</td></tr>
<tr><td></td><td>S-metolachlor 10 µM</td><td>0.046978073</td><td>0.004127987</td><td>7.4759</td><td>0.6569</td><td>Yes</td></tr>
<tr><td></td><td>S-metolachlor 30 µM</td><td>0.054376733</td><td>0.002486991</td><td>8.6533</td><td>0.3958</td><td>Yes</td></tr>
</table></td><td>Anonymous (34), 2014,</td></tr>
</table>

Type of study/data	**Test substance, purity**	**Relevant information about the study (as applicable)**	**Observations**	**Reference**
			Results for mCAR and rCAR: (table below)	

Results for mCAR and rCAR:

Construct	Treatment	Normalised Luciferase activity	SD	Fold Change	SD	Statistical significance ($p<0.01$)
Empty Vector	DMSO	0.011601867	0.001689407	1.0000	0.1456	
	S-metolachlor 30 µM	0.008282301	0.004001144	0.7139	0.3449	No
mCAR3	DMSO	0.011443407	0.000769916	1.0000	0.0673	
	PB 1mM	0.023631748	0.002029814	2.0651	0.1774	Yes
	TCPOBOP 0.5 µM	0.518724797	0.020691201	45.3296	1.8081	Yes
	S-metolachlor 1 µM	0.096709989	0.004178678	8.4512	0.3652	Yes
	S-metolachlor 3 µM	0.171585113	0.005805368	14.9942	0.5073	Yes
	S-metolachlor 10 µM	0.281709139	0.014659835	24.6176	1.2811	Yes
	S-metolachlor 30 µM	0.307933035	0.027386899	26.9092	2.3932	Yes
rCAR3	DMSO	0.005454521	0.000447746	1.0000	0.0821	
	PB 1mM	0.032469909	0.001828858	5.9528	0.3353	Yes
	CLOT 10 µM	0.520540006	0.015926700	95.4328	2.9199	Yes
	S-metolachlor 1 µM	0.049952778	0.001797251	9.1581	0.3295	No
	S-metolachlor 3 µM	0.119770083	0.015447592	21.9579	2.8321	No
	S-metolachlor 10 µM	0.279788451	0.070675039	51.2948	12.9572	Yes
	S-metolachlor 30 µM	0.311426195	0.091897838	57.0951	6.8480	Yes

Type of study/data	Test substance, purity	Relevant information about the study (as applicable)	Observations	Reference
Enzyme and DNA synthesis induction in cultured female rat hepatocytes No GLP	S-metolachlor, 98.8 %, batch number: CAB2H12058, 87.4 % S-enantiomeric content	Sprague Dawley rats (out-bred Crl:CD(SD) Hepatocytes from two independent perfusions were pooled. Concentrations: 1, 5, 10, 20, 40 and 75 µM Exposure for 96 hours Positive control: phenobarbital sodium (PB; 10, 100 and 1000 µM) and epidermal growth factor (EGF)	No increase in PROD activity, BROD activity slightly increased up to 1.3-fold, Cell proliferation significantly increases (up to 1.9-fold) Phenobarbital induced increased cell proliferation (up to 1.64-fold) and increased PROD activity up to 2.8-fold as well as BROD activity up to 4.7-fold. (ATP as indicator for cytotoxicity)	Anonymous (10), 2014

Treatment	ATP (luminescence units released) [a]	S-phase labelling index (%) [b]	PROD (pmol resorufin/min/mg) [c]	BROD (pmol resorufin/min/mg) [c]
Vehicle control (0.5 % v/v DMSO)	99051 ± 7968 (100.0 ± 8.0)	6.49 ± 1.24 (100.0 ± 19.1)	0.405 ± 0.091 (100.0 ± 22.6)	2.42 ± 0.22 (100.0 ± 9.3)
PB 10 µM	77206 ± 3949** (78.0 ± 4.0)	10.39 ± 1.09** (160.0 ± 16.8)	0.488 ± 0.085 (120.5 ± 21.0)	3.50 ± 0.94 (144.5 ± 38.8)
PB 100 µM	89463 ± 7925 (90.3 ± 8.0)	10.59 ± 1.08** (163.1 ± 16.6)	0.921 ± 0.059** (227.2 ± 14.6)	7.08 ± 0.11** (292.3 ± 4.7)
PB 1000 µM	95601 ± 5474 (96.5 ± 5.5)	10.64 ± 0.85** (164.0 ± 13.1)	1.134 ± 0.057** (279.9 ± 14.1)	11.48 ± 0.79** (474.1 ± 32.6)
S-metolachlor 1 µM	93673 ± 7611 (94.6 ± 7.7)	11.88 ± 0.974** (183.0 ± 15.0)	0.350 ± 0.041 (86.4 ± 10.1)	2.77 ± 0.06 (114.6 ± 2.6)
S-metolachlor 5 µM	96460 ± 12271 (97.4 ± 12.4)	12.43 ± 1.54** (191.5 ± 23.7)	0.369 ± 0.054 (91.0 ± 13.3)	2.76 ± 0.12 (114.1 ± 4.9)
S-metolachlor 10 µM	89418 ± 5537* (90.3 ± 5.6)	12.34 ± 1.42** (190.1 ± 21.8)	0.398 ± 0.002 (98.2 ± 0.5)	3.23 ± 0.17** (133.7 ± 6.9)
S-metolachlor 20 µM	88500 ± 2530* (89.3 ± 2.6)	12.01 ± 2.03** (185.0 ± 31.2)	0.440 ± 0.082 (108.6 ± 20.2)	3.35 ± 0.29* (138.6 ± 12.2)
S-metolachlor 40 µM	93104 ± 3650 (94.0 ± 3.7)	9.73 ± 1.29** (149.9 ± 19.8)	0.448 ± 0.077 (110.5 ± 19.0)	3.12 ± 0.19* (128.7 ± 7.9)
S-metolachlor 75 µM	57907 ± 3394** (58.5 ± 3.4)	11.96 ± 0.70** (184.3 ± 10.8)	0.377 ± 0.092 (93.1 ± 22.7)	3.20 ± 0.21* (132.3 ± 8.5)

<table>
<tr><th>Type of study/data</th><th>Test substance, purity</th><th>Relevant information about the study (as applicable)</th><th>Observations</th><th>Reference</th></tr>
<tr><td></td><td></td><td></td><td>
<table>
<tr><td>EGF
25 ng/mL</td><td>-</td><td>26.23 ± 1.14**
(404.1 ± 17.6)</td><td>-</td><td>-</td></tr>
</table>
Values are mean ± SD. Values in parenthesis are mean % control ± SD. A, n = 6 per group, b n = 5 per group, c n = 3 per group. Statistically different from control *p<0.05; **p<0.01 (Student's t-test , 2-sided)
</td><td></td></tr>
<tr><td>Enzyme and DNA synthesis induction in cultured female human hepatocytes

No GLP</td><td>S-metolachlor, 98.8 %, batch number: CAB2H12058, 87.4 % S-enantiomeric content</td><td>Human female hepatocytes from one donor.

Concentrations: 1, 5, 10, 20, 40 and 75 µM

Exposure for 96 hours

Positive control: phenobarbital sodium (PB; 10, 100 and 1000 µM) and epidermal growth factor (EGF, 25 ng/ml)</td><td>
No effect on cell proliferation. No effect on CYP enzyme activity. (ATP as indicator for cytotoxicity)
<table>
<tr><th>Treatment</th><th>ATP (luminescence units released) [a]</th><th>S-phase labelling index (%) [b]</th><th>PROD (pmol resorufin/min/mg) [c]</th><th>BROD (pmol resorufin/min/mg) [c]</th></tr>
<tr><td>Vehicle control
(0.5 % [v/v] MSO)</td><td>110849 ± 2851
(100.0 ± 2.6)</td><td>0.31 ± 0.06
(100.0 ± 18.8)</td><td>0.133 ± 0.052
(100.0 ± 39.1)</td><td>0.484 ± 0.073
(100.0 ± 15.0)</td></tr>
<tr><td>PB 10 µM</td><td>100370 ± 3668**
(90.5 ± 3.3)</td><td>0.36 ± 0.14
(117.1 ± 45.2)</td><td>0.100 ± 0.034
(75.0 ± 25.6)</td><td>0.758 ± 0.047**
(156.8 ± 9.7)</td></tr>
<tr><td>PB 100 µM</td><td>106086 ± 7328
(95.7 ± 6.6)</td><td>0.35 ± 0.13
(114.9 ± 41.4)</td><td>0.238 ± 0.044
(178.7 ± 33.0)</td><td>0.734 ± 0.021**
(151.9 ± 4.4)</td></tr>
<tr><td>PB 1000 µM</td><td>93842 ± 9505**
(84.7 ± 8.6)</td><td>0.27 ± 0.07
(86.3 ± 24.3)</td><td>0.298 ± 0.057*
(223.4 ± 43.2)</td><td>1.487 ± 0.252**
(307.5 ± 52.0)</td></tr>
<tr><td>S-metolachlor
1 µM</td><td>99434 ± 5363**
(89.7 ± 4.8)</td><td>0.31 ± 0.12
(99.3 ± 38.1)</td><td>0.225 ± 0.019*
(169.0 ± 14.5)</td><td>0.226 ± 0.058**
(46.8 ± 11.9)</td></tr>
<tr><td>S-metolachlor
5 µM</td><td>101606 ± 7659*
(91.7 ± 6.9)</td><td>0.35 ± 0.05
(113.5 ± 15.9)</td><td>0.087 ± 0.030
(65.1 ± 22.9)</td><td>0.357 ± 0.091
(73.9 ± 18.8)</td></tr>
<tr><td>S-metolachlor
10 µM</td><td>97038 ± 3326**
(87.5 ± 3.0)</td><td>0.43 ± 0.15
(140.6 ± 48.8)</td><td>0.109 ± 0.031
(81.9 ± 23.6)</td><td>0.312 ± 0.117
(64.4 ± 24.1)</td></tr>
<tr><td>S-metolachlor
20 µM</td><td>92539 ± 5387**
(83.5 ± 4.9)</td><td>0.39 ± 0.07
(126.4 ± 23.9)</td><td>0.075 ± 0.020
(56.2 ± 14.7)</td><td>0.205 ± 0.110*
(42.4 ± 22.8)</td></tr>
<tr><td>S-metolachlor
40 µM</td><td>83329 ± 4112**
(75.2 ± 3.7)</td><td>0.35 ± 0.04
(114.5 ± 13.1)</td><td>0.082 ± 0.007
(61.3 ± 5.6)</td><td>0.167 ± 0.036**
(34.5 ± 7.3)</td></tr>
<tr><td>S-metolachlor
75 µM</td><td>48926 ± 4280**
(44.1 ± 3.9)</td><td>0.21 ± 0.07*
(67.3 ± 22.4)</td><td>0.077 ± 0.024
(58.1 ± 17.8)</td><td>0.096 ± 0.004**
(19.9 ± 0.8)</td></tr>
<tr><td>EGF</td><td>-</td><td>2.99 ± 0.21**</td><td>-</td><td>-</td></tr>
</table>
</td><td>Anonymous (11), 2014</td></tr>
</table>

<table>
<tr><th>Type of study/data</th><th>Test substance, purity</th><th>Relevant information about the study (as applicable)</th><th>Observations</th><th>Reference</th></tr>
<tr><td></td><td></td><td></td><td>
<table>
<tr><td>25 ng/mL</td><td></td><td>(969.9 ± 67.2)</td><td></td><td></td></tr>
<tr><td colspan="5">Values are mean ± SD. Values in parenthesis are mean % control ± SD. [a] n = 6 per group, [b] n = 5 per group, [c] n = 3 per group
Statistically different from control *$p<0.05$; **$p<0.01$ (Student's t-test , 2-sided).</td></tr>
</table>
</td><td></td></tr>
<tr><td>Enzyme and DNA synthesis induction in cultured female human hepatocytes

No GLP</td><td>S-metolachlor, 98.1 % w/w,</td><td>Human female hepatocytes from two donors

Concentrations: 1, 5, 10, 20, 40 and 75 µM

Positive control: phenobarbital sodium (PB; 10, 100 and 1000 µM) and epidermal growth factor (EGF, 25 ng/ml)

Exposure for 96 hours</td><td>
No induction of analysed CYP enzyme activity (PROD & BROD)

No induction of cell proliferation

positive control (phenobarbital sodium salt) did not show an increase in PROD and signs of cytotoxicity were seen (ATP ↓)

no analysis of CYP enzymes, which could give insight in involvement of other nuclear receptors than CAR

Donor 1:
<table>
<tr><th>Treatment</th><th>ATP (luminescence units released)[A]</th><th>S-phase labelling index (%)[B]</th><th>PROD (pmol resorufin/min/mg)[C]</th><th>BROD (pmol resorufin/min/mg)[D]</th></tr>
<tr><td>Vehicle control (0.1 % [v/v] DMSO)</td><td>141825 ± 5596
(100.0 ± 3.9)</td><td>0.24 ± 0.04
(100.0 ± 16.8)</td><td>0.192 ± 0.045
(100.0 ± 23.6)</td><td>0.382 ± 0.106
(100.0 ± 27.8)</td></tr>
<tr><td>PB
10 µM</td><td>163945 ± 15690***
(115.6 ± 11.1)</td><td>0.23 ± 0.03
(93.3 ± 12.5)</td><td>0.129 ± 0.078
(67.0 ± 40.8)</td><td>0.409 ± 0.061
(106.9 ± 16.0)</td></tr>
<tr><td>PB
100 µM</td><td>153081 ± 7604
(107.9 ± 5.4)</td><td>0.18 ± 0.04
(72.6 ± 16.8)</td><td>0.126 ± 0.099
(65.4 ± 51.4)</td><td>0.500 ± 0.084
(130.7 ± 22.1)</td></tr>
<tr><td>PB
1000 µM</td><td>132696 ± 4898
(93.6 ± 3.5)</td><td>0.21 ± 0.06
(85.0 ± 25.9)</td><td>0.199 ± 0.029
(103.5 ± 15.1)</td><td>0.749 ± 0.224
(195.9 ± 58.7)**</td></tr>
<tr><td>S-metolachlor
1 µM</td><td>151941 ± 7888
(107.1 ± 5.6)</td><td>0.20 ± 0.03
(84.4 ± 13.7)</td><td>0.203 ± 0.035
(105.8 ± 18.3)</td><td>0.429 ± 0.045
(112.2 ± 11.9)</td></tr>
<tr><td>S-metolachlor
5 µM</td><td>148163 ± 6318
(104.5 ± 4.5)</td><td>0.29 ± 0.05
(120.3 ± 21.0)</td><td>0.178 ± 0.048
(92.7 ± 24.9)</td><td>0.388 ± 0.061
(101.5 ± 15.9)</td></tr>
<tr><td>S-metolachlor
10 µM</td><td>153680 ± 9124
(108.4 ± 6.4)</td><td>0.23 ± 0.06
(96.4 ± 25.2)</td><td>0.167 ± 0.057
(86.9 ± 29.7)</td><td>0.307 ± 0.005
(80.4 ± 1.2)</td></tr>
<tr><td>S-metolachlor
20 µM</td><td>154326 ± 8528
(108.8 ± 6.0)</td><td>0.25 ± 0.04
(102.7 ± 16.5)</td><td>0.152 ± 0.033
(79.4 ± 17.0)</td><td>0.369 ± 0.082
(96.5 ± 21.6)</td></tr>
</table>
</td><td>Anonymous (5), 2019</td></tr>
</table>

S-metolachlor 40 µM	128301 ± 8685 (90.5 ± 6.1)	0.26 ± 0.04 (108.8 ± 16.1)	0.106 ± 0.010 (55.2 ± 5.2)	0.203 ± 0.040 (53.1 ± 10.5)
S-metolachlor 75 µM	91310 ± 5442*** (64.4 ± 3.8)	#	0.116 ± 0.030 (60.7 ± 15.6)	0.211 ± 0.067 (55.3 ± 17.6)
EGF 25 ng/ml	-	1.09 ± 0.08 (450.1 ± 33.2)***	-	-

Donor 2:

Treatment	ATP (luminescence units released)^{A}	S-phase labelling index (%)^{B}	PROD (pmol resorufin/min/mg)^{C}	BROD (pmol resorufin/min/mg)^{D}
Vehicle control (0.1 % [v/v] DMSO)	264108 ± 26706 (100.0 ± 10.1)	0.09 ± 0.03 (100.0 ± 36.7)	0.174 ± 0.055 (100.0 ± 31.7)	0.978 ± 0.127 (100.0 ± 13.0)
PB 10 µM	259361 ± 19415 (98.2 ± 7.4)	0.09 ± 0.03 (101.0 ± 37.6)	0.179 ± 0.047 (103.0 ± 26.9)	1.072 ± 0.106 (109.6 ± 10.8)
PB 100 µM	270500 ± 29342 (102.4 ± 11.1)	0.07 ± 0.00 (82.7 ± 1.4)	0.170 ± 0.037 (97.8 ± 21.5)	1.423 ± 0.345 (145.5 ± 35.3)*
PB 1000 µM	251500 ± 18783 (95.2 ± 7.1)	0.10 ± 0.04 (116.8 ± 46.3)	0.224 ± 0.083 (128.9 ± 47.9)	2.017 ± 0.057 (206.2 ± 5.8)***
S-metolachlor 1 µM	270560 ± 29478 (102.4 ± 11.2)	0.09 ± 0.03 (99.6 ± 35.8)	0.186 ± 0.047 (107.2 ± 27.1)	0.940 ± 0.042 (96.2 ± 4.3)
S-metolachlor 5 µM	285569 ± 28434 (108.1 ± 10.8)	0.09 ± 0.03 (100.7 ± 37.7)	0.182 ± 0.032 (104.8 ± 18.3)	1.071 ± 0.120 (109.5 ± 12.3)
S-metolachlor 10 µM	257934 ± 30972 (97.7 ± 11.7)	0.07 ± 0.00 (82.7 ± 1.2)	0.163 ± 0.022 (94.2 ± 12.4)	1.229 ± 0.019 (125.7 ± 2.0)
S-metolachlor 20 µM	244527 ± 28360 (92.6 ± 10.7)	0.09 ± 0.03 (100.4 ± 36.8)	0.155 ± 0.033 (89.2 ± 19.2)	1.251 ± 0.099 (127.9 ± 10.1)
S-metolachlor 40 µM	198703 ± 16272 (75.2 ± 6.2)***	0.09 ± 0.03 (98.8 ± 37.09)	0.187 ± 0.044 (107.7 ± 25.1)	1.100 ± 0.063 (112.5 ± 6.5)
S-metolachlor 75 µM	104011 ± 11863 (39.4 ± 4.5)***	#	0.135 ± 0.032 (77.8 ± 18.7)	0.914 ± 0.205 (93.5 ± 20.9)
EGF 25 ng/ml	-	0.65 ± 0.05 (739.2 ± 55.7) ***	-	-

A: Values are Mean ± SD. Values in parenthesis are mean % control ± SD; n = 6 per group. One way ANOVA was performed on the results, followed by a Dunnett's multiple comparison test *** statistically different from control *** p<0.001.

<table>
<tr><th>Type of study/data</th><th>Test substance, purity</th><th>Relevant information about the study (as applicable)</th><th>Observations</th><th>Reference</th></tr>
<tr><td></td><td></td><td></td><td>B: Values are Mean ± SD. n = 5 per group. Statistical analysis was performed using a one way analysis of variance (DMSO control compared to S-metolachlor or PB) or a 2-tailed Student's t-test (DMSO control compared to EGF); *** statistically different from control p<0.001. # not counted due to cytotoxicity.
C: Values are Mean ± SD. n = 3 per group. Statistical analysis was performed using a one-way analysis of variance followed by a Dunnett's multiple comparison test. No statistically significant differences were observed.
D: Values are Mean ± SD. n = 3 per group. Statistical analysis was performed using a one way analysis of variance followed by a Dunnett's multiple comparison test; * statistically different from control p<0.05; ** p<0.01; *** p<0.001.</td><td></td></tr>
<tr><td>Comparative study of human and mouse pregnane X receptor agonistic activity

No GLP</td><td>Metolachlor, purity >97 %</td><td>COS-7 simian kidney cells, expression plasmids of pSG5-hPXR and pSG5-mPXR encoding the full-length receptor protein
Positive controls: Rifampicin and PCN</td><td>Metolachlor is an agonist of human PXR as well as of mice PXR
<table>
<tr><td>Compound</td><td>hPXR assay</td><td></td><td>mPXR assay</td><td></td></tr>
<tr><td></td><td>REC$_{20}$ [a] (M)</td><td>RLA[b] (%)</td><td>REC$_{20}$ [a] (M)</td><td>RLA[b] (%)</td></tr>
<tr><td>Rifampicin</td><td>4.3 × 10^{-7}</td><td>100[c]</td><td>N.D.[d]</td><td></td></tr>
<tr><td>PCN</td><td>N.D.</td><td></td><td>5.7 × 10^{-8}</td><td>100[e]</td></tr>
<tr><td>Metolachlor</td><td>5.0 × 10^{-7}</td><td>81</td><td>2.7 × 10^{-6}</td><td>32</td></tr>
</table>
[a] 20 % relative effective concentration; the concentration of the test compound showing 20 % of the agonistic activity of 1×10^{-5} M rifampicin via hPXR, or 1×10^{-5} M PCN via mPXR. Each REC20 value represents the mean of three independent experiments.
[b] Relative luciferase activity; percentage response at a concentration of 1×10^{-5} M with 100 % activity defined as the activity achieved with 1×10^{-5} M rifampicin or 1×10^{-5} M PCN. Each RLA value is expressed as mean from at least three independent experiments performed in triplicate.
[c] RLA of rifampicin for hPXR is represented as the activity at a concentration of 1×10^{-5} M.
[d] Not detectable (no effect or REC20 >1×10^{-5} M).
[e] RLA of PCN for mPXR is represented as the activity at a concentration of 1×10^{-5} M.</td><td>Kojima, 2011</td></tr>
</table>

<table>
<tr><th>Type of study/data</th><th>Test substance, purity</th><th>Relevant information about the study (as applicable)</th><th>Observations</th><th>Reference</th></tr>
<tr><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>Screening assay for human CAR activators
No GLP</td><td>Metolachlor, purity not specified</td><td>C3A hepatoma cells
reporter assays for hAhR, hCAR and hPXR
Positive controls: FL81, rifampicin and omeprazole</td><td>Activation of human CAR and PXR by metolachlor
The relative extent of receptor activation (Emax) and selectivity for best hCAR activators identified in the screening process
<table>
<tr><td></td><td></td><td colspan="3">Emax (%)</td><td colspan="2">Selectivity Ratio</td></tr>
<tr><td>Compound name</td><td>µM</td><td>hCAR</td><td>hPXR</td><td>hAhR</td><td>hCAR/hPXR</td><td>hCAR/hAhR</td></tr>
<tr><td>Metolachlor</td><td>10</td><td>42.29±5.46*</td><td>53.52±2.00*</td><td>5.91±0.96</td><td>0.79</td><td>7.15</td></tr>
<tr><td>FL81</td><td>10</td><td>100*</td><td>26.67±0.29*</td><td>1.71±0.11</td><td>3.75</td><td>58.48</td></tr>
<tr><td>Rifampicin</td><td>10</td><td>25.80±3.15</td><td>100*</td><td>3.80±0.23</td><td>0.26</td><td>6.79</td></tr>
<tr><td>Omeprazole</td><td>10</td><td>18.75±3.05</td><td>23.33±4.60</td><td>100*</td><td>0.80</td><td>0.19</td></tr>
</table>The data is presented as relative fold-activation when positive control is set as 100, Mean ± S.E.M. (n = 3). * p<0.05 vs. vehicle (DMSO) control.</td><td>Kuelbeck, 2011</td></tr>
<tr><td>Screening assay for arylhydrocarbon receptor agonistic activity
No GLP</td><td>Metolachlor, purity > 95 %</td><td>Mouse hepatoma Hepa1c1c7 cells
hAhR-reporter plasmid</td><td>No activation of human AhR in vitro</td><td>Takeuchi, 2008</td></tr>
</table>

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

Animal studies:

No long-term studies are available with S-metolachlor. As bridging from metolachlor to S-metolachlor is accepted, the results from the long-term studies conducted with metolachlor are taken to conclude on S-metolachlor to avoid further animal studies. Two studies on chronic toxicity and carcinogenicity of metolachlor in rats (Anonymous (39), 1983) and mice (Anonymous (38), 1982) are available. However, the study conducted in mice was considered not acceptable due to deviations (i.e. too high mortality). Body weight loss was observed in the long-term studies in both rats and mice at the top dose. No tumours were observed in the surviving animals of the not acceptable mice study. However, in rats, liver was shown to be the target organ. In females and males total incidences of foci (sum of eosinophilic, clear and basophilic foci) were statistically significant increased at the top dose of 150 mg/kg bw/d. Also the number of animals with foci was increased in both sexes, albeit only for females statistically significant. For the dose-dependent increase of neoplastic nodules a positive trend was observed in both sexes: incidences for control and 3 treatment groups: m: 0/0/0/4 (6.7%), f: 0/0/1/4 (6.7%) (Cochrane-Armitage, $p < 0.01$). Hepatocellular carcinoma showed also a positive trend in females (incidences for control and 3 treatment groups: f: 0/0/0/2 (3.3%), Cochrane-Armitage, $p < 0.01$) and the combined incidence of “nodules + carcinoma” was statistically significant increased in females at the top dose of 150 mg/kg bw/d (incidence for control and 3 treatment groups: 0/0/1/6). Also in males a dose-dependent increase of the rate of “nodules + carcinoma” was observed ((incidence for control and 3 treatment groups: 2/1/3/6, Cochrane-Armitage, $p < 0.01$) and overall 10% of males and females were affected at the top dose. (c.f. Table 15) Incidences of neoplastic nodules and carcinomas for both sexes at 150 mg/kg bw/d were above the HCD, which consisted of only two control groups from the same study. Reported incidences of the two historical control groups were as follows: proliferative foci – females: 1/47 (2.1%), 0/46; males: 0/45, 2/45 (4.4%) and hepatocellular carcinoma: females: 0/47, 1/46 (2.2%); males: 0/45, 1/45 (2.2%) (c.f. Table 16) It should be mentioned that the HCD was of questionable quality as it consisted of only two control groups from the same study. Besides the original evaluation of tumour incidences in the liver, also a re-evaluation is available. In the re-evaluation, also an increase of total nodules and carcinoma was reported. No reasoned arguments for the re-evaluation were presented, but, nonetheless, it confirmed the previous outcome.

Additional neoplastic findings at the top dose of 150 mg/kg bw/d in females were a dose-dependent and statistically significant increase of adenoma (incidences for control and 3 treatment groups: 11/ 20/ 20/ 31) as well as of carcinoma (incidence for control and 3 treatment groups: 0/ 1 (3.7 %) /1 (3.7 %) /5 (12.8 %)) of the pituitary (no data from historical controls available).

Follicular cell adenoma of the thyroid (incidence for control and 3 treatment groups: 0/ 0/ 2 (3.5 %) /3 (5 %)) were also increased at the highest dose in females (Cochrane-Armitage, $p < 0.05$) As historical control data only one study with two groups of 46 and 47 animals was provided; incidences of 0 and 1, corresponding to a maximum of 2.1 %, were reported. The maximum of this low quality HCD is exceeded by the the observed incidences at the two upper dose levels.

Table 15: Tumour incidences pituitary, brain, thyroid and liver (original evaluation from the study report)

Dose (ppm)	**0**		**30**		**300**		**3000**	
	M	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Pituitary								
– number examined (terminal sacrifice)	32	25	32	27	34	31	27	39
Adenoma (not otherwise	18	11	22	20	15	20	19	31$^{\#**}$

Dose (ppm)	**0**		**30**		**300**		**3000**	
specified)								
Carcinoma	0	0	1	1 (3.7 %)	0	1 (3.2 %)	0	$5^{\#}$ (12.8 %)
Brain								
– number examined (terminal sacrifice)	33	33	34	30	25	29	34	40
Invasive carcinoma: pituitary	0	0	0	2	0	2 (6.9 %)	$1^{\#}$	4 (10 %)
Thyroid								
– number examined (terminal kill & died on test/moribund)	58	57	58	59	57	57	59	60
Adenoma: clear cell	4	4	3	2	5	2	2	7
Carcinoma: clear cell	1	2	1	0	0	1	1	1
Adenoma: follicular cell	0	0	3 (5.2 %)	0	3 (5.3 %)	2 (3.5 %)	1 (1.7 %)	$3^{\#}$ (5 %)
Liver								
- Number examined (terminal kill & died on test/moribund)	59	60	59	60	60	60	60	60
Foci of cellular alteration								
- eosinophilic	10	4	15	7	14	5	21	23*
- clear	6	4	12	6	11	9	9	12
- basophilic	5	7	5	5	0	10	5	11
Total incidences foci[a]	21	15	32	18	25	24	35^{*}	46^{*}
Total number of animals with foci	19 (32.2 %)	13 (21.7 %)	24 (40.7 %)	15 (25 %)	22 (36.7 %)	18 (30 %)	29 (48.3 %)	34* (56.7 %)
Proliferative foci (neoplastic nodules)	0	0	0	0	0	1 (1.7%)	$4^{\#\#}$ (6.7 %)	$4^{\#\#}$ (6.7 %)
Hepatocellular carcinoma	2 (3.4 %)	0	1 (1.7 %)	0	3 (5 %)	0	2 (3.3 %)	$2^{\#\#}$ (3.3 %)
Total nodules+carcinoma (%)	2 (3.4 %)	0	1 (1.7 %)	0	3 (5 %)	1 (1.7 %)	$6^{\#\#}$ (10 %)	$6^{**\#\#}$ (10 %)

[a]: foci of any type (eosinophilic+clear+basophilic), statistical significance at $p<0.05$:*Fisher's exact test ,$^{\#}$Cochrane-Armitage Trend-Test, one-sided], at $p<0.01$, **Fisher's exact test, $^{\#\#}$Cochrane-Armitage Trend-Test, one-sided

Table 16: Historical control data – combination of animals died on test/moribund and terminal sacrifice (based on data from only one available study (1982). In the eight month of the study an outbreak of Sialodacroadenitis virus occurred, according to the study director without unusual findings.

	Control 1		**Control 2**	
	M	**F**	**M**	**F**
Liver lesions				
Number of organs examined	45	47	45	46

	Control 1		Control 2	
Proliferative foci	0	1 (2.1 %)	2 (4.4 %)	0
Hepatocellular Carcinoma	0	0	1 (2.2 %)	1 (2.2 %)
Survival	36 %	49 %	62 %	47 %
Thyroid				
Number of organs examined	43	47	45	46
Follicular cell adenoma	2 (4.7 %)	1 (2.1 %)	1 (2.2 %)	0

Nasal turbinates have been shown target organs for the structurally similar chloroacetanilide Alachlor. It was investigated whether metolachlor had similar tumour-promoting characteristics. In contrast to Alachlor, which induced a marked and dose-related increase of nasal turbinate tumours, rats treated with metolachlor exhibited no significant increase of malignant tumours when performing a group-wise comparison to controls. The incidence of observed adenocarcinoma was 2/69 males or 1/59 males in the group exposed to 3000 ppm in the original report and the re-evaluation, respectively. Nevertheless a positive trend (Cochrane-Armitage, $p<0.05$) was observed and the incidence in the high dose group was above the historical control data, where no neoplastic findings in 2 examined nasal turbinates out of nearly 400 animals were observed (c.f. Table 17, Table 18). It should be mentioned that in animals from the historic control data base, only those with macroscopic lesions were examined, therefore the informative value of the provided HCD for nasal turbinate tumours might be limited.

Overall, a NOAEL for carcinogenicity was set at 15 mg/kg bw/d.

Table 17: Nasal tumour incidence – original evaluation and re-evaluation

Original report				Re-evaluation[2]		
Males						
Feeding Level ppm	Adenomatous Polyp	Adenocarcinoma	Fibroadenoma	Polypoid Adenoma[3]	Adenocarcinoma[4]	Neurofibro-sarcoma[5]
0	1/67[1]	0/67	0/67	1/57	0/57	0/57
30	0/59	0/59	0/59	0/59	0/59	0/59
300	0/53	0/53	0/53	0/53	0/53	0/53
3000	0/69	2/69[#]	1/69	1/59	1/59[#]	1/59
Females						
Feeding Level ppm	Adenoma Papilloma	Squamous cell Papilloma	Odontoma Adenoma	Adenoma Papilloma[3]	Squamous Papilloma[6]	Odontoma[7]
0	0/67	0/67	1/67	0/57	0/57	1/57
30	0/58	1/58	0/58	0/57	0/57	0/57
300	1/59	0/59	0/59	1/59	0/59	0/59
3000	0/69	1/69	0/69	0/59	1/59	0/59

[1] including animals of interim sacrifice
[2] animals of 1-year interim sacrifice were not re-examined for males
[3] Tumours of this type associated with the respiratory epithelium
[4] Tumours of this type associated with nasal glands
[5] Tumour associated with peripheral nerve
[6] Tumours of this type associated with buccal mucosa
[7] Tumour associated with teeth
[#] statistically different at $p<0.05$ level, ,[#]Cochrane-Armitage Trend-Test, one-sided

Table 18: **Historical Control Data for Nasal turbinate tumour incidence**

	104-Week Studies[a]			
	Covance - Madison[b,]			
	Males		**Females**	
Total number of animals examined	397		398	
Nasal Turbinates Number examined [c]	2		2	
	Total Incidence (%)	Range (%)[d]	Total Incidence (%)	Range (%)
Neoplastic findings	0/2 (0.0)	0.0 - 0.0	0/2 (0.0)	0.0 - 0.0

a Historical control data for this table was not reviewed by QA and is not GLP compliant.
b Data from 6 studies conducted at Covance - Madison (formerly Raltech Scientific Services, Inc and Hazleton Laboratories America, Inc) from June 1975 through June 1987.
c In the six Covance - Madison studies with microscopic data available, nasal turbinates were not required to be examined (per protocol) and were only examined when macroscopic lesions were present.
d Range (%) represents the lowest and highest group incidence across studies.

Epidemiological studies, human data:

Epidemiological studies are a source for human information on carcinogenicity of metolachlor (c.f. Table 13). The largest epidemiological study of pesticide exposure and health outcomes is the Agricultural Health Study (AHS), which was conducted in the U.S. Federal States of Iowa and North Carolina. The AHS is a prospective cohort study, composed of about ~ 57,000 licensed private and commercial pesticide applicators. Recruitment of the cohort occurred between 1993 and 1997 and a plenty of publications have resulted from the data of this study. Rusiecki et al. (2006,) evaluated cancer incidences from applicators exposed to metolachlor (n=22,781) of the period 1993-2002 of the AHS. Low-metolachlor exposed applicators were taken as the referent and two different lifetime metolachlor exposure metrics were investigated. Only for the metric ”lifetime exposure days”, but not for the ”intensity weighted lifetime days exposure” an increased risk for lung cancer (RR = 2.37; 95 % CI, 0.97-5.82, p-trend = 0.03) was observed in the highest category ($T3_U$) of use. Among a total number of 680 cases of all cancers, 12 cases of lung cancer in the $T3_U$-category were reported, 46 cases for all tertiles. Silver et al., (2015,) evaluated cancer incidences from the AHS for a longer follow-up period through 2010 (North Carolina) or 2011 (Iowa) for applicators exposed to metolachlor (n=26,505) and saw no increase for lung cancer in any of the exposure quartiles. However, for liver cancer and follicular cell lymphoma positive associations were reported and a positive trend for liver cancer was observed for both lifetime days (p<0.01) and intensity-weighted lifetime days (p=0.03) at higher categories of use (with unexposed person-time as the referent): for Q3 and Q4 the RR were 3.06 (95 % CI, 1.05 – 8.9) and 3.99 (95 % CI, 1.43 – 11.1) for lifetime days. For intensity-weighted lifetime days an RR of 3.14 (95 % CI, 1.11 – 8.88) and 3.18 (95 % CI, 1.1 – 9.22) was reported for the two highest quartiles of use. For follicular cell lymphoma also a positive trend was observed (p=0.03, lifetime days and p= 0.04 intensity-weighted lifetime days) and significant increases were reported. Alavanja et al. (2004) analysed a similar AHS-period as Rusiecki et al. (2006) and again, a significantly increased risk for lung cancer was identified, based on data obtained in the AHS between 1993 and 2001. For the highest category of lifetime exposure days (> 457) an Odds ratio of 4.1 (95 % CI, 1.6 – 10.4) was reported when “no exposure” was the referent group and an OR of 5.0 (95 % CI, 1.7 – 14.9) when low exposure was taken as referent group. Positive trends were seen for both referent group analyses.

Andreotti et al. (2009) analysed association of pesticides and pancreatic cancer in the AHS cohort (1993 – 2004) and found no effect of metolachlor.

When the risk of colorectal cancer in the AHS cohort (1993-2002) was analysed by Lee et al. (2007), no increased risk for metolachlor users regarding colorectal cancer (total incidences as well as separated incidences for colon and rectum) was seen. A positive association for metolachlor use and colon cancer was observed, when the body weight of users was taken into account (AHS, 1993 -2005): at a BMI of 30 (= obese) or above the HR was significantly elevated (HR = 2.91, 95% CI, 1.42 - 5.96) (Andreotti et al., 2010).

For prostate cancer, a decreased risk for metolachlor users was observed according to the assessment of the data from the AHS cohort (1993-2002) by Rusiecki et al. (2006). Koutros et al. (2010) reported an OR of 1.47 (95 % CI, 1.08-2) for the risk for prostate cancer for highly exposed users of the AHS, who already bear a genetic risk factor for prostate cancer. However, Barry et al. (2011) observed a significant negative association of high metolachlor use and prostate cancer among the entire AHS cohort, when genetic risk factors were not taken into account (OR=0.77, 95 % CI, 0.6 – 0.99, p=0.0.2). The publication by De Ross et al. (2003) based on a different data set than the AHS and focussed on non-Hodgkin lymphoma (NHL). For NHL and metolachlor no significant association was observed, ORs were decreased.

Lee et al. (2005) used telephone interviews to analyse pesticide exposure and risk of glioma. Data were presented for metolachlor itself and metolachlor as component of a herbicide group, an acetanilide group and a nitrosatable pesticides group. For all groups no significant association was reported to develop glioblastoma multiforme, astrocytoma or other glioma, however, the OR was about two-fold increased for glioblastoma multiforme in the herbicide group and for glioblastoma multiforme and astrocytomas in the acetanilide group as well as in the nitrosatable pesticides group. Differing ORs for the association of metolachlor as well as for the analysed groups of pesticides and brain cancer for self-responders and proxy responders were reported. Proxy responders showed in all cases the strongest positive associations, while for self-responders inversed ORs were reported. The proxy-responder OR for brain-cancer for metolachlor was 2.6 (95 %CI, 0.6-11.3), while an OR=0.4 (95 %CI, 0.1-2.3) for self-responder was observed. Overall, the OR was 1.2 (95 %CI; 0.4-3.6). The authors are aware, that the observed higher positive associations for proxy responders raise concern of recall bias. Three studies are available regarding a potential association of childhood cancer and use of metolachlor. Flower et al. (2004) reported no positive association for paternal use of metolachlor and childhood cancer among private pesticides applicators of the AHS cohort (OR=0.69, 95 %CI, 0.26-1.84). Among the children of exposed applicators (n=3,032), 5 cases of cancer occurred. Thorpe & Shirmohammadi (2005) investigated a potential correlation of four types of childhood cancers in 689 cases (bone and brain, leukemia, non-Hodgkin lymphoma) and exposure to selected pesticides and nitrates via groundwater in Maryland (US). According to the authors exposure to low-levels of metolachlor and, more pronounced, to mixtures of metolachlor with further pesticides (+nitrate/atrazine and +nitrate/simazine/alachlor) significantly increased the risk for the four analysed types of childhood cancer (Crude ORs: 1.54, 7.56, 5.31). Positive associations were reported for bone cancer and metolachlor (Crude OR=2.26, 95 % CI, 0.97-5.24), as well as leukemia and metolachlor (Crude OR=1.48, 95 % CI, 0.93-2.36). The authors are aware, that there are several limitations of the study (e.g. amount of tap water consumption per day, other routes of pesticides exposure, distance of residence to herbicide application sites). Metayer et al. (2013) investigated an association between exposure to herbicides (including metolachlor) via house dust and childhood acute lymphoblastic leukemia (ALL). As in cases of ALL (n=252) no metolachlor was detected in dust, no association could be observed.

Overall, in epidemiological studies some associations of metolachlor exposure with increased likelihoods to develop certain tumours were reported (lung cancer, colon cancer, liver cancer, follicular cell lymphoma). Most interesting was the positive exposure-response association between liver cancer and metolachlor use (Silver et al., 2015) identified in the AHS cohort for a follow-up period through 2010/20, as also in the rat long-term study liver tumours had been observed. Regarding the risk of developing prostate cancer, negative associations were observed. Nevertheless, these associations need to be balanced against the fact that these were mainly seen from evaluations of a single cohort. Although data were stratified for confounders, it needs to be kept in mind that participants were also exposed to additional compounds. It may be concluded that there is limited evidence of carcinogenicity of metolachlor in humans which is, however, partly complimentary to what was observed in a study in rats and might support a need for classification.

Mechanistic studies:

Mechanistic studies were conducted to elucidate a potential mechanism or mode of action of proliferative changes in livers. Available studies and results are summarised in detail in Table 14. Metolachlor and S-metolachlor induced S-phase replicative DNA synthesis in rats at doses starting at 500 mg/kg bw/day after 72 hours (4.3-fold in males at 500 mg/kg bw, 2.9-fold in females at 1000 mg/kg bw) and 15, 38 hours, respectively (Anonymous (17), 1994, Anonymous (22), 1995b), but metolachlor as well as S-metolachlor did not result in replicative liver DNA synthesis after 7 or 28 days of treatment (Anonymous (35), 1995). However, in this negative 7-day and 28-day study, no positive controls were included.

In cultured female rat hepatocytes, cell proliferation (up to 1.9-fold) was shown (Anonymous (10), 2014), albeit no clear dose-response was obvious. In this in vitro system BROD activity was only slightly increased up to 1.3-fold and no increase of PROD activity was seen. In vivo an increase in CAR-dependent enzyme activity in response to S-metolachlor/metolachlor was seen after different periods of treatment: after 14 days of treatment with S-metolachlor (5000 ppm) PROD activity in female rats was increased 9-fold and BROD activity about 13-fold (Anonymous (27), 2006). After 28 days a 10-fold induction of PROD activity was observed for S-metolachlor (5000 ppm) as well as metolachlor (5000 ppm) in male rats, while in females

PROD was induced 62-fold in response to S-metolachlor and 45-fold in response to metolachlor (Anonymous (35), 1995).
Direct activation of CAR from different species (rat, mouse, human) was shown in a transactivation assay (Anonymous (34), 2014): at the highest dose rCAR3 was induced 57-fold (pos. control, clotrimazole: 95 -fold), mCAR3 27-fold (pos. control, TCPOBOP: 45-fold), and hCAR3 9-fold (pos. control, CITCO: 10-fold). Valuable experiments with CAR-knockout hepatocytes and humanized-CAR animals are missing.
Other mechanisms possibly involved in hepatic tumour formation were not investigated and the impact of other receptors/signaling pathways cannot be assessed. For example, AhR implication cannot be excluded from the available data, as in most of the in vivo mechanistic studies no enzyme activity indicative for AhR activity was measured. When EROD activity was analysed, an induction was observed, albeit, in comparison to CAR-associated CYP enzymes, to a lesser extent: 3-fold/2.5-fold after 28-day of S-metolachlor and metolachlor treatment in males/females, 2-fold after 60 days of metolachlor treatment (Anonymous (35), 1995; Anonymous (27), 2006). However, in vitro no activation of human AhR could be demonstrated (Takeuchi, 2008; Kuelbeck, 2011). In contrast, further in vitro analysis revealed that metolachlor is an agonist of human PXR, as well as mice PXR, and human CAR (Kojima, 2011; Kuelbeck, 2011).
Two studies on enzyme and DNA synthesis induction in cultured female human hepatocytes (Anonymous (11), 2014, Anonymous (5), 2019) are available. The study by Anonymous (11), 2014, is based on only one donor and the results are therefore of limited validity: PROD induction in response to treatment with S-metolachlor was only seen at the lowest dose. For BROD a decrease was observed. This result was confirmed in the study by Anonymous (5), 2019: results from two female donors were presented, but one of the females was under chemotherapy just before the hepatocytes were prepared and it is questionable if such data should be used. Moreover, the positive control showed no response for PROD and cytotoxicity was observed, questioning if the selected doses were appropriate. No cell proliferation in response to PB or S-metolachlor was observed in the human hepatocytes, while EGF induced cell proliferation at least 4-fold.
Due to the above summarized findings and lack of further experiments to exclude other possible mechanisms responsible for tumour formation than CAR activation, the DS concludes that a potential non-relevance of different mechanism for liver tumours is not sufficiently demonstrated.

Table 19: Compilation of factors to be taken into consideration in the hazard assessment

Species and strain	**Multi-site response**	**Tumour type and background incidence**	**Progression of lesions to malignancy**	**Reduced tumour latency**	**Responses in single or both sexes**	**Confounding effect by excessive toxicity?**	**Route of exposure**	**MoA and relevance to humans**
Rat, Sprague Dawley, (Crl:CD(SD)BR)	Yes	Liver carcinoma HCD incidence (Max.): 2.2 %	Yes (arising in adenoma)	unknown	Both sexes	No excessive toxicity	oral	relevant for humans
		Pituitary carcinoma	Yes (arising in adenoma)	unknown	Single (female)	No excessive toxicity	oral	relevant for humans
		Nassal turbinate adenocarcinoma HCD incidence (Max.): 0 %	unknown	unknown	Single (male)	No excessive toxicity	oral	relevant for humans

10.9.2 Comparison with the CLP criteria

The following criteria for classification for carcinogenicity are given in the CLP regulation:

Table 20: CLP criteria for classification of carcinogenicity

CLP criteria
A substance is classified in Category 1 (known or presumed human carcinogens) for carcinogenicity on the basis of epidemiological and/or animal data. A substance may be further distinguished as: Category 1A, known to have carcinogenic potential for humans, classification is largely based on human evidence, or Category 1B, presumed to have carcinogenic potential for humans, classification is largely based on animal evidence. The classification in Category 1A and 1B is based on strength of evidence together with additional considerations (see section 3.6.2.2). Such evidence may be derived from: - human studies that establish a causal relationship between human exposure to a substance and the development of cancer (known human carcinogen); or - animal experiments for which there is sufficient (1) evidence to demonstrate animal carcinogenicity (presumed human carcinogen). In addition, on a case-by-case basis, scientific judgement may warrant a decision of presumed human carcinogenicity derived from studies showing limited evidence of carcinogenicity in humans together with limited evidence of carcinogenicity in experimental animals. The placing of a substance in Category 2 (suspected human carcinogens) is done on the basis of evidence obtained from human and/or animal studies, but which is not sufficiently convincing to place the substance in Category 1A or 1B, based on strength of evidence together with additional considerations (see section 3.6.2.2). Such evidence may be derived either from limited (1) evidence of carcinogenicity in human studies or from limited evidence of carcinogenicity in animal studies. […] 3.6.2.2.3. Strength of evidence involves the enumeration of tumours in human and animal studies and determination of their level of statistical significance. Sufficient human evidence demonstrates causality between human exposure and the development of cancer, whereas sufficient evidence in animals shows a causal relationship between the substance and an increased incidence of tumours. Limited evidence in humans is demonstrated by a positive association between exposure and cancer, but a causal relationship cannot be stated. Limited evidence in animals is provided when data suggest a carcinogenic effect, but are less than sufficient. The terms 'sufficient' and 'limited' have been used here as they have been defined by the International Agency for Research on Cancer (IARC) and read as follows: (a) Carcinogenicity in humans The evidence relevant to carcinogenicity from studies in humans is classified into one of the following categories: - sufficient evidence of carcinogenicity: a causal relationship has been established between exposure to the agent and human cancer. That is, a positive relationship has been observed between the exposure and cancer in studies in which chance, bias and confounding could be ruled out with reasonable confidence; - limited evidence of carcinogenicity: a positive association has been observed between exposure to the agent and cancer for which a causal interpretation is considered to be credible, but chance, bias or confounding could not be ruled out with reasonable confidence. (b) Carcinogenicity in experimental animals Carcinogenicity in experimental animals can be evaluated using conventional bioassays, bioassays that employ genetically modified animals, and other in-vivo bioassays that focus on one or more of the critical stages of carcinogenesis. In the absence of data from conventional long-term bioassays or from assays with neoplasia as the end-point, consistently positive results in several models that address several stages in the multistage process of carcinogenesis should be considered in evaluating the degree of evidence of carcinogenicity in experimental animals. The evidence relevant to carcinogenicity in experimental animals is classified into one of the following categories: - sufficient evidence of carcinogenicity: a causal relationship has been established between the agent and an increased incidence of malignant neoplasms or of an appropriate combination of benign and malignant neoplasms in (a) two or more species of animals or (b) two or more independent studies in one species carried out at different times or in different laboratories or under different protocols. An increased incidence of tumours in both sexes of a single species in a well-conducted study, ideally conducted under Good Laboratory Practices, can also provide sufficient evidence. A single study in one species and sex might be considered to provide sufficient evidence of carcinogenicity when malignant neoplasms occur to an unusual degree with regard to incidence, site, type of tumour or age at onset, or when there are strong findings of tumours at

CLP criteria
multiple sites; - limited evidence of carcinogenicity: the data suggest a carcinogenic effect but are limited for making a definitive evaluation because, e.g. (a) the evidence of carcinogenicity is restricted to a single experiment; (b) there are unresolved questions regarding the adequacy of the design, conduct or interpretation of the studies; (c) the agent increases the incidence only of benign neoplasms or lesions of uncertain neoplastic potential; or (d) the evidence of carcinogenicity is restricted to studies that demonstrate only promoting activity in a narrow range of tissues or organs. 3.6.2.2.4. Additional considerations (as part of the weight of evidence approach (see 1.1.1)). Beyond the determination of the strength of evidence for carcinogenicity, a number of other factors need to be considered that influence the overall likelihood that a substance poses a carcinogenic hazard in humans. The full list of factors that influence this determination would be very lengthy, but some of the more important ones are considered here. 3.6.2.2.5. The factors can be viewed as either increasing or decreasing the level of concern for human carcinogenicity. The relative emphasis accorded to each factor depends upon the amount and coherence of evidence bearing on each. Generally, there is a requirement for more complete information to decrease than to increase the level of concern. Additional considerations should be used in evaluating the tumour findings and the other factors in a case-by-case manner. 3.6.2.2.6. Some important factors which may be taken into consideration, when assessing the overall level of concern are: (a) tumour type and background incidence; (b) multi-site responses; (c) progression of lesions to malignancy; (d) reduced tumour latency; (e) whether responses are in single or both sexes; (f) whether responses are in a single species or several species; (g) structural similarity to a substance(s) for which there is good evidence of carcinogenicity; (h) routes of exposure; (i) comparison of absorption, distribution, metabolism and excretion between test animals and humans; (j) the possibility of a confounding effect of excessive toxicity at test doses; (k) mode of action and its relevance for humans, such as cytotoxicity with growth stimulation, mitogenesis, immunosuppression, mutagenicity. Mutagenicity: it is recognised that genetic events are central in the overall process of cancer development. Therefore, evidence of mutagenic activity in vivo may indicate that a substance has a potential for carcinogenic effects.

Based on the limited evidence from epidemiological studies (increased likelihood to develop certain tumours e.g. lung cancer, colon cancer, liver cancer, follicular cell lymphoma) no classification for Carc. Cat. 1A is proposed based on the available data and information. However, the limited evidence from epidemiological studies, which is partly complementary to carcinogenic effects observed in rats, supports a need for classification that is based on animal data and classification into Cat. 1B or 2 can be considered, based on strength of evidence. On the one hand, carcinogenic long-term study findings were observed only in the rat and not in the mice, however, the available study in mice showed high mortality (> 50 %) and was considered not acceptable and is therefore only of limited value. Accordingly, from animal studies only evidence for one species is available. However, a multi-site tumour formation was evident in rats as the organs liver and pituitary were affected and tumours in the nasal turbinates were observed Moreover, a progression to malignancy was observed with adenoma and carcinoma in the pituitary of females, and increased incidences of neoplastic nodules and carcinomas in the liver of males and females. Of the adenocarcinoma in nasal turbinates of male rats, one was identified by the pathologist as arising from a subepithelial nasal gland and no associated preneoplastic lesions were identified. Fibroadenoma and squamous cell papilloma occurred at unrelated localisation. A high frequency of inflammation in the nasal epithelium was reported for all dose groups.

Mechanistic data could not sufficiently demonstrate that CAR activation is the only mechanism involved in liver-tumour formation and the non-relevance for humans of the observed tumours was not sufficiently shown. Furthermore, also in the large cohort of the AHS a positive exposure-response association between liver cancer and metolachlor use was reported for applicators. Overall, there is limited evidence for carcinogenicity from animal and epidemiological studies and this criteria warrants classification of S-metolachlor as a suspected human carcinogen (Carc. Category 2) according the Guidance on the Application of the CLP Criteria (V5.0 – July 2017, Tab. 3.6.1). However, at the pesticides peer review meeting, experts discussed if a classification into Carc. Cat. 1B might be more appropriate.

10.9.3 Conclusion on classification and labelling for carcinogenicity

Classification into Carc. Cat. 1A or B is currently not considered to be appropriate. Limited evidence for a carcinogenic potential in rats is provided. In addition, there is limited evidence for a carcinogenic potential of metolachlor in humans, which is, however, partly complimentary to what was observed in rats Therefore, classification into Carc. Cat. 2 (H351) is proposed.

10.10

Reproductive toxicity

10.10.1Adverse effects on sexual function and fertility

10.10.2 Short summary and overall relevance of the provided information on adverse effects on sexual function and fertility

The reproductive toxicity of metolachlor was assessed in a two-generation study in rats. Results of this study are summarised in Table 21. Further details regarding study design, guideline (and deviations, if any) and information on incidences and severities of findings and extent of changes relative to controls are given in the text below. For additional information, reference is made to Volume 3 Chapter B.6 of the RAR.

Table 21: Summary of reproductive toxicity studies

Study type, compound, guideline, deviations if any	**Dose levels**	**NO(A)EL**	**Critical effects**	**Reference**
2-generation study in rats (CD rats) metolachlor (batch FL800362, purity 95.4%, enantiomeric content: 47.7% w/w of each of the enantiomers) study performed according "guidelines established in 43 FR 37336, Part 183.83-4". Design is similar to OECD 416 with some deviations (see text below) GLP (self-certification of the performing laboratory, internal quality assurance system)	0, 30, 300, 1000 ppm (corresponding to 1.8, 17.7 and 54.9 mg/kg bw/d)	parental: 300 ppm (17.7 mg/kg bw/d) offspring: 300 ppm (17.7 mg/kg bw/d) reproductive: 1000 ppm (54.9 mg/kg bw/d)	parental: Food intake ↓ (F1 females), rel. liver & thyroid wt ↑; offspring: body weight in F1 and F2 pups ↓ no effects on reproduction or fertility	Anonymous (33), 1981 acceptable

The study is similar to OECD 416 but has some deviations: Food intake was measured only during the pre-mating period and the calculation of mean daily substance intake has been performed on that basis. Conversion values for ppm to mg/kg bw/d were based on food consumption during week 10 in F0 males. In addition, organ weights of parental animals were only determined in the F1 but not in the F0 generation. Oestrus cycle of the female rats was not investigated and the age of vaginal opening of preputial separation in F1 weanlings selected for further breeding were not determined. It is only stated that the pups were examined for "developmental anomalies at birth and again at weaning." The latter deficiencies are clearly due to the age of the study. When pups were weighed, sexes were regarded separately only on day 21 but not before.

There were no unscheduled deaths and no clinical signs of toxicity among the parental F0 animals up to the highest tested dose of 54.9 mg/kg bw/d. The same holds true for the F1 parental males. One mid dose and one high dose F1 female were found dead at an age of 32 or 52 days, respectively. The cause of these deaths could not be clearly established but, due to their isolated occurrence and to the absence of further clinical signs, a relation to treatment is unlikely. In addition, one control and one mid dose female were sacrificed in moribund condition for humane reasons, both at 170 days of age.

Body weight and body weight gain in the parental animals were not altered in any generation at any dose level. Food consumption was not compromised in the F0 generation. In the F1 generation high dose females displayed significant reductions as compared to the control group for 8 of the 17 measurement intervals (pre-mating weeks) whereas such differences were only occasionally seen in the other dose groups. Thus, an adverse effect of the test substance on food intake at the top dose level became apparent.

Organ weight determinations in F1 parental animals revealed an increase in relative thyroid weight in males receiving 54.9 mg/kg bw/d (+ 26 %) which was, however, not accompanied by histopathological findings.

Likewise, the relative liver weight was increased in both sexes at this dose (males: + 11 %, females: + 9 %), but, again, not related to histological changes. Gross and histopathological examination of other organs did not reveal findings that could be attributed to treatment.

Male fertility was further investigated by histology of the testes, which failed to demonstrate any adverse effect on spermatogenesis. Atrophy of spermatic cells and, in one case, also aspermia were noted at the low dose level in 2 out of 15 F0 males but were not confirmed at higher dose levels or in the F1 generation. Thus, these isolated findings were considered spontaneous.

In the F0 generation, the mating index appeared somewhat lower at 54.9 mg/kg bw/d group as compared to the control group (63.6 % vs. 81.1 %) but the difference was not statistically significant. Fertility and gestation index or average gestation length were not altered. No evidence of any differences in the reproductive parameters was observed in the F1 generation. The reproductive success in terms of litter size or number of totally delivered viable pups was not compromised.

10.10.3 Comparison with the CLP criteria

Table 22: Toxicological results concerning adverse effects on sexual function and fertility

Toxicological result	**CLP criteria**
2-generation reproduction study in rats, metolachlor administered via diet (Anonymous (33), 1981): No effects on fertility or reproduction observed up to highest dose tested (1000 ppm, 54.9 mg/kg bw/d)	Category 1A: Known human reproductive toxicant Category 1B: Presumed human reproductive toxicant largely based on data from animal studies - clear evidence of an adverse effect on sexual function and fertility in the absence of other toxic effects, or - the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effects Category 2: Suspected human reproductive toxicant - some evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on sexual function and fertility and - where the evidence is not sufficiently convincing to place the substance in Category 1 (deficiencies in the study). - the adverse effect on reproduction is considered not to be a secondary non-specific consequence of the other toxic effects

No human data on adverse effects on sexual function and fertility are available, hence no classification with Cat. 1A according to CLP regulation is proposed.

In the submitted multigeneration study, no findings with relevance for classification for adverse effects on sexual function and fertility were reported. Nevertheless, important parameters such as cyclicity, ovarian follicles or developmental landmarks in the offspring have not been investigated. Overall, no classification with Cat. 1B or 2 according to the CLP regulation is proposed.

10.10.4 Adverse effects on development

10.10.5 Short summary and overall relevance of the provided information on adverse effects on development

The developmental toxicity of S-metolachlor is assessed based on four teratology studies in rats and rabbits using metolachlor or S-metolachlor and one two-generation study using metolachlor in rats. An additional available teratology study in rats (Anonymous (15), 1976) was not taken into account: the study was considered not acceptable due to several deficiencies (non guideline-conform, test item was not adequately described, too low top dose level, no justification for dose setting, deficient study report). The results of the four acceptable/supplementary studies are summarised in Table 23. Further details regarding study design, guideline (and deviations, if any) and information on incidences and severities of findings and extent of changes relative to controls are given in the text below. For additional information, reference is made to Volume 3 Chapter B.6 of the RAR. For the pesticides procedure also information from published literature was assessed. The majority of these studies was performed using a formulated product containing S-metolachlor or metolachlor and is therefore not reported here, but results of one study using metolachlor are reported below.

For the pesticides procedure additional studies regarding developmental toxicity of two environmental metabolites of S-metolachlor were assessed. Both metabolites are primary metabolites in the environment and were not or only to an extent of 0.14 % recovered in rat excreta. They are not expected to enhance the toxicity of S-metolachlor and results of toxicity testing are therefore not reported here.

Table 23: Summary of developmental toxicity studies

Study type, compound, guideline, deviations if any	Dose levels	NO(A)EL	Critical effects	Reference
Developmental toxicity study, rat (Tif:RAIf) S-metolachlor (purity: 95.6%; batch no.: V4673/7; S-enantiomeric content: 84% w/w, R-enantiomeric content: 11.1% w/w) OECD 414, administration only from days 6 through 15 post coitum. GLP	0, 5, 50, 500, 1000 mg/kg bw/d	maternal: 50 mg/kg bw/d developmental: 500 mg/kg bw/d	maternal: clinical signs, body weight ↓, body weight gain ↓, food consumption ↓ developmental: dumbbell shaped cervical vertebral centers↑	Anonymous (24), 1995 acceptable

Study type, compound, guideline, deviations if any	**Dose levels**	**NO(A)EL**	**Critical effects**	**Reference**
Developmental toxicity study, rat (Crl:COBS CD (SD) BR) metolachlor (purity: 96.4%, batch number FL-841697, enantiomeric content not reported) No guideline given in the study. Study design is similar to OECD 414 but pathological examination of the dams was rather limited and uterine weights were not determined at termination. Administration only from days 6 through 15 post coitum GLP	0, 30, 100, 300, 1000 mg/kg bw/d	maternal: 100 mg/kg bw/d developmental: 300 mg/kg bw/d	maternal: body weight ↓, body weight gain ↓, food consumption ↓, clinical signs (convulsions, salivation, lacrimation, urine-stained abdominal fur) ↑ developmental: fetal weight ↓, delayed ossification ↑	Anonymous (26), 1985 Acceptable

Study type, compound, guideline, deviations if any	**Dose levels**	**NO(A)EL**	**Critical effects**	**Reference**
Developmental toxicity study, rabbit (New Zealand White rabbits (Har:PF/CF(NZW)BR) S-metolachlor purity: 89.6%, batch: FL830813, S-enantiomeric content of 93.7%, OECD 414, stability of the test substance in the dosing formulation was not determined during the study, but confirmed years after when the test report was prepared. For this additional analytical work, a different lot of the test substance (FL-941255 with a purity of 94.4 %) was used. Administration only from day 7 through 19 of presumed gestation. GLP	0, 20, 100, 500 mg/kg bw/d	maternal: 100 mg/kg bw/d developmental: 100 mg/kg bw/d	maternal: body weight (gain) ↓, food consumption ↓ developmental: fetal malformations & variations ↑, fetal weight↓	Anonymous (16), 1995 Acceptable

Study type, compound, guideline, deviations if any	**Dose levels**	**NO(A)EL**	**Critical effects**	**Reference**
Developmental toxicity study, rabbit (New Zealand White rabbits, DLI:NZW) metolachlor (purity 95.4%, batch:-FL-791174, contains 47.7% w/w of the R- and S-enantiomer) No guideline given in the study. Study design is similar to OECD 414, administration from day 6 through day 18 of presumed gestation. No precise data on food consumption, terminal sacrifice on day 30. No clear discrimination between malformations and variations. non GLP	0, 36, 120, 360 mg/kg bw/d	maternal: 120 mg/kg bw/d developmental: 120 mg/kg bw/d	maternal: clinical signs ↑ (miosis, vaginal discharge), abortions ↑, body weightloss , food consumption ↓ developmental: malformations ↑	Anonymous (25), 1980 supplementary

In both studies in rats maternal toxicity occurred at the two top dose levels of 500 and 1000 mg/kg bw/d S-metolachlor, or 300 and 1000 mg/kg bw/d metolachlor, respectively. Dose-related reductions in body weight (up to 8 % in the study using S-metolachlor and 5 % in the study using metolachlor on day 21 after treatment with 1000 mg/kg bw/d), bw gain, (-45 % compared to control for days 6-11) and food consumption were observed (Table 24, Table 25). Anonymous (26) (1985) reported clinical signs including clonic and/or tonic convulsions, excess salivation and/or lacrimation and urine-stained abdominal for which became severe at the limit dose of metolachlor, four dams died at this dose (Table 25). In Anonymous (24) (1995) all dams from 500 and 1000 mg/kg bw/d and nine dams from 50 mg/kg bw/d groups exhibited discomfort after S-metolachlor administration (pushing head through bedding for up to one hour following dosing). The NOAEL for maternal toxicity was set at 50 mg/kg bw/d (S-metolachlor) and 100 mg/kg bw/d (metolachlor), respectively.

Table 24: Maternal findings - study in rats using S-metolachlor (Anonymous (24), 1995)

Dose (mg/kg bw/day)	**0**	**5**	**50**	**500**	**1000**
Mean body weight (g with SD) Day 6 Day 9 Day 16 Day 21	226.5 ± 10.3 241.5 ± 10.9 296.5 ± 16.6 375.2 ± 25.6	225.5 ± 10.5 241.7 ± 12.7 295.0 ± 18.6 369.8 ± 27.7	229.5 ± 12.0 243.0 ± 12.6 296.6 ± 16.4 377.3 ± 29.4	227.7 ± 9.5 236.6 ± 11.5 284.5 ± 14.1* (-4%) 357.9 ± 28.1	225.0 ± 9.9 229.3 ± 9.6** (-5%) 275.5 ± 14.7** (-7%)

Dose (mg/kg bw/day)	**0**	**5**	**50**	**500**	**1000**
				(-5%)	345.3 ± 29.0** (-8%)
Mean bw gain (g with SD) Days 6 -11 Days 11-16 Days 16-21 Days 6 -16 Days 6 – 21	28.5 ± 4.7 41.5 ± 6.7 78.7 ± 13.3 70 ± 9.4 148.7 ± 20.2	29.5 ± 5.0 40.1 ± 7.7 74.8 ± 12.5 69.6 ± 10.5 144.2 ± 21.3	27.6 ± 4.4 39.5 ± 8.0 81.2 ± 18.3 67.1 ± 9.6 148 ± 25.1	23.5 ± 6.0* (-17%) 33.3 ± 4.8** (-20%) 73.4 ± 19.3 (-7%) 56.8 ± 9.5** (-19%) 130.2 ± 23.5 * (-12%)	15.8 ± 5.3** (-45%) 34.7 ± 7.7* (-16%) 69.9 ± 18.1 (-11%) 50.5 ± 11.9** (-28%) 120.4 ± 27.5** (-19%)
Food consumption (g/animal/day, with SD) Days 6-11 Days 11-16 Days 16-21	26.0 ± 1.8 27.8 ± 2.7 26.9 ± 3.0	25.9 ± 2.3 27.8 ± 3.0 27.5 ± 3.7	25.3 ± 2.3 27.1 ± 2.2 28.2 ± 3.0	22.5 ± 2.3** (-13%) 25.6 ± 1.8* (-8%) 28.4 ± 2.6	20.3 ± 1.7** (-22%) 25.1 ± 2.4** (-10%) 27.3 ± 2.2

*p<0.05, **p<0.01, Anova + Dunnett-test

$^{\#}$ only pregnant females included in calculations

Table 25: Maternal findings - study in rats using metolachlor (Anonymous (26), 1985)

Dose (mg/kg bw/day)	**0**	**30**	**100**	**300**	**1000**
Number of rats per group	25	25	25	25	25
Mortality	0	0	0	0	4**
Clonic or tonic convulsions (affected females/ observations$^{\#}$)	0	0	0	0	11 / 11**
Excess salivation (affected females/observations $^{\#}$)	0	0	0	16 / 61**	25 / 214**
Excess lacrimation (affected females/observations $^{\#}$)	0	0	0	0	8$^{(21)**}$
Urine-stained fur (affected females/observations $^{\#}$)	0	0	0	0	19 / 140**
Body weight§ (g, ± SD), day 6	276.6 ± 13.6	275.5 ± 13.3	277.0 ± 12.4	276.9 ± 18.0	272.6 ± 13.1
Body weight (g, ± SD), day 9	288.0 ± 14.8	290.0 ± 13.2	288.5 ± 13.1	285.7 ± 18.4	276.9 ± 17.9* (-4%)
Body weight (g, ± SD), day 15	325.3 ± 19.4	329.8 ± 14.5	325.0 ± 18.0	323.5 ± 25.9	309.0 ± 15.5* (-5%)
Body weight (g, ± SD), day 20	403.0 ± 25.8	409.4 ± 21.4	403.6 ± 23.0	397.4 ± 32.6	380.8 ± 18.6** (-6%)
Mean bw gain (g, ± SD), days 6-11	25.0 ± 6.0	28.2 ± 8.0	24.7 ± 7.2	20.5 ± 6.3* (-18%)	13.6 ± 7.7** (-46%)

Dose (mg/kg bw/day)	**0**	**30**	**100**	**300**	**1000**
Mean bw gain (g, ± SD), days 6-15	48.7 ± 11.0	54.2 ± 11.8	48.0 ± 12.5	46.6 ± 11.5 (-4%)	39.2 ± 10.1[*] (-20%)
Mean bw gain (g, ± SD), days 6-20	126.3 ± 18.7	134.0 ± 18.8	126.6 ± 17.5	120.5 ± 20.1 (-5%)	111.2 ± 15.5[*] (-12%)
Food consumption (g, ± SD), days 6-11	87.2 ± 7.1	86.5 ± 7.7	84.5 ± 7.1	81.4 ± 9.7[*] (-7%)	77.1 ± 10.4[**] (-12%)

[*] $p<0.05$, [**] $p<0.01$ in Dunett's test

[#] frequency of observation of this sign, summed up over the entire study period and all animals

[§] body weight data exclude non-pregnant animals

The number of pregnant females and the mean number of Corpora lutea did not differ between the treated groups and the control and all rats, which were pregnant, had litters with viable fetuses.

Under the conditions of the teratology study conducted by Anonymous (24) (1995) using S-metolachlor, some external and visceral anomalies were reported which were mainly considered as not treatment-related as no dose response was apparent (Table 26). Skeletal anomalies mainly comprised irregular, poor or absent ossification of cranial bones, sternebrae, vertebral centres, ribs or phalanges and fused, asymmetric or bipartite sternebrae. A remarkable finding was a dose-dependent increase in the incidence of dumbbell shaped cervical vertebral centres gaining statistical significance at the top dose level of 1000 mg/kg bw/d for numbers of affected litters as well as fetuses. Both, the fetal incidence of 4.7 % as well as the litter incidence of 27.3 % was within the respective historical control ranges (0.6 - 8.4 % for fetuses and 4.2 - 47.8 % for litters), however, the respective means of 2.7 % or 15.4 % were clearly exceeded. The historical control database as provided as part of the study report included 5068 fetuses from 680 litters, which were produced in 20 studies in the same rat stock. These studies had been run between 1 January 1988 and 31 October 1994, and, thus, covered the in-life phase of the study under evaluation. The NOAEL for developmental toxicity was set at 500 mg/kg bw/d.

Table 26: Fetal findings – study in rats using S-metolachlor (Khalil, 1995)

Dose (mg/kg bw/day)	**0**	**5**	**50**	**500**	**1000**
Number of litters	**22**	**23**	**23**	**21**	**22**
Mean number of live fetuses per litter (with SD)	**15.0 ± 2.6**	**13.7 ± 2.8**	**14.9 ± 3.0**	**13.1 ± 4.0**	**12.8 ± 4.8**
Mean fetal weight (g), males/females	**5.4 / 5.1**	**5.5 / 5.1**	**5.4 / 5.1**	**5.5 / 5.2**	**5.4 / 5.2**
Fetal external anomalies (% litter incidence)	**4.5**	**0**	**4.3**	**4.8**	**0**
Fetal visceral anomalies (% litter incidence)	**31.8**	**30.4**	**26.1**	**23.8**	**38.1**
Fetal skeletal anomalies (% litter incidence)	**40.9**	**34.8**	**26.1**	**47.6**	**22.7**
Dumbbell-shaped cervical vertebral centers (fetuses/fetuses evaluated)	**1 / 168** (0.6%)	**1 / 163** (0.6%)	**2 / 177** (1.1%)	**3 / 142** (2.1%)	**7[*] / 148** (4.7%)
Dumbbell-shaped cervical vertebral centers (litters/litters evaluated)	**1 / 22** **(4.5%)**	**1 / 23** **(4.3%)**	**2 / 23** **(8.7%)**	3 / 21 (14.3%)	**6[§] / 22** **(27.3%)**

[*] $p<0.05$, Anova + Dunnett-test; [§] fetal and litter incidence within historical control range, above the means

Under the conditions of the teratology study conducted by Anonymous (26) (1985) using metolachlor, a slightly lowered fetal weight (- 4 %) was reported for the top dose group. Malformations included one hydrocephalus (vehicle control), one *Spina bifida* and exencephaly (at 300 mg/kg/day in the same litter) and one micrognathia (at 1000 mg/kg/d). These isolated findings were considered chance events because of their rareness, the lack of statistical significance and (apart from micrognathia that was confined to the highest dose) because there was no dose response. Visceral and skeletal variations did not occur often and were not dose-related. The only significant difference (p<0.01) was achieved for a variation that is considered to indicate some retardation in development: at the top dose level of 1000 mg/kg bw/d, an incompletely ossified ischium was observed in two fetuses in two litters. In the control or the other treated groups, this finding was not present. The NOAEL for developmental toxicity was set at 300 mg/kg bw/d.

Under the condition of the two-generation study in rats conducted by Anonymous (33) (1981) developmental effects were observed in terms of reduced fetal bodyweight. During lactation pup survival was not altered. However, mean pup body weight was lower in both generations at the top dose level of 54.9 mg/kg bw/d: at PND 21 in the F2 generation a statistically significant decrease of 8 % in females and 7 % in males was observed and in the F1 body weight was reduced about 8 % in females and 9 % in males In female F2 pups, there was a statistically significant reduction (-6 %) in body weight on day 21 also in the mid dose group.

Asimilar tendency was observed in the F1 generation but the difference had not achieved statistical significance (Table 27). Decreased body weight was already observed from day 4 (F2) and day 14 (F1) on at the top dose, however, at this time points no differentiation was made between sexes. Survival and normal morphological and functional development were not altered.

Table 27: Developmental effects of metolachlor in a 2-generation study in rats (Anonymous (33) (1981), pup weights at selected time points

	Dietary concentration (ppm)							
	F1				F2			
	0	30	300	1000	0	30	300	1000
Mean pup weight day 4	9.7	9.7	9.8	9.8	9.9	9.4*	9.7	9.2* (-7%)
Mean pup weight day 14	27.6	27.8	27.7	26.4* (-4%)	27.3	26.4	26.5	25.9** (-5%)
Mean male pup weight day 21	46.2	45.9	45.1	41.9** (-9%)	44.2	42.6	42.6	41.0** (-7%)
Mean female pup weight day 21	43.9	43.9	41.6 (-5%)	40.5** (-8%)	42.7	41.8	40.3* (-6%)	39.2** (-8%)

* Significantly different from control $P < 0.05$
** Significantly different from control $P < 0.01$

The results of two teratology studies with New Zealand White Rabbits were assessed. Effects of S-metolachlor (Har:PF/CF(NZW)BR) and metolachlor (DLI:NZW) were investigated. Under the conditions of the teratology study conducted by Anonymous (16) (1995) using S-metolachlor, maternal toxicity occurred at the top dose level (500 mg/kg bw/d) and reduced body weight, body weight gain and food intake were reported. Maternal findings are summarized inTable 28. Four unscheduled deaths were reported. One doe in the top dose group was found dead on day 25, following a period of body weight loss. Even though occurring after cessation of treatment, this death is considered treatment-related. In the low dose group treated with 20 mg/kg bw/d, one doe was sacrificed because it had aborted (day 21) and another one was found dead on day 28 showing also evidence of abortion. These cases were most likely not related to treatment since there were no further abortions at higher dose levels. The fourth animal, this time from the mid dose group receiving 100 mg/kg bw/d, was sacrificed on day 15 for humane reasons because of a fractured hindlimb. This isolated event was probably also not related to test substance administration. Gastrointestinal disturbances became apparent at 100 and 500 mg/kg bw/d since soft stool and/or reduced defecation were observed to occur more frequently as in the control and low dose groups. The difference achieved statistical significance. In addition, these signs were observed in the high dose group during the entire treatment period and not only or more frequently towards the end of the study. In 16 does of this group, they were observed for the first time on days 8 or 9 already whereas first observations were reported in the control group on day 17, in the low dose group on day 18, and in the mid dose group (but in one animal only) on day 11. There were no further clinical signs of toxicity and necropsy did not reveal any gross lesions that could be allocated to treatment.

Table 28: Maternal findings, study in rabbits using S-metolachlor (Anonymous (16), 1995)

Dose (mg/kg bw/day)	**0**	**20**	**100**	**500**
Found dead or sacrificed before scheduled termination	0	2	1	1
Abortion	0	$1 (2)^{\#}$	0	0
Reduced or soft stool	6	11	14^{**}	19^{**}
Mean body weight (g, ±SD) Day 7	3952 ± 75	3956 ± 101	4080 ± 90	3963 ± 62

Dose (mg/kg bw/day)	0	20	100	500
Day 14	3993 ± 76	4020 ± 103	4145 ± 95	3841 ± 60
Day 19	4101 ± 86	4142 ± 110	4226 ± 95	3782 ± 68*
Day 29	4225 ± 92	4213 ± 94	4330 ± 97	4097 ± 74
Bw gain (g, ±SD)				
Days 7-14	42 ± 19	64 ± 10	64 ± 22	-122 ± 34*
Days 14-19	108 ± 18	123 ± 14	81 ± 13	- 59 ± 27*
Days 19-21	33 ± 9	37 ± 7	30 ± 11	75 ± 19**
Days 21-25	79 ± 10	39 ± 25	68 ± 16	153 ± 18*
Food consumption (g, ±SD)				
Day 6	172 ± 5	182 ± 12	180 ± 8	174 ± 7
Day 7	167 ± 5	176 ± 12	183 ± 10	75 ± 7* (-55%)
Day 11	158 ± 6	178 ± 9	167 ± 9	91 ± 13* (-42%)
Day 19	149 ± 10	165 ± 7	153 ± 10	78 ± 15* (-48%)
Day 21	146 ± 7	152 ± 10	145 ± 11	149 ± 13
Day 28	86 ± 8	89 ± 13	98 ± 13	133 ± 9*

*p<0.05, **p<0.01, Anova or covariance analysis
$^{\#}$one case confirmed, the other only presumed

The NOAEL for maternal toxicity was set at 100 mg/kg bw/d.
The mean number of Corpora lutea was similar among all the groups. There was no impact of treatment on resorptions or on the mean number of live foetuses. The fetal sex ratio was not affected. Fetal weight appeared slightly lower at the top dose level of 500 mg/kg bw/d even though the difference to the control was not statistically significant. A slightly higher litter incidence of external, visceral and skeletal malformations was observed in the high dose group receiving 500 mg/kg bw/d. The only variation which gained statistical significance was a skeletal one described as “fully formed ribs” (see Table 29). Most malformations, including all cleft palates, all cases of abnormally flexed limbs/paws, one of two cases of hydrocephalus, reduced trachea size (sometimes considered rather a variation) and all skeletal findings were found in the same litter (see Table 30) of the high dose group. The external limb malformations are most likely related to the skeletal finding of short and bowed ulna/radius. The heavily affected litter (BT14) consisted of five fetuses (four males and one female) whereas the median litter size in the same dose group was 8 and the mean 7.9. All five foetuses had multiple malformations. Also in this litter only, the variation of a curled tongue was noted in three foetuses. The doe producing this litter consumed only very little food over the whole treatment period (0 – 57 g on the individual days with less than 10 g on most of them) and had the lowest body weight in the high dose group between days 14 and 25.
The only skeletal malformation (agenesis of a vertebral centrum and its associated ribs) observed at 100 mg/kg bw/day did not exhibit a dose response and is, therefore and because of its isolated occurrence, not considered treatment-related.

Table 29: Fetal data – body weight, (litter) incidences of anomalies, study in rabbits using S-metolachlor (Anonymous (16), 1995)

	Dose (mg/kg bw/d)			
	0	20	100	500
Litters evaluated	19	15	16	18
Mean body weight of male/female fetuses (g)	43.0 / 41.8	43.5 / 44.4	44.4 / 42.3	39.8 / 40.3 (-7%) / (-4%)

Malformations (litter incidence) external / visceral / skeletal	0 / 0 / 0	0 / 0 / 0	0 / 0 / 1	1 / 2 / 1
Variations (litter incidence) visceral / skeletal	1 / 15	1 / 7	0 / 12	3 / 15
Single variation “Fully formed ribs” (affected / total number of fetuses)	49 / 161	18 / 107	29 / 129	72** / 143

$^{*}p<0.05$, $^{**}p<0.01$, Anova or covariance analysis

Table 30: Summary of fetal malformations (litter incidence in brackets), study in rabbits using S-metolachlor (Anonymous (16), 1995)

Type	**Finding**	**Dose (mg/kg bw/day)**			
		0	**20**	**100**	**500**
Fetuses evaluated		161	107	129	143
Litters evaluated		19	15	16	18
External	Abnormal limb flexure	0	0	0	4 (1)
Visceral	Cleft palate	0	0	0	4 (1)
	Hydrocephalus	0	0	0	2 (2) 1.4% (11.1%)
	Trachea size reduced				1 (1)
Skeletal	Agenesis of vertebral centrum or of ribs	0	0	1	0
	Short cranial bones (zygomas/squamosals)	0	0	0	5 (1)
	Wavy clavicle	0	0	0	4 (1)
	Short and bowed ulna/radius	0	0	0	5 (1)
	Bowed scapula	0	0	0	1

For the observed malformation hydrocephalus the DS requested historical control data during the pesticides procedure. Anonymous (29) (2017b) provided historical control data for NZW rabbits, obtained in the time period between 1983 and 1987, from the laboratory where the study by Anonymous (16) (1995) has been conducted in 1983 even though it was reported only in 1995. The historical database comprised 12 studies with a total of 196 litters and 1586 fetuses. In two out of these 12 studies, hydrocephalus was observed: in one study 1 out of 145 fetuses showed hydrocephalus and in a second study in total 2 out of 143 fetuses from two different litters were affected. These HCD demonstrates, that hydrocephalus is a very rare malformation. The observed incidence of hydrocephalus in the high dose group is far above the mean valuefrom the HCD, albeit the maximum was not exceeded.

The NOAEL for developmental toxicity was set at 100 mg/kg bw/d, based on observed anomalies at 500 mg/kg bw/d.

Under the conditions of the teratology study conducted by Anonymous (25) (1980) using metolachlor, two premature deaths were reported. A top dose female receiving 360 mg/kg bw/d died on day 29 following incomplete delivery of its litter. The two delivered fetuses were dead and malformed with hydrocephalus and small encephalocele. This doe had exhibited a strongly reduced food intake and body weight loss (by 9.9 %) from the beginning of treatment until death. One animal treated with 36 mg/kg bw/d was found dead on day 24, following a long-lasting period of reduced food consumption beginning on day 12 that continued also after cessation of treatment. The doe also exhibited body weight loss (5.6 % as compared to day 6) but had 8 fetuses, which were presumed to have been alive at the time of death of the mother. Hemorrhagic erosions and focal congestion of the stomach mucosa were noted. Since there was no dose response, the clinical signs and eventually the death of this female rabbit were not considered treatment-related. Two abortions were observed: in one animal treated with the top dose of 360 mg/kg bw/d on day 17 (one fetus aborted, 8 implantations sites found at sacrifice on day 20), i.e., during the treatment period, and in one female treated with 120 mg/kg bw/d on day 25 which aborted one early absorption but had no further fetuses. Taking into account these circumstances, the top dose case might be attributed to treatment but not the other. The aborting doe in the top dose group had exhibited clear signs of maternal toxicity (lower food consumption from day 10 onwards, body weight loss). A rather unusual and rare clinical sign was pupil constriction (miosis) observed in animals treated with 120 and 360 mg/kg bw/d within one hour after dosing, disappearing gradually thereafter. This sign that might be considered to indicate a vagotonic response was seen on at least one day but in a few animals occurred on up to 6 days during the treatment period. Another sign that could be due to parasympathetic activation was excess salivation in one of the developmental studies on rats. Necropsy and (limited) histopathology did not reveal evidence of treatment-related adverse findings.

In the absence of precise data, no meaningful conclusion with regard to food consumption is possible. The only parameter given in the report is the number of days on which the individual animals consumed less than one-half of the offered amount of diet. In the control group, such a lower food intake was seen in 8 females, mostly in the post-observation period (apart from 2 does with occasional reduction during the treatment period). In the low dose group, 11 animals were affected. In five of them, this finding was noted during the administration period already. Similarly, 10 mid dose females had a lower food intake with six of them during treatment already. At the top dose level, such a low food consumption was quite common with 12 females affected and 11 of them showing the effect for the first time (and mostly frequently) during the administration period. Thus, at least in the group receiving 360 mg/kg bw/d, an adverse effect on food consumption can be assumed. Mean absolute body weight and body weight gain were significantly reduced in this dose group during the treatment period but normalised thereafter. In the dose groups receiving 120 and 360 mg/kg bw/d, body weight gain was markedly higher after cessation of treatment. Maternal findings are summarised in Table 31.

Table 31: Maternal findings - study in rabbits using metolachlor (Anonymous (25), 1980)

Dose (mg/kg bw/day)	**0**	**36**	**120**	**360**
Premature deaths	-	1	-	1
Abortions	-	-	1	1
Vaginal bleeding	0/16	0/16	0/16	4/16
Miosis (at least once during treatment period)	0/16	0/16	8/16	10/16
Mean body weight (kg, ± SD)$^{\#}$				
Day 6	4.53 ± 0.36	4.36 ± 0.38	4.53 ± 0.38	4.48 ± 0.33
Day 12	4.54 ± 0.35	4.35 ± 0.42	4.50 ± 0.43	4.40 ± 0.33**
Day 18	4.57 ± 0.33	4.39 ± 0.41	4.55 ± 0.45	4.32 ± 0.34**
Day 30	4.53 ± 0.38	4.42 ± 0.39	4.73 ± 0.41	4.48 ± 0.35
Mean bw gain (kg)				
Day 6 - 12	0.01	-0.01	-0.03	-0.08**
Day 6 – 18	0.04	0.03	0.02	-0.16**
Day 0 – 30	0.06	0.07	0.21	0.05
Day 6 - 30	0.03	0.04	0.15	-0.01

**p<0.01, Covariance analysis; $^{\#}$ pregnant animals only considered

The NOAEL for maternal toxicity was set at 120 mg/kg bw/d.

Table 32: Cesarean section data - study in rabbits using metolachlor (Anonymous (25), 1980

Dose (mg/kg bw/day)	0	36	120	360
Litters evaluated	14	13	12	12
Fetuses evaluated	83	92	78	65
Mean litter size	5.8	7.0	6.5	5.2
Mean fetal weight (g), male/female fetuses	52.5 / 50.2	50.7 / 46.9	52.2 / 53.2	53.6 / 50.5
Hydrocephalus (litter)	0	0	0	2 (1) 3.1% (8.3%)

There were no significant differences among the control and treatment groups with regard to Corpora lutea, implantations, resorption rate or litter size. Mean fetal weight or sex ratio of fetuses were not affected by treatment. There was no difference with regard to the frequency of external, visceral and skeletal malformations but for one exception: two delivered dead pups of the same litter in the group treated with 360 mg/kg bw/d had hydrocephalus with exencephaly and incompletely ossified, highly domed parietals. As described above the food and body weight of the doe was reduced. According to the author of the study, the historical control incidence in the performing laboratory was 1:1000 litters. This very low historical control incidence is not appropriate to exclude that the observed findings was treatment-related. To support the evaluation of these malformations, further historical control data on the spontaneous incidence of hydrocephaly was requested by the DS during the pesticides procedure and was provided by the applicant: Anonymous (28) (2017a) reported historical control data from the laboratory where the study by Anonymous (25) (1980, run in 1979) has been conducted from studies performed later (1980-1990) in the same rabbit strain. A total of 99 studies (1463 litters) were reported, in only 16 of them hydrocephalus was observed. In 15 of these studies one fetus was affected (number of fetuses affected/number of fetuses: 1/136, 1/94, 1/97, 1/132, 1/133, 1/150, 1/150, 1/138, 1/138, 1/112, 1/87, 1/111, 1/98, 1/140, 1/40) while in the remaining study, using unusualdosing via interuterine device during gestation, 3 fetuses in two litters displayed hydrocephalus 3/111). Therefore, the observed incidences of hydrocephalus in the high dose group are far above the mean value from the HCD.

In a study from Greenlee et al. (2004), isolated embryos from CD-1 mice were incubated with 0.1 µg/ml metolachlor. The percentage of developing blastocysts was unaltered, the percentage of apoptosis was significantly increased and the mean number of cells per embryo was significantly reduced by 10 %.
No human data on adverse effects on development are available.

10.10.6 Comparison with the CLP criteria

Table 33: Toxicological results concerning adverse effects on development

Toxicological result	**CLP criteria**
Teratology study, rat, S-metolachlor (Anonymous (24), 1995) Maternal toxicity: lower body weight, body weight gain and food intake at 500 and 1000 mg/kg bw/d Developmental effects: Some external, visceral and skeletal anomalies in foetuses, which were not considered as adverse due to missing dose-response. Higher incidence of dumbbell shaped cervical vertebral centers at the highest dose (1000 mg/kg bw/d).	Category 1A: Known human reproductive toxicant Category 1B: Presumed human reproductive toxicant largely based on data from animal studies - clear evidence of an adverse effect on sexual function and fertility in the absence of other toxic effects, or - the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effects Category 2: Suspected human reproductive toxicant - some evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on sexual function and fertility and - where the evidence is not sufficiently convincing to place the substance in Category 1 (deficiencies in the study). - the adverse effect on reproduction is considered not to be a secondary non-specific consequence of the other toxic effects
Teratology study, rat, metolachlor (Anonymous (26), 1985) Maternal toxicity: lower body weight, body weight gain, food intake and clinical signs at 300 and 1000 mg/kg bw/d Developmental effects at the highest dose (1000 mg/kg bw/d): lower fetal weight and ossification delay	
2-generation reproduction study in rats, metolachlor administered via diet (Anonymous (33), 1981): Parental tocixity at 54.9 mg/kg bw/d: food intake ↓ (F_1 females), relative liver and thyroid wt ↑ No effects on fertility or reproduction observed up to highest dose tested (54.9 mg/kg bw/d) Developmental effects at 54.9 mg/kg bw/d: reduced mean pup body weigh	
Teratology study, rabbit, S-metolachlor (Anonymous (16), 1995) Maternal toxicity: lower body weight(gain), food intake at 500 mg/kg bw/d Developmental effects at the highest dose (500 mg/kg bw/d): lower fetal weight, higher incidence of fetal variation and malformations, mostly observed in a single litter. Two cases of hydrocephalus in different litters	
Teratology study, rabbit, metolachlor (Anonymous (25), 1980) Maternal toxicity: lower body weight (gain), food intake, clinical signs, abortions, death (presumed) at 360 mg/kg bw/d Developmental effects at 360 mg/kg bw/d: two pups (from the same litter) with hydrocephalus	

There are no appropriate epidemiological studies available on developmental effects in humans. Hence, classification with Category 1A according to CLP regulation is not possible.

The prenatal developmental toxicity of (S-)metolachlor was investigated in rats and rabbits complying with international test guidelines and GLP.

In rabbits, maternal toxicity (lower body weight, body weight gain, feed intake, clinical signs, abortions, death) was observed in animals treated with 360 mg/kg bw/d metolachlor and 500 mg/kg bw/d S-metolachlor. No treatment-related differences in the reproductive parameters number of pregnant females, mean number of Corpora lutea and live-births were reported. Developmental toxicity was confined to dose levels of 360 mg/kg bw/d metolachlor and 500 mg/kg bw/d S-metolachlor. After treatment with S-metolachlor fetal weight tended to be lowered and there was a higher incidence of a certain skeletal variation (fully formed ribs) and multiple malformations were observed in a single litter at the top dose level. Two cases of hydrocephalus were reported, one of them occurred in the heavily affected litter where several malformations were observed, the other one in a litter where no further malformations were observed. In the rabbit teratology study with metolachlor, also two cases of hydrocephalus were reported after treatment with 360 mg/kg bw/d. They were from the litter of a dam that died on day 29. Overall, 4 hydrocephaly were reported in three litters at the top dose levels tested. During the pesticides procedure, DS requested further historical control data on the spontaneous incidence of hydrocephaly to support the evaluation of this fetal malformation. This was provided by the applicants for both teratology studies in rabbits. On one hand, the historical control data suggest that the hydrocephalus in rabbit fetuses after administration of (S-)metolachlor might have occurred by chance, as it was reported in similar incidences, albeit in only a very low number of studies from the HCD. On the other hand, it must not be ignored that the same malformation was observed in two independent studies and that the mean incidence of the historical control database was exceeded.

During the pesticides procedure, the applicant provided, as part of the “additional information”, further considerations on the developmental toxicity and the resulting classification proposal (Anonymous (42), 2019) which have been copied in full length, into Vol. 3, B.6.6.2, just for transparency and to allow informed discussion.

One of the applicant’s arguments against classification is, again, the historical control data which, as discussed above, are not sufficient to put the study results into question even though they might raise some doubts.

Then, it is emphasised that there is no increase in further developmental findings in the rabbit but malformations may be, and often are, substance-specific and an increase in one severe finding might be sufficient for classification, in particular when, as in this case, it was observed in two independent studies. Malformations can be, and often are, also species-specific and, normally, it is not known which species is the better model for humans. Therefore, the additional argument that no teratogenic findings were observed in the rat must be rejected as well.

The applicant advocates separate and independent assessment of the two studies instead of combining the study results. However, this is just the approach taken by the DS. Occurrence of hydrocephalus in two separate and independent studies is a major argument for evaluation.

Occurrence of the same type of malformation in different strains is even of more concern.

Furthermore, the applicant argues that, in the study by Anonymus (25) (1980), in three litters sired by the same buck, various malformations were observed, including the high dose litter with two fetuses with hydrocephalus. A possible (genetic) impact of the male cannot be excluded but remains speculative. It must not be ignored that hydrocephalus was seen only at the highest dose level but not in the respective litters in the control or in the mid dose group even though the buck was the same. An effect of the test substance appears at least likely.

Eventually, the applicant questioned the examination method in these old studies. If this is true, the acceptability of the studies in general must be put into question. Inadequate technique could have also resulted in overlooking of critical findings.

Overall, in one animal species, i.e., the rabbit, there was evidence of a teratogenic effect since hydrocephalus was observed in a small number of fetuses and litters in two independent studies in different strains at high dose levels. In principle, Carc. Cat. 1B might be considered but the developmental effects have been observed only in the presence of overt maternal toxicity. In the Guidance on the Application of CLP Criteria (Version 5.0 - July 2017), the following is stated: ”*Classification shall not automatically be discounted for substances that produce developmental toxicity only in association with maternal toxicity, even if a specific maternally-mediated mechanism has been demonstrated*.” The latter is not the case here, i.e., a possible association between maternal toxicity and teratogenicity has not been sufficiently investigated. However, as it is further said: "*In such a case, classification in Category 2 may be considered more appropriate than in Category 1*." It seems that this approach would be most appropriate for S-metolachlor taking into account, in addition, that effects worth for classification were seen at a low incidence only and that, indeed, historical control data might raise some doubts. Accordingly, Category 2 for developmental toxicity (H361d, "Suspected of damaging the unborn child") is proposed.

10.10.7Adverse effects on or via lactation

No data are available to judge whether there are specific effects on or via lactation (H362).

10.10.8 Conclusion on classification and labelling for reproductive toxicity

In summary, classification with Repr. 2 (H361d) is considered appropriate.

10.11 Specific target organ toxicity-single exposure

This endpoint is not addressed in this CLH report and is outside the scope of the public consultation.

10.12 Specific target organ toxicity-repeated exposure

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

The specific target organ toxicity of S-metolachlor upon repeated exposure has been investigated in several regulatory 28 day and 90-day oral studies in rats, in 90-day and 1-year oral studies in dogs and in a 21-day dermal study in rabbits. Albeit deviations from the current test guidelines were noted, most of the studies could be considered for risk assessment. Results of this study are summarised in Table. Further details regarding study design, guideline (and deviations, if any) and information on incidences and severities of findings and extent of changes relative to controls are given in the text below or in the Volume 3 B.6 of the Renewal Assessment Report (RAR) provided as supplementary material. Two additional available studies regarding the specific target organ toxicity in rat and dog (Anonymous (7), 1974a and Anonymous 8, 1974b) were not taken into account: The studies were considered not acceptable due to several deficiencies (non guideline-, intermediary administration of higher doses to pre-treated animals, no overt signs of toxicity at top dose, incomplete report). For the pesticides procedure also studies regarding short-term toxicity of two environmental metabolites of S-metolachlor were assessed. Both metabolites are primary metabolites in the environment and were not or only to an extent of 0.14 % recovered in rat excreta. They are not expected to enhance the toxicity of S-metolachlor and results of toxicity testing are not reported here.

Table 34: Summary table of animal studies on STOT RE

<table>
<tr><th>Study type, compound, guideline, deviations if any</th><th>Dose levels</th><th>NOAEL mg/kg bw/d (M/F)</th><th>Results / Critical effects</th><th>Reference</th></tr>
<tr><td>28d, p.o. , rat, S-metolachlor (purity: 95.6%, batch: V4673/7, S-enantiomeric content: 78%)
test method B.8 of 92/69/EEC
some investigations were skipped (e.g., FOB regarding neuro toxicological properties, detailed clinical assessment, determination of several organ weights, histopathological evaluation of several organs)
non GLP</td><td>0, 30, 300, 3000, 5000 ppm (equal to 0, 2.65, 24.5, 242, 426.0 mg/kg bw/d (M) and 0, 2.73, 26.4, 257.0, 435.0 mg/kg bw/d (F)</td><td>24.5/26.4</td><td>liver weight ↑; centrilobular hypertrophy
<table>
<tr><td>Dose (mg/kg bw/d)</td><td>0</td><td>0</td><td>2.65</td><td>2.73</td><td>24.5</td><td>26.4</td><td>242</td><td>257</td><td>426</td><td>435</td></tr>
<tr><td>sex</td><td>M</td><td>F</td><td>M</td><td>F</td><td>M</td><td>F</td><td>M</td><td>F</td><td>M</td><td>F</td></tr>
<tr><td>absolute liver weight (wk 4)</td><td>15.14</td><td>8.33</td><td>14.63</td><td>8.34</td><td>15.14</td><td>8.09</td><td>16.72</td><td>9.42*</td><td>15.91</td><td>8.74</td></tr>
<tr><td>relative liver weight (bw) (wk 4)</td><td>46.74</td><td>40.91</td><td>45.42</td><td>40.67</td><td>46.95</td><td>40.08</td><td>52.93*</td><td>46.01*</td><td>55.94*</td><td>44.43</td></tr>
<tr><td>histopathology liver[a]</td><td>0/5</td><td>0/5</td><td>0/5</td><td>0/5</td><td>0/5</td><td>0/5</td><td>5/5</td><td>2/5</td><td>4/5</td><td>3/5</td></tr>
</table>
*: statistically significant (Wilcoxon test (p<0.05) or Jonckeere test (p < 0.01));
[a]: incidence of animals with slight hepatic centrilobular hypertrophy</td><td>Anonymous (12), 1995</td></tr>
<tr><td>28d, p.o. , rat, metolachlor (purity: 97.3%, batch: P111072, 48.8% w/w of each of the enantiomers)
test method B.8 of</td><td>0, 3000, 5000 ppm (equal to 0, 265, 447 mg/kg bw/d (M) and 0, 264, 433 mg/kg</td><td>Not established</td><td>liver weight ↑; centrilobular hypertrophy
<table>
<tr><td>Dose (mg/kg bw/d)</td><td>0</td><td>0</td><td>265</td><td>264</td><td>447</td><td>433</td></tr>
<tr><td>sex</td><td>M</td><td>F</td><td>M</td><td>F</td><td>M</td><td>F</td></tr>
</table></td><td>Anonymous (12), 1995</td></tr>
</table>

<table>
<tr><th>Study type, compound, guideline, deviations if any</th><th>Dose levels</th><th>NOAEL mg/kg bw/d (M/F)</th><th>Results / Critical effects</th><th>Reference</th></tr>
<tr><td>92/69/EEC
some investigations were skipped (e.g., FOB regarding neuro toxicological properties, detailed clinical assessment, determination of several organ weights, histopathological evaluation of several organs)
non GLP</td><td>bw/d (F)</td><td></td><td>
<table>
<tr><td>absolute liver weight (wk 5)</td><td>15.14</td><td>8.331</td><td>14.90</td><td>9.262</td><td>19.20*</td><td>10.12*</td></tr>
<tr><td>relative liver weight (bw) (wk 5)</td><td>46.74</td><td>40.91</td><td>48.54</td><td>45.07*</td><td>57.87*</td><td>47.09*</td></tr>
<tr><td>histopathology liver[a]</td><td>0/5</td><td>0/5</td><td>5/5</td><td>0/5</td><td>3/5</td><td>5/5</td></tr>
</table>
*: statistically significant (Wilcoxon test (p<0.05) or Jonckeere test (p < 0.01));
a: incidence of animals with slight hyaline cytological changes (kidney) and hepatic centrilobular hypertrophy (liver)</td><td></td></tr>
<tr><td>90d, p.o., rat, S-metolachlor (purity: 89.6%, batch: FL830813)
Protocol in compliance with test method B.26 of directive 92/69/EEC. Some investigations were skipped (e.g., determination of several organ weights, FOB regarding neuro toxicological properties, detailed clinical assessment).</td><td>0, 30, 300, 3000, 10000 ppm. Achieved doses males: 0, 1.9, 18.5, 187.9, 624.7 mg/kg bw and females: 0, 2.3, 24, 237.8, 763.9 mg/kg bw</td><td>18.5/24</td><td>↓b.w. and b.w. gain; altered clinical chemistry parameters; liver wt ↑ & histopathology, kidney wt
<table>
<tr><td>Dose (mg/kg bw/d)</td><td colspan="2">0</td><td>1.9</td><td>2.3</td><td>18.5</td><td>24</td><td>187.9</td><td>237.8</td><td>624.7</td><td>763.9</td></tr>
<tr><td></td><td>M</td><td>F</td><td>M</td><td>F</td><td>M</td><td>F</td><td>M</td><td>F</td><td>M</td><td>F</td></tr>
<tr><td>body weight (wk 13)</td><td>551.5</td><td>290.0</td><td>553.4</td><td>273.3</td><td>543.6</td><td>279.3</td><td>502.8*</td><td>260.4</td><td>477.9**</td><td>240.3</td></tr>
<tr><td>bw gain (wk 13; % wk 0)</td><td>140.08</td><td>68.66</td><td>140.27</td><td>64.44</td><td>133.00</td><td>66.6</td><td>117.33*</td><td>55.82**</td><td>104.15**</td><td>40.74**</td></tr>
<tr><td>feed consumption</td><td>26.4</td><td>18.8</td><td>27.0</td><td>17.7</td><td>25.9</td><td>18.7</td><td>24.7*</td><td>17.6</td><td>23.5**</td><td>16.4**</td></tr>
</table></td><td>Anonymous (4), 1995</td></tr>
</table>

<table>
<tr><th>Study type, compound, guideline, deviations if any</th><th>Dose levels</th><th>NOAEL mg/kg bw/d (M/F)</th><th>Results / Critical effects</th><th>Reference</th></tr>
<tr><td>non GLP</td><td></td><td></td><td>
<table>
<tr><td>(mean; g/day</td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td colspan="11">Haematology</td></tr>
<tr><td>leukocytes</td><td>19.38</td><td>9.83</td><td>13.49</td><td>9.27</td><td>14.69</td><td>8.51</td><td>13.34</td><td>8.16</td><td>13.35</td><td>5.89**</td></tr>
<tr><td colspan="11">Blood chemistry</td></tr>
<tr><td>SGOT</td><td>110.4</td><td>79.5</td><td>81.3</td><td>81.3</td><td>93.9</td><td>81.1</td><td>93.5</td><td>67</td><td>68.1**</td><td>60.9**</td></tr>
<tr><td>SGPT</td><td>30.4</td><td>39.7</td><td>29.3</td><td>34.6</td><td>23</td><td>30.1</td><td>19.6**</td><td>20.6**</td><td>16.1**</td><td>15.9**</td></tr>
<tr><td>γGT</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0.2</td><td>0</td><td>3.1**</td><td>2.7**</td></tr>
<tr><td>AP</td><td>56</td><td>35</td><td>61.3</td><td>36.1</td><td>48.6</td><td>25.5</td><td>43.3**</td><td>30.8</td><td>37.9**</td><td>34.8</td></tr>
<tr><td>cholesterol</td><td>79</td><td>77.8</td><td>69.9</td><td>67.1</td><td>66.1</td><td>74.6</td><td>67.6</td><td>75.7</td><td>74.0</td><td>89.2</td></tr>
<tr><td>bilirubin</td><td>0.298</td><td>0.255</td><td>0.196</td><td>0.191*</td><td>0.223</td><td>0.215*</td><td>2.9</td><td>0.181*</td><td>.23*</td><td>.213*</td></tr>
<tr><td>prot</td><td>6.75</td><td>7.18</td><td>6.91</td><td>7.05</td><td>6.93</td><td>7.04</td><td>7.19**</td><td>7.32</td><td>7.44**</td><td>7.54*</td></tr>
<tr><td>A/G-ratio</td><td>1.94</td><td>2.03</td><td>1.68**</td><td>1.96</td><td>1.73**</td><td>1.97</td><td>1.64**</td><td>1.84*</td><td>1.47**</td><td>1.66**</td></tr>
<tr><td colspan="11">Urinalysis: no compound related effect</td></tr>
<tr><td colspan="11">Organ weight (wk 13)</td></tr>
<tr><td>Liver (absolute)</td><td>13.8</td><td>8.3</td><td>17.2</td><td>9</td><td>18.3</td><td>5</td><td>14.3</td><td>9.3</td><td>15.2</td><td>7.9</td></tr>
<tr><td>Liver (relative)</td><td>2.543</td><td>2.834</td><td>3.111*</td><td>3.288</td><td>3.347**</td><td>2.87</td><td>2.842*</td><td>3.579**</td><td>3.274**</td><td>3.367**</td></tr>
<tr><td>Kidney (absolute)</td><td>2.8</td><td>1.9</td><td>3.2</td><td>1.9</td><td>3.1</td><td>1.8</td><td>3.3**</td><td>2</td><td>3.3**</td><td>1.8</td></tr>
<tr><td>Kidney (relative)</td><td>0.521</td><td>0.642</td><td>0.577</td><td>0.678</td><td>0.570</td><td>0.654</td><td>0.654**</td><td>0.767**</td><td>0.717**</td><td>0.773**</td></tr>
</table>
</td><td></td></tr>
</table>

<table>
<tr><th>Study type, compound, guideline, deviations if any</th><th>Dose levels</th><th>NOAEL mg/kg bw/d (M/F)</th><th>Results / Critical effects</th><th>Reference</th></tr>
<tr><td></td><td></td><td></td><td>
<table>
<tr><td>Adrenals (absolute)</td><td>0.054</td><td>0.064</td><td>0.057</td><td>0.065</td><td>0.052</td><td>0.064</td><td>0.049</td><td>0.061</td><td>0.042**</td><td>0.053*</td></tr>
<tr><td>Adrenals (relative)</td><td>0.010</td><td>0.022</td><td>0.010</td><td>0.024</td><td>0.010</td><td>0.023</td><td>0.010</td><td>0.024</td><td>0.009</td><td>0.023</td></tr>
<tr><td colspan="11">Histopathology liver (wk 13)</td></tr>
<tr><td>glycogen accumulation</td><td>1</td><td>0</td><td>6</td><td>4</td><td>9</td><td>2</td><td>0</td><td>0</td><td>1</td><td>2</td></tr>
<tr><td>eosinophilic inclusions</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>1</td><td>0</td><td>7</td><td>0</td></tr>
</table>
statistically significant change (*, p<0.05 or **, p<0.01);
</td><td></td></tr>
<tr><td>90d, p.o., rat, S-metolachlor (purity: 98.5%, batch: P501001, S-enantiomer content: 87.2% w/w)

Protocol in compliance with test method B.26 of directive 92/69/EEC. Some investigations were skipped (e.g., FOB regarding neuro toxicological properties, detailed clinical assessment).</td><td>30, 300, 3000 ppm. Achieved doses in males: 0, 1.90, 20.4, 208 mg/kg bw/d and in females: 0, 2.13, 23.9, 236 mg/kg bw/d</td><td>20.4/23.9</td><td>liver weight ↑; ↑; kidney weight ↑, b.w. gain ↓, altered clinical chemistry and urine analysis parameters (i.e. leukocyturia)
<table>
<tr><td>Dose (mg/kg bw/d)</td><td colspan="2">0</td><td>1.9</td><td>2.13</td><td>20.4</td><td>23.9</td><td>208</td><td>236</td></tr>
<tr><td></td><td>M</td><td>F</td><td>M</td><td>F</td><td>M</td><td>F</td><td>M</td><td>F</td></tr>
<tr><td colspan="9">Haematology</td></tr>
<tr><td>MetHb</td><td>0.008</td><td>0.008</td><td>0.008</td><td>0.008</td><td>0.008</td><td>0.009</td><td>0.009*</td><td>0.009*</td></tr>
<tr><td colspan="9">Blood chemistry</td></tr>
<tr><td>glucose</td><td>7.399</td><td>7.309</td><td>7.127</td><td>6.786</td><td>6.747*</td><td>7.932</td><td>6.327**</td><td>7.357</td></tr>
<tr><td>urea</td><td>4.921</td><td>5.557</td><td>5.076</td><td>4.990</td><td>5.280</td><td>5.412</td><td>5.543**</td><td>5.387</td></tr>
<tr><td>creatinine</td><td>21.44</td><td>23.98</td><td>22.55</td><td>21.42</td><td>21.23</td><td>23.29</td><td>18.47**</td><td>22.49</td></tr>
<tr><td>cholesterol</td><td>1.705</td><td>1.994</td><td>1.936</td><td>2.065</td><td>1.644</td><td>2.121</td><td>2.272*</td><td>2.014</td></tr>
</table>
</td><td>Anonymous (13), 1999</td></tr>
</table>

<table>
<tr><th>Study type, compound, guideline, deviations if any</th><th>Dose levels</th><th>NOAEL mg/kg bw/d (M/F)</th><th>Results / Critical effects</th><th>Reference</th></tr>
<tr><td>non GLP</td><td></td><td></td><td>
<table>
<tr><td>bilirubin</td><td>1.807</td><td>2.373</td><td>1.790</td><td>2.786</td><td>1.664</td><td>2.276</td><td>1.484*</td><td>1.698**</td></tr>
<tr><td>ASAT (GOT)</td><td>80.41</td><td>84.76</td><td>76.51</td><td>106.8</td><td>79.89</td><td>64.01*</td><td>66.74*</td><td>60.59**</td></tr>
<tr><td>globulin</td><td>36.91</td><td>35.85</td><td>37.39</td><td>35.61</td><td>37.47</td><td>36.90</td><td>40.48**</td><td>37.91*</td></tr>
<tr><td>A/G-ratio</td><td>0.951</td><td>1.117</td><td>0.928</td><td>1.143</td><td>0.919</td><td>1.088</td><td>0.882*</td><td>1.049**</td></tr>
<tr><td colspan="9">Urinalysis</td></tr>
<tr><td>leukocyturia</td><td>82.50</td><td>15.79</td><td>55.00</td><td>20.00</td><td>77.50</td><td>27.50</td><td>212.5*</td><td>30.00</td></tr>
<tr><td colspan="9">Organ weight (wk 13)</td></tr>
<tr><td>Liver abs (g)</td><td>18.68</td><td>10.26</td><td>17.21</td><td>9.945</td><td>19.1</td><td>9.954</td><td>20.29</td><td>10.3</td></tr>
<tr><td>Liver rel (‰)</td><td>38.94</td><td>38.04</td><td>37.08</td><td>37.56</td><td>39.37</td><td>36.34*</td><td>45.24**</td><td>40.89**</td></tr>
<tr><td>Kidney abs (g)</td><td>3.265</td><td>1.952</td><td>2.942</td><td>2.026</td><td>3.347</td><td>2.01</td><td>3.49</td><td>1.985</td></tr>
<tr><td>Kidney rel (‰)</td><td>6.803</td><td>7.282</td><td>6.356</td><td>7.662</td><td>6.908</td><td>7.351</td><td>7.807**</td><td>7.523</td></tr>
<tr><td>Spleen abs (g)</td><td>0.756</td><td>0.558</td><td>0.784</td><td>0.716</td><td>0.853*</td><td>0.567</td><td>0.781</td><td>0.513</td></tr>
<tr><td>Spleen rel (‰)</td><td>1.577</td><td>2.083</td><td>1.695</td><td>2.707</td><td>1.775*</td><td>2.082</td><td>1.755**</td><td>2.033</td></tr>
</table>
statistically significant change (Wilcoxon test or Lepage test or Jonckheere test: *, p<0.05 or **, p<0.01);
</td><td></td></tr>
<tr><td>90d, p.o., rat, metolachlor (purity: 97.7%, batch: P111072, 48.8% w/w of each of the enantiomers)
Protocol in compliance with test method B.26 of directive 92/69/EEC. Some investigations</td><td>0, 30, 300, 3000 ppm. Achieved doses in males: 0, 1.99, 20.2, 210.4 mg/kg bw/d and in females: 0, 2.32, 23.4, 259.3 mg/kg bw/d</td><td>20.2/23.4</td><td>↓b.w. and b.w. gain, leukocyturia
<table>
<tr><td>Dose (mg/kg bw/d)</td><td colspan="2">0</td><td>1.9</td><td>2.13</td><td>20.4</td><td>23.9</td><td>208</td><td>236</td></tr>
<tr><td></td><td>M</td><td>F</td><td>M</td><td>F</td><td>M</td><td>F</td><td>M</td><td>F</td></tr>
<tr><td colspan="9">Haematology</td></tr>
<tr><td>MetHb</td><td>0.008</td><td>0.008</td><td>0.008</td><td>0.008</td><td>0.008</td><td>0.008</td><td>0.008</td><td>0.009</td></tr>
<tr><td colspan="9">Blood chemistry</td></tr>
</table>
</td><td>Anonymous (14), 1999</td></tr>
</table>

<table>
<tr><th>Study type, compound, guideline, deviations if any</th><th>Dose levels</th><th>NOAEL mg/kg bw/d (M/F)</th><th>Results / Critical effects</th><th>Reference</th></tr>
<tr><td>were skipped (e.g., FOB regarding neuro toxicological properties, detailed clinical assessment).

non GLP</td><td></td><td></td><td>
<table>
<tr><td>glucose</td><td>7.050</td><td>7.793</td><td>6.787</td><td>6.462**</td><td>6.812</td><td>7.845</td><td>7.138</td><td>7.627</td></tr>
<tr><td>urea</td><td>5.401</td><td>6.077</td><td>5.012</td><td>5.902</td><td>5.642</td><td>6.485</td><td>5.723</td><td>5.989</td></tr>
<tr><td>creatinine</td><td>21.77</td><td>26.58</td><td>22.56</td><td>21.83**</td><td>20.86</td><td>24.91</td><td>22.41</td><td>24.27*</td></tr>
<tr><td>cholesterol</td><td>1.779</td><td>2.398</td><td>1.773</td><td>2.296</td><td>1.951</td><td>2.125</td><td>2.049*</td><td>2.249</td></tr>
<tr><td>bilirubin</td><td>1.651</td><td>2.153</td><td>1.684</td><td>2.791**</td><td>1.439</td><td>2.568**</td><td>1.43*</td><td>1.855</td></tr>
<tr><td>ASAT (GOT)</td><td>77.11</td><td>76.44</td><td>78.82</td><td>74.76</td><td>80.73</td><td>74.46</td><td>63.41**</td><td>60.76</td></tr>
<tr><td>ALAT (GPT)</td><td>34.20</td><td>32.86</td><td>38.66</td><td>34.68</td><td>43.80</td><td>28.66</td><td>30.74</td><td>23.80*</td></tr>
<tr><td>globulin</td><td>37.64</td><td>37.08</td><td>36.67</td><td>37.04</td><td>37.38</td><td>34.93</td><td>39.29</td><td>36.65</td></tr>
<tr><td>A/G-ratio</td><td>0.934</td><td>1.098</td><td>0.938</td><td>1.122</td><td>0.951</td><td>1.169*</td><td>0.901</td><td>1.097</td></tr>
<tr><td colspan="9">Urinalysis</td></tr>
<tr><td>leukocyturia</td><td>102.5</td><td>17.5</td><td>111.1</td><td>17.5</td><td>117.5</td><td>17.5</td><td>172.5*</td><td>32.5*</td></tr>
<tr><td colspan="9">Organ weight (wk 13)</td></tr>
<tr><td>Liver abs (g)</td><td>18.27</td><td>11.00</td><td>18.59</td><td>9.737*</td><td>19.46*</td><td>9.631</td><td>18.36</td><td>10.48</td></tr>
<tr><td>Liver rel (‰)</td><td>38.83</td><td>38.32</td><td>37.50</td><td>36.79</td><td>40.99*</td><td>37.21</td><td>40.22</td><td>41.65**</td></tr>
<tr><td>Kidney abs (g)</td><td>3.233</td><td>2.021</td><td>3.366</td><td>2.027</td><td>3.477</td><td>2.021</td><td>3.285</td><td>2.004</td></tr>
<tr><td>Kidney rel (‰)</td><td>6.889</td><td>7.037</td><td>6.798</td><td>7.653*</td><td>7.338</td><td>7.806**</td><td>7.185</td><td>7.976**</td></tr>
<tr><td>Spleen abs (g)</td><td>0.758</td><td>0.567</td><td>0.801</td><td>0.541</td><td>0.834</td><td>0.560</td><td>0.742</td><td>0.544</td></tr>
<tr><td>Spleen rel (‰)</td><td>1.622</td><td>1.974</td><td>1.633</td><td>2.053</td><td>1.772</td><td>2.155</td><td>1.625</td><td>2.167</td></tr>
</table>
statistically significant change (Wilcoxon test or Lepage test or Jonckheere test: *, p<0.05 or **, p<0.01);</td><td></td></tr>
<tr><td>90d, p.o., dog, S-metolachlor (purity: 95.4%, batch: FL941255, S-</td><td>0, 300, 500, 1000, 2000 ppm. Achieved</td><td>15.1/17.2</td><td>relative liver weight ↑, body weight ↓, food consumption ↓, histopathological findings in liver and epididymis</td><td>Anonymous (3), 1995</td></tr>
</table>

<table>
<tr><th>Study type, compound, guideline, deviations if any</th><th>Dose levels</th><th>NOAEL mg/kg bw/d (M/F)</th><th>Results / Critical effects</th><th>Reference</th></tr>
<tr><td>enantiomer content: 84.3% w/w, R-enantiomer content: 11.1% w/w)
OECD 409
GLP</td><td>doses: males: 0, 9.0, 15.1, 31.1, 62 mg/kg bw/d and females: 0, 10.0, 17.2, 31.5, 74 mg/kg bw/d</td><td></td><td>
<table>
<tr><td>Dose (mg/kg bw/d)</td><td colspan="2">0</td><td>9</td><td>10</td><td>15.1</td><td>17.2</td><td>31.1</td><td>31.5</td><td>62</td><td>74</td></tr>
<tr><td></td><td>M</td><td>F</td><td>M</td><td>F</td><td>M</td><td>F</td><td>M</td><td>F</td><td>M</td><td>F</td></tr>
<tr><td>body weight (wk 13)</td><td>11.33</td><td>9.88</td><td>10.83</td><td>9.45</td><td>11.23</td><td>9.1</td><td>9.7</td><td>9.25</td><td>11.55</td><td>9.25</td></tr>
<tr><td>bw gain (wk 13; % wk 0)</td><td>116.3</td><td>123.5</td><td>109.7</td><td>119.2</td><td>112.3</td><td>115.9</td><td>96.5</td><td>113.8</td><td>118.9</td><td>117.1</td></tr>
<tr><td colspan="11">Organ weight (wk 13)</td></tr>
<tr><td>Liver (absolute)</td><td>310.61</td><td>286.203</td><td>293.848</td><td>260.718</td><td>336.63</td><td>239.738</td><td>316.598</td><td>230.268*</td><td>358.095</td><td>285.218</td></tr>
<tr><td>Liver (relative)</td><td>2.688</td><td>2.791</td><td>2.683</td><td>2.755</td><td>2.917</td><td>2.603</td><td>3.263*</td><td>2.579</td><td>2.968</td><td>3.112</td></tr>
<tr><td colspan="11">Histopathology (wk 13)</td></tr>
<tr><td colspan="11">Liver: Perivascular infalmmation, acute</td></tr>
<tr><td>R lateral lobe</td><td>0/4</td><td>1/4</td><td>0/4</td><td>0/4</td><td>1/4</td><td>1/4</td><td>0/4</td><td>0/4</td><td>0/4</td><td>3/4</td></tr>
<tr><td>L lateral lobe</td><td>0/4</td><td>1/4</td><td>0/4</td><td>0/4</td><td>1/4</td><td>0/4</td><td>0/4</td><td>0/4</td><td>0/4</td><td>3/4</td></tr>
<tr><td colspan="11">Epididymis, degenerative cells in tubules</td></tr>
<tr><td>Right</td><td>0/4</td><td></td><td>0/4</td><td></td><td>0/4</td><td></td><td>0/4</td><td></td><td>1/4</td><td></td></tr>
<tr><td>Left</td><td>0/4</td><td></td><td>0/4</td><td></td><td>0/4</td><td></td><td>0/4</td><td></td><td>3/4</td><td></td></tr>
</table>
*significant different from control, p<=0.05, Dunnet’s t-Test, two tailed</td><td></td></tr>
<tr><td>3 month, p.o., dog, S-metolachlor (purity: 98.5%, batch: P.501001, S-enantiomer content: 87.2% w/w)</td><td>200 mg/kg bw/d (only one dose tested)</td><td>LOAEL: 200 mg/kg bw/d</td><td>liver weight ↑, AP ↑, GGT ↑
<table>
<tr><td>Dose (mg/kg bw/d)</td><td colspan="2">0</td><td colspan="2">200</td></tr>
<tr><td></td><td>M</td><td>F</td><td>M</td><td>F</td></tr>
</table></td><td>Anonymous (43), 1999</td></tr>
</table>

<table>
<tr><th>Study type, compound, guideline, deviations if any</th><th>Dose levels</th><th>NOAEL mg/kg bw/d (M/F)</th><th>Results / Critical effects</th><th>Reference</th></tr>
<tr><td></td><td></td><td></td><td>
<table>
<tr><td>ALP activity (U/L) – week 7</td><td>74.8</td><td>63.75</td><td>265.4*</td><td>211.9*</td></tr>
<tr><td>ALP activity (U/L) – week 13</td><td>68.35</td><td>62.08</td><td>308.7*</td><td>256.1*</td></tr>
<tr><td>GGT activity (U/L) – week 7</td><td>2.750</td><td>2.825</td><td>6.350*</td><td>5.250*</td></tr>
<tr><td>GGT activity (U/L) – week 13</td><td>3.575</td><td>1.800</td><td>13.01*</td><td>8.525*</td></tr>
<tr><td>Liver weight (g)</td><td>344.2</td><td>288.1</td><td>470.2*</td><td>429.5*</td></tr>
<tr><td>Liver:body weight ratio %</td><td>30.81</td><td>26.03</td><td>40.10</td><td>40.02*</td></tr>
</table>
* Wilcoxon p < 0.04
+/- Jonckheere p < 0.01</td><td></td></tr>
<tr><td>6 month, p.o. , dog, metolachlor; purity not reported, batch: FL-781314)
Study was performed prior to the publication of regulatory guidelines
non GLP</td><td>0, 100, 300, 1000 ppm. Achieved doses:
males: 0, 2.92, 9.71, 29.61 mg/kg bw/d and
females: 0, 2.97, 8.77, 29.42 mg/kg bw/d</td><td>2.92 / 2.97</td><td>body weight ↓, body weight gain ↓, AP ↑, haematological changes
<table>
<tr><th rowspan="3">Week</th><th colspan="8">Dietary concentration (ppm)</th></tr>
<tr><td colspan="4">Males</td><td colspan="4">Females</td></tr>
<tr><td>0</td><td>100</td><td>300</td><td>1000</td><td>0</td><td>100</td><td>300</td><td>1000</td></tr>
<tr><td colspan="9">Body weight and body weight gain</td></tr>
<tr><td>1</td><td>11.6</td><td>11.8</td><td>10.7 (92%)</td><td>11.1 (96%)</td><td>8.4</td><td>8.7</td><td>8.6</td><td>8.6</td></tr>
<tr><td>4</td><td>12.3</td><td>12.5</td><td>11.1 (90%)</td><td>11.5 (94%)</td><td>9.2</td><td>9.3</td><td>9.3</td><td>9.1</td></tr>
<tr><td>8</td><td>12.8</td><td>12.6</td><td>11.9 (93%)</td><td>12.1 (95%)</td><td>9.5</td><td>9.5</td><td>9.9</td><td>9.5</td></tr>
<tr><td>16</td><td>13.4</td><td>13.1</td><td>12.6</td><td>12.2</td><td>10.0</td><td>10.2</td><td>10.6</td><td>9.8</td></tr>
</table>
</td><td>Anonymous (44), 1980,</td></tr>
</table>

<table>
<tr><th>Study type, compound, guideline, deviations if any</th><th>Dose levels</th><th>NOAEL mg/kg bw/d (M/F)</th><th>Results / Critical effects</th><th>Reference</th></tr>
<tr><td></td><td></td><td></td><td>
<table>
<tr><td></td><td></td><td></td><td>(94%)</td><td>(91%)</td><td></td><td></td><td></td><td></td></tr>
<tr><td>24</td><td>13.6</td><td>13.0</td><td>12.6
(93%)</td><td>12.3
(90%)</td><td>10.1</td><td>10.3</td><td>10.4</td><td>9.7</td></tr>
<tr><td>26</td><td>14.2</td><td>13.6</td><td>12.8
(90%)</td><td>12.4
(87%)</td><td>10.5</td><td>10.8</td><td>10.5</td><td>10.0</td></tr>
<tr><td>30</td><td>12.5</td><td>-</td><td>-</td><td>11.5
(92%)</td><td>10.1</td><td>-</td><td>-</td><td>8.5</td></tr>
<tr><td>1–13</td><td>1.60</td><td>1.25
(78%)</td><td>1.57
(98%)</td><td>0.75
(47%)</td><td>1.39</td><td>1.08</td><td>1.58
(114%)</td><td>0.95
(68%)</td></tr>
<tr><td>1–26</td><td>2.44</td><td>1.80
(74%)</td><td>2.18
(89%)</td><td>1.34
(55%)</td><td>2.06</td><td>2.08</td><td>1.88
(91%)</td><td>1.40
(68%)</td></tr>
<tr><td colspan="9">No statistically significant differences from control values; % of control</td></tr>
<tr><td colspan="9">ALP activity (IU/L)</td></tr>
<tr><td>Month 6</td><td>56</td><td>77</td><td>78**
(139%)</td><td>87*
(155%)</td><td>69</td><td>96</td><td>83
(120%)</td><td>100
(145%)</td></tr>
<tr><td colspan="9">* Statistically significant difference from control group mean, p<0.05, ** Statistically significant difference from control group mean, p<0.01, % of control</td></tr>
</table>
</td><td></td></tr>
<tr><td>1 year p.o., dog, metolachlor; purity: 97%, batch: FL861768

Protocol partly in compliance with test method B.30 of directive 92/69/EEC. Bodyweight variation</td><td>0, 100, 300, 1000 ppm. Achieved doses: 3.5, 9.7, 32.7 mg/kg bw/d in males and 3.6, 9.7, 33.0 mg/kg bw/d in</td><td>3.5/3.6</td><td>AP ↑; kidney wt ↓
<table>
<tr><td rowspan="2">Dose (mg/kg bw/d)</td><td colspan="2">0</td><td>3.5</td><td>3.6</td><td>9.7</td><td>9.7</td><td>32.7</td><td>33.0</td></tr>
<tr><td>M</td><td>F</td><td>M</td><td>F</td><td>M</td><td>F</td><td>M</td><td>F</td></tr>
<tr><td colspan="9">Body weight change</td></tr>
<tr><td>Bw (kg) Baseline</td><td>7.03</td><td>6.45</td><td>6.86</td><td>5.98</td><td>7.19</td><td>6.24</td><td>6.77</td><td>6.34</td></tr>
<tr><td>Bw (kg) Day 364</td><td>9.8</td><td>8.98</td><td>10.83</td><td>8.53</td><td>9.75</td><td>8.4</td><td>9.58</td><td>8.45</td></tr>
</table>
</td><td>Anonymous (20), 1989</td></tr>
</table>

<table>
<tr><th>Study type, compound, guideline, deviations if any</th><th>Dose levels</th><th>NOAEL mg/kg bw/d (M/F)</th><th>Results / Critical effects</th><th>Reference</th></tr>
<tr><td>at start of trial > 20 %, no GT dosages
GLP</td><td>females</td><td></td><td>
<table>
<tr><td>% Change Bw</td><td>100</td><td>100</td><td>143.1</td><td>100.7</td><td>92.5</td><td>85.4</td><td>101.6</td><td>83.3</td></tr>
<tr><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>Kidney (absolute, day 365)</td><td>56.022</td><td>35.71</td><td>47.007</td><td>37.912</td><td>45.580* (-19%)</td><td>36.192</td><td>41.007** (-27%)</td><td>35.572</td></tr>
<tr><td>Kidney (relative to bw, % bw, day 365)</td><td>0.563</td><td>0.400</td><td>0.444* (-21%)</td><td>0.449</td><td>0.481 (-15%)</td><td>0.439</td><td>0.443* (-21%)</td><td>0.429</td></tr>
<tr><td>Kidney (relative to brain, % brain, day 365)</td><td>65.063</td><td>45.844</td><td>55.121* (-15%)</td><td>46.675</td><td>54.235* (-17%)</td><td>44.397</td><td>50.075** (-23%)</td><td>44.409</td></tr>
<tr><td colspan="9">Mean Alkaline Phosphatase (U/L)</td></tr>
<tr><td>Day -16</td><td>119</td><td>115</td><td>129</td><td>126</td><td>135</td><td>107</td><td>124</td><td>109</td></tr>
<tr><td>Day 82</td><td>80</td><td>73</td><td>81</td><td>82</td><td>90</td><td>77</td><td>99</td><td>103*</td></tr>
<tr><td>Day 180</td><td>50</td><td>41</td><td>50</td><td>55</td><td>62</td><td>58</td><td>71</td><td>75*</td></tr>
<tr><td>Day 278</td><td>35</td><td>34</td><td>39</td><td>46</td><td>45</td><td>44</td><td>53</td><td>56*</td></tr>
<tr><td>Day 358</td><td>37</td><td>56</td><td>43</td><td>55</td><td>49</td><td>46</td><td>60</td><td>72</td></tr>
</table>
*0.01<P<= 0.05, two tailed Dunnet t-Test on raw data.
**P<= 0.01, two tailed Dunnet t-Test on raw data.
</td><td></td></tr>
<tr><td>21d, dermal, rabbit, metolachlor (purity: 96.4%, batch: FL841697)
Protocol partially in compliance with test method B.9 of directive 92/69/EEC. 21 days instead of 28</td><td>0, 10, 100, 1000 mg/kg bw</td><td>Systemic: 100/100
Local: < 10/<10</td><td>liver weight ↑, local dermal effects at 10 mg/kg bw/d
<table>
<tr><td rowspan="3"></td><td colspan="8">Dose (mg/kg bw/d)</td></tr>
<tr><td colspan="2">0</td><td colspan="2">10</td><td colspan="2">100</td><td colspan="2">1000</td></tr>
<tr><td>M</td><td>F</td><td>M</td><td>F</td><td>M</td><td>F</td><td>M</td><td>F</td></tr>
<tr><td colspan="9">Clinical chemistry</td></tr>
<tr><td>Bilirubin (mg/dL, day 19)</td><td>0.240</td><td>0.142</td><td>0.214</td><td>0.172</td><td>0.262</td><td>0.238</td><td>0.248</td><td>0.2440</td></tr>
</table>
</td><td>Anonymous (30), 1987</td></tr>
</table>

<table>
<tr><th>Study type, compound, guideline, deviations if any</th><th>Dose levels</th><th>NOAEL mg/kg bw/d (M/F)</th><th>Results / Critical effects</th><th>Reference</th></tr>
<tr><td>days treatment
GLP</td><td></td><td></td><td>
<table>
<tr><td></td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0*</td><td>0</td><td>*</td></tr>
<tr><td colspan="9">Organ weights</td></tr>
<tr><td>Liver (absolute, g)
(% of control)</td><td>82.6
(100)</td><td>83.4
(100)</td><td>98.0
(119)</td><td>84.0
(101)</td><td>101.9
(123)</td><td>68.2
(82)</td><td>135.8 *
(164)</td><td>108.2
(130)</td></tr>
<tr><td>Liver (relative to bw, %bw)
(% of control)</td><td>2.579
(100)</td><td>2.428
(100)</td><td>3.042
(118)</td><td>2.610
(107)</td><td>3.283
(127)</td><td>2.310
(95)</td><td>4.132 *
(160)</td><td>3.307
(136)</td></tr>
<tr><td>Liver (relative to brain wt, %brain wt)
(% of control)</td><td>921
(100)</td><td>863
(100)</td><td>1064
(116)</td><td>944
(109)</td><td>1174
(127)</td><td>712
(83)</td><td>1531 *
(166)</td><td>1132
(131)</td></tr>
<tr><td>Kidney (absolute, g)
(% of control)</td><td>18.5
(100)</td><td>17.0
(100)</td><td>17.5
(95)</td><td>16.7
(98)</td><td>17.8
(96)</td><td>15.2
(89)</td><td>21.8
(118)</td><td>19.8
(116)</td></tr>
<tr><td>Kidney (relative to bw, %bw)
(% of control)</td><td>0.5818
(100)</td><td>0.5006
(100)</td><td>0.5401
(93)</td><td>0.5295
(106)</td><td>0.5779
(99)</td><td>0.5193
(104)</td><td>0.6674
(115)</td><td>0.6120 *
(122)</td></tr>
<tr><td>Kidney (relative to brain wt, %brain wt)
(% of control)</td><td>205.6
(100)</td><td>175.1
(100)</td><td>177.7
(86)</td><td>186.9
(107)</td><td>203.0
(99)</td><td>159.2
(91)</td><td>246.1
(120)</td><td>208.1
(119)</td></tr>
<tr><td colspan="9">Gross pathology'</td></tr>
<tr><td>erythema</td><td>0</td><td>0</td><td>5</td><td>5</td><td>5</td><td>5</td><td>5</td><td>5</td></tr>
<tr><td>dry skin</td><td>0</td><td>0</td><td>5</td><td>5</td><td>5</td><td>5</td><td>5</td><td>5</td></tr>
<tr><td>fissuring</td><td>0</td><td>0</td><td>0</td><td>1</td><td>2</td><td>2</td><td>5</td><td>5</td></tr>
<tr><td>wrinkles</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>5</td><td>5</td></tr>
<tr><td colspan="9">Histopathology (dermis, back)'</td></tr>
<tr><td>Hyperkeratosis (minimal)</td><td>0</td><td>0</td><td>5^{*}</td><td>3</td><td>5^{*}</td><td>5^{*}</td><td>5^{*}</td><td>5^{*}</td></tr>
</table>
</td><td></td></tr>
</table>

<table>
<tr><th>Study type, compound, guideline, deviations if any</th><th>Dose levels</th><th>NOAEL mg/kg bw/d (M/F)</th><th>Results / Critical effects</th><th>Reference</th></tr>
<tr><td></td><td></td><td></td><td>
<table>
<tr><td>Parakeratosis (minimal)</td><td>0</td><td>0</td><td>1</td><td>3</td><td>3</td><td>4*</td><td>2</td><td>5*</td></tr>
<tr><td>subacute lymphocytic inflammation (focal)</td><td>0</td><td>0</td><td>0</td><td>3</td><td>3</td><td>3</td><td>5*</td><td>4*</td></tr>
<tr><td>Congestion (focal)</td><td>0</td><td>0</td><td>1</td><td>3</td><td>3</td><td>4</td><td>5*</td><td>5*</td></tr>
</table>
$^{'}$: incidence on 5 animals/examined tissues; significance: Fisher=s exact test * $p<0.05$
</td><td></td></tr>
</table>

In the short-term 28-day toxicity studies in the rat, liver was detected as being the primary target organ, as reflected by modifications of clinical chemistry parameters such as increased cholesterol, protein and globulin levels, and decreased A/G-ratio from 242 mg/kg bw/d on. At this feeding level, an increased liver weight was observed, while histopathology revealed a slight hepatic centrilobular hypertrophy. A similar toxicological profile was established for metolachlor within the same study. In the 90 day feeding study in the rat, hepatotoxicity starting at doses of approx. 188 mg/kg bw/d was confirmed by the modifications of cholesterol, protein and A/G-levels, and by increased γGT-activity at approx. 625 mg/kg bw/d. Other signs of toxicity included liver and kidney weight increase, and lowered body weight, body weight gain and food consumption from 188 mg/kg bw/d onwards. The histopathological appearance of eosinophilic hepatocytic inclusions at this dose was confined to male animals, suggesting a higher susceptibility for liver injury in this sex. Increased leucocyturia was observed in both sexes.

In dogs, the main effects reported at doses from approx. 9 mg/kg bw/d were decreased body weight, increased AP-activity (6-month and 1-year study) and decreased kidney weight (1-year study). Increased liver weight was observed from approx. 31 mg/kg bw/d (90-d study). The dog was detected as being the most sensitive species.

Dermal systemic toxicity in the rabbit confirmed the liver as the target organ, with increased weight at 1000 mg/kg bw/d. In females, an increase of relative kidney weight was observed at 1000 mg/kg bw/d. However, significant local effects including dry skin and erythema (generally Draize score 1 besides to one occurrence of score 2 in one male in mid dose group, test day 5) and fissuring were seen in treated skin of all dose groups starting at 10 mg/kg bw/d. At this dose erythema were observed as early as test day 6, dry skin as early as test day 9, fissuring as early as test day 11. Wrinkling of the skin was observed in animals receiving 1000 mg/kg bw/d from day 6 on. Histopathological skin lesions included hyper- and parakeratosis at all dose-levels in both sexes. Additionally, congestion and subacute lymphocytic dermal inflammation of the dermis was observed in both sexes.

Human data on adverse effects after repeated dermal exposure is not available.

10.12.2 Comparison with the CLP criteria

After oral administration, no effects of sufficient severity were reported in available studies that would lead to a classification for STOT RE; the results of these studies are therefore not included in the comparison with the CLP criteria in Table 35.

Table 35: Toxicological results concerning adverse effects after repeated dermal exposure

Toxicological results	**CLP criteria**
21-day dermal toxicity study in rabbits (Anonymous (30), 1987) skin effects (erythema, dry skin, fissuring, minimal hyperkeratosis and parakeratosis, focal subacute lymphocytic inflammation and focal congestion) starting at dose levels of 10 mg/kg bw/d	Category 1 (H372): Substances that have produced significant toxicity in humans or that, based on evidence from studies in experimental animals, can be presumed to have the potential to produce significant toxicity in humans following repeated exposure. Substances are classified in Category 1 for target organ toxicity (repeat exposure) on the basis of: reliable and good quality evidence from human cases or epidemiological studies; or observations from appropriate studies in experimental animals in which significant and/or severe toxic effects, of relevance to human health, were produced at generally low exposure concentrations. Equivalent guidance values for 28-day and 90-day studies: Dermal, rat: 28-day: ≤ 60 mg/kg bw/d 90-day: ≤ 20 mg/kg bw/d
	Category 2 (H373):

Toxicological results	CLP criteria
	Substances that, based on evidence from studies in experimental animals can be presumed to have the potential to be harmful to human health following repeated exposure. Substances are classified in category 2 for target organ toxicity (repeat exposure) based on observations from appropriate studies in experimental animals in which significant toxic effects, of relevance to human health, were produced at generally moderate exposure concentrations. In exceptional cases, human evidence can also be used to place a substance in Category 2. Equivalent guidance values for 28-day and 90-day studies: Dermal, rat: 28-day: ≤ 600 mg/kg bw/d 90-day: ≤ 200 mg/kg bw/d

After dermal administration, effects in skin were seen in rabbits at dose levels of 10 mg/kg bw/d and above. Effects included erythema (generally Draize score 1 besides to one occurrence of score 2 in one male in mid dose group), dry skin and fissuring after gross examination and after histopathological examination minimal hyperkeratosis, minimal parakeratosis, focal subacute lymphocytic inflammation and focal congestion. At higher dose levels (100 or 1000 mg/kg bw/d) higher incidences of animals were affected but the severity grading did not aggravate. There are no appropriate epidemiological studies available on specific target organ toxicity from repeated exposure in humans. According to the criteria in CLP regulation, severe effects observed at generally low exposure dose levels (below 20 mg/kg bw/d for a 90-day study and below 60 mg/kg bw/d for a 28-day study) need to be considered for a categorisation into Cat. 1, while significant effects observed at generally moderate exposure dose levels (between 20 and 200 mg/kg bw/d for a 90-day study and between 60 to 600 mg/kg bw/d for a 28-day study) need to be considered for a categorisation into Cat. 2.

While effects were observed already at dose levels compatible with Cat. 1, the reported effects (especially fissuring, inflammation and congestion) are considered more as signs of significant toxicity than those of severe toxicity. Hence, a classification with STOT RE 2 (skin) is proposed.
As bridging from metolachlor to S-metolachlor is accepted, the observed effects of metolachlor in the 21-day dermal toxicity study in rabbits are also taken to conclude on classification with STOT RE for S-metolachlor. While metolachlor is a 50:50 mixture of the S- and R-isomer, S-metolachlor contains the S-isomer at higher levels, typically >84 % and the R-isomer at lower levels, typically <13 %. Even when assuming that the observed effects was caused only by the R-isomer and considering the difference in R-isomer content between metolachlor and S-metolachlor, a conversion of the observed effect dose of 10 mg/kg bw/d metolachlor would result in an extrapolated dose of 40 mg/kg bw/d for S-metolachlor. In view of the same reasoning as for metolachlor, this also leads to a proposal of classification into Cat. 2 (STOT RE 2 (skin)).

10.12.3 Conclusion on classification and labelling for STOT RE

In summary, classification with STOT RE 2 (H373) is considered appropriate for skin effects.

Aspiration hazard
This endpoint is not addressed in this CLH report and is outside the scope of the public consultation.

11 EVALUATION OF ENVIRONMENTAL HAZARDS

All the information on ready biodegradability are 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).

11.1 Rapid degradability of organic substances

Table 36: Summary of relevant information on rapid degradability

<table>
<tr><th>Method</th><th>Results</th><th>Remarks</th><th>Reference</th></tr>
<tr><td>OECD 301/B</td><td>Ready biodegradability
mineralization of S-metolachlor under the test conditions was 0 % in 29 days
S-Metolachlor is not readily degradable</td><td>The study is considered acceptable

Reliability 1</td><td>Grade (1996)</td></tr>
<tr><td>OECD 111</td><td>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</td><td></td><td>Keller (1996)</td></tr>
<tr><td>OECD 309</td><td>Aerobic mineralisation in surface water:
<i>S-Metolachlor:</i>
DT_{50} values are normalised to 20 °C
<table>
<tr><th>System*</th><th>pH water</th><th>pH sed^{a)}</th><th>DT_{50} whole sys</th><th>St. (χ^2)</th><th>DT_{50} Water</th><th>St. (χ^2)</th><th>Method of calculation</th></tr>
<tr><td>10 µg/L</td><td>8.6</td><td>7.6</td><td>74 d</td><td></td><td>NA</td><td></td><td>SFO</td></tr>
<tr><td>95 µg/L</td><td>8.6</td><td>7.6</td><td>97 d</td><td></td><td>NA</td><td></td><td>SFO</td></tr>
</table>
* Fresh water plus suspended sediment
a) Measured in calcium chloride solution
b) Temperature of incubation=temperature that the environmental media was collected or std temperature of 20 °C
NA: not applicable

<i>Metabolite CGA40172:</i>
Max in total system 9.1 % after 58 days.
DT_{50}-values were not applicable

<i>Mineralisation:</i>
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

<i>Non-extractable residues:</i>
Not detected in both systems</td><td>The study is considered acceptable

Reliability 1</td><td>Crabtree (2014)</td></tr>
<tr><td>BBA Guideline Part IV; 5-1</td><td>Degradation in water/sediment system:
<i>S-Metolachlor:</i>
<table>
<tr><th>Water / sediment system*</th><th>pH water phase</th><th>pH sed</th><th>DT_{50} / DT_{90} whole sys.</th><th>St. (χ2)</th><th>DT_{50} / DT_{90} water</th><th>DT_{50} /DT_{90} sed</th><th>St. (χ2)</th><th>Method of calculation</th></tr>
<tr><td>River (Rhine), sandy loam (<i>Mamouni</i>)</td><td>7.7</td><td>8.3</td><td>54.8 d/
182 d</td><td>1.9</td><td>NA</td><td>NA</td><td>--</td><td>SFO</td></tr>
<tr><td>Pond (Ormalingen), silt loam (<i>Mamouni</i>)</td><td>7.3</td><td>8.1</td><td>42.0 d/
140 d</td><td>3.5</td><td>NA</td><td>NA</td><td>--</td><td>SFO</td></tr>
</table></td><td>The study is considered acceptable

S-Metolachlor is not readily biodegradable in the tested systems

Reliability 1</td><td>Mamouni (1997)
Seyfried (1997)</td></tr>
</table>

<table>
<tr><th>Method</th><th>Results</th><th>Remarks</th><th>Reference</th></tr>
<tr><td></td><td>
<table>
<tr><td>River (Rhine), sandy loam (Seyfried)</td><td>7.7</td><td>8.3</td><td>45.4 d/ 151 d</td><td>3.1</td><td>NA</td><td>NA</td><td>--</td><td>SFO</td></tr>
<tr><td>Pond (Ormalingen), silt loam (Seyfried)</td><td>7.3</td><td>8.1</td><td>33.6 d/ 112 d</td><td>3.8</td><td>NA</td><td>NA</td><td>--</td><td>SFO</td></tr>
<tr><td colspan="3">Geometric mean at 20 °C</td><td>43.3 d</td><td></td><td></td><td></td><td></td><td></td></tr>
</table>
* temperature during study: 20 °C
NA: not applicable

Metabolites
<u>CGA41507:</u>
Max in water 8.2 % after 175 d. max. sed 12.1 % after 271 d). max. in total system 17.8 % after 175 days.
Under anaerobic conditions: max. 54.7 % after 271 days in total system

<u>CGA51202 (OXA):</u>
Max in water 16.8 % after 362 d. Max in total system 21.2 % after 362 days

<u>CGA354743 (ESA):</u>
Max in water 6.7 % after 362 d. Max in total system 8.5 % after 362 days.

<u>CGA217498:</u>
Max in water 2.7 % after 362 d. Max in total system 5.6 % after 362 days.

Mineralisation and non-extractable residues:
<table>
<tr><td>Water / sediment system</td><td>Mineralisation (end of the study).</td><td>Non-extractable residues in sed (max)</td><td>Non-extractable residues in sed. (end of the study)</td></tr>
<tr><td>River (Rhine), sandy loam (study Mamouni)</td><td>max 4.5 % after 362 d</td><td>max. 40.3 % after 175 d</td><td>max 39.7 % after 362 d</td></tr>
<tr><td>Pond (Ormalingen), silt loam (study Mamouni)</td><td>max 1.8 % after 362 d</td><td>max 58.8 % after 175 d</td><td>max 60.8 % after 362 d</td></tr>
<tr><td>River (Rhine), sandy loam (study Seyfried)</td><td>max 3.1 % after 180 d</td><td>max 35.8 % after 91 d</td><td>max 34.8 % after 180 d</td></tr>
<tr><td>Pond (Ormalingen), silt loam (study Seyfried)</td><td>max 2.0 % after 180 d</td><td>max 52.9 % after 91 d</td><td>max 56.5 % after 180 d</td></tr>
</table>
</td><td></td><td></td></tr>
<tr><td>EPA guideline No. 162-1

SETAC (1995)

BBA Part IV, 4-1

Dutch Registration Guideline, Section</td><td>
Degradation in soil: Aerobic degradation (Laboratory studies)
S-Metolachlor:
<table>
<tr><td>Soil type</td><td>parent</td><td>pH^{a)}</td><td>t. °C / % MWHC</td><td>DT₅₀ / DT₉₀ (d)</td><td>DT₅₀ (d) 20 °C pF2/10kPa ^{b)}</td><td>St. (χ²)</td><td>Method of calculation</td></tr>
<tr><td>Sandy clay loam (18 Acres)</td><td>S-metolachlor</td><td>5.7</td><td>20, pF2</td><td>97.2</td><td>91.2</td><td>1.4</td><td>HS ^{d)}</td></tr>
</table>
</td><td>The studies are considered acceptable

Reliability 1</td><td>Clark (1995);

Morgenroth (1997);

Kitschmann (1997a)

Keller (1997)

Simmonds & Simmonds (2013 &</td></tr>
</table>

Method	**Results**								**Remarks**	**Reference**
G.1 US-EPA OPPTS 835.4100 (2008) OECD 307 FOCUS Kinetics guidance (2006)	Sandy clay loam (18 Acres)	S-metolachlor	6.3	20, pF2	84.8	84.8	1.9	HS d)		2014) Hardy (2014, 2014a & 2014b) Lucas (1996) Hein (2007) Phaff, (2001)
	Geometric mean 18 Acres				90.8	**87.9**				
	Loamy sand (Birkenheide)	S-metolachlor	5.6 (KCl)	20, 40 % MWHC	38.6	**24.5**	4.2	FOMC d)		
	Loamy sand (Borstel)	S-metolachlor	5.3	20, pF2	175	173	1.0	SFO		
	Loamy sand (Borstel)	S-metolachlor	6.1	20, pF2	221	221	1.7	HS d)		
	Geometric mean Borstel (n=2)				196.7	**195.5**				
	Sandy loam (Buckeystown)	S-metolachlor	8.0 c)	25, 75 % 1/3 bar	13.2	15.3	4.9	FOMC d)		
	Sandy loam (Buckeystown)	metolachlor	8.0 c)	25, 75 % 1/3 bar	10.1	11.7	4.3	FOMC d)		
	Geometric mean Buckeystown (n=2)				11.5	**13.4**				
	Sandy loam (Collombey)	Metolachlor	7.4 (KCl)	20, 40 % MWHC	11.2	**11.2**	3.6	SFO		
	Silt loam (Gardner)	S-metolachlor	7.6	20, pF2	91.6	79.5	1.0	SFO		
	Sandy loam (Gardner)	S-metolachlor	7.5	20, pF2	91.7	91.7	3.5	SFO		
	Geometric mean Gardner (n=2)				91.6	**85.4**				
	Loam (Gartenacker)	S-metolachlor	7.3 (KCl)	20, 75 % 1/3 bar	13.2	12.6	10.3	SFO		
	Loam (Gartenacker)	Metolachlor	7.3 (KCl)	20, 75 % 1/3 bar	15.2	14.6	4.6	SFO		
	Loam (Gartenacker)	S-metolachlor	7.3	20, pF2	26.2	24.7	3.2	Lag phase, overall DT_{50} HS		
	Silt loam (Gartenacker)	S-metolachlor	7.5	20, pF2	35.5	30.8	3.2	Lag phase overall DT_{50} HS		

Method	Results	Remarks	Reference

Silt loam (Gartenacker)	S-metolachlor	7.3 (KCl)	20, 60 % FC	16.3	12.5	6.8	SFO
Geometric mean Gartenacker (n=5)				19.8	**17.7**		
Loamy sand (Standard soil 2.2)	S-metolachlor	5.7 c)	20, 40 % MWHC	48.8	48.8	5.3	FOMC d)
Loamy sand (Standard soil 2.2)	metolachlor	5.7 (KCl)	20, 40 % MWHC	24	24	2.5	FOMC d)
Geometric mean German standard soil 2.2 (n=2)				34.2	**34.2**		
Sandy loam (Lorsch)	S-metolachlor	5.2 (KCl)	20, 40 % MWHC	49.9	**32.9**	6.9	SFO
Sandy loam (Pappelacker)	S-metolachlor	7.6 c)	20, 40 % MWHC	25.3	**15.3**	4.5	FOMC d)
Sandy loam (Weide)	Metolachlor	7.6 (KCl)	20, 40 % MWHC	11.8	11.8	4.3	SFO
Sandy loam (Weide)	S-metolachlor	7.6 c)	20, 40 % MWHC	16.4	10.3	4.5	FOMC d)
Geometric mean Weide (n=2)				13.9	**11.0**		
Geometric mean (n = 11)					**30.1**		
pH dependence					**no**		

* geometric mean of DT_{50} (same soils)

a) Measured in H_2O unless otherwise stated

b) Normalized using a Q10 of 2.58 and Walker equation coefficient of 0.7

c) medium not stated

d) HS: slow phase DT_{50}; FOMC: DT_{50}= DT_{90}/3.32

NA: not applicable

Metabolites

CGA354743/CGA380168 (ESA): max. 21.3 % (20 °C) after 42 d, (n= 19); 23.6 % (10 °C) after 120 d (n=1)

CGA51202 / CGA351916 (OXA): max. 21.1 % after 153 d, (n = 19)

CGA40172: max. 6.5 % after 14 d

CGA50720: max. 8.2 % after 3 month

CGA368208: max. 7.6 % after 120 d

CGA37735: max. 7.1 % after 181 d

NOA436611: max. 9.1 % after 153 d

CGA357704: max. 21.9 % after 28 d

Mineralisation after 100 days:

<table>
<tr><th>Method</th><th colspan="9">Results</th><th>Remarks</th><th>Reference</th></tr>
<tr><td></td><td colspan="9">0.3 – 29.0 % after 3 month, (n = 19)
<i>Non-extractable residues after 100 days:</i>
4.6 – 44.5 % after 3 month d, (n= 19)</td><td></td><td></td></tr>
<tr><td rowspan="17">FAO guidelines on producing pesticide residue data from supervised trails (1990)
BBA part IV, 4-1
IVA Guidelines on residue studies (1994)
SETAC (1995)
FOCUS Degradation Kinetics (2006, 2011, 2014)</td><td colspan="9">Degradation in soil: Aerobic degradation (Field studies)
<i>S-Metolachlor:</i></td><td rowspan="17">The studies are considered acceptable (new evaluation according to FOCUS Degradation Kinetics [2006, 2011 and 2017] by Ford [2014] and RMS [2016])
Reliability 1</td><td rowspan="17">Mostert (1996a, 1997, 1997c, 1997h, 1997i, 1997n, 1997o and 1997r)
Stolze (1997a and amendment)
Stolze (1997b)
Evans (2004, 2004a)
Ford (2014)</td></tr>
<tr><td>Soil type</td><td>Location</td><td>pH</td><td>Depth (cm)</td><td>DT$_{50}$ (d) actual</td><td>DT$_{90}$(d) actual</td><td>St. (χ^2)</td><td>DT$_{50}$ (d) Norm.</td><td>Method calculation</td></tr>
<tr><td>Silt loam (bare)</td><td>DE</td><td>6.5$^{a)}$</td><td>0-30</td><td>24.1</td><td>183</td><td>6.28</td><td>NA</td><td>FOMC</td></tr>
<tr><td>Sandy loam (bare)</td><td>CH</td><td>7.4$^{a)}$</td><td>0-30</td><td>3.55</td><td>50.4</td><td>4.77</td><td>NA</td><td>HS</td></tr>
<tr><td>Sandy loam (maize cover)</td><td>CH</td><td>7.5$^{a)}$</td><td>0-30</td><td>22.9</td><td>76.1</td><td>3.79</td><td>NA</td><td>SFO</td></tr>
<tr><td>Silt loam (bare)</td><td>CH</td><td>7.9$^{a)}$</td><td>0-30</td><td>18.6</td><td>61.9</td><td>1.65</td><td>NA</td><td>SFO</td></tr>
<tr><td>Sandy loam (maize cover)</td><td>CH</td><td>7.8$^{a)}$</td><td>0-30</td><td>11.4</td><td>37.9</td><td>1.96</td><td>NA</td><td>SFO</td></tr>
<tr><td>Loam (bare)</td><td>FR</td><td>7.15$^{a)}$</td><td>0-30</td><td>30.8</td><td>102</td><td>4.45</td><td>NA</td><td>SFO</td></tr>
<tr><td>Silt clay loam (bare)</td><td>FR</td><td>7.45$^{a)}$</td><td>0-30</td><td>12.8</td><td>256</td><td>21.7</td><td>NA</td><td>DFOP</td></tr>
<tr><td>Silty sand (bare)</td><td>DE</td><td>6.1$^{a)}$.</td><td>0-30</td><td>26.1</td><td>86.8</td><td>11.7</td><td>NA</td><td>SFO</td></tr>
<tr><td>Loamy silt (bare)</td><td>DE</td><td>7.4$^{a)}$</td><td>0-30</td><td>4.62</td><td>27.6</td><td>10.7</td><td>NA</td><td>FOMC</td></tr>
<tr><td>Silt loam (bare)</td><td>IT</td><td>7.6$^{b)}$</td><td>0-20</td><td>43.9</td><td>146</td><td>13</td><td>NA</td><td>SFO</td></tr>
<tr><td>Clay loam (bare)</td><td>FR</td><td>7.3$^{b)}$</td><td>0-30</td><td>21</td><td>69.9</td><td>15</td><td>NA</td><td>SFO</td></tr>
<tr><td>Sandy loam (bare)</td><td>DE</td><td>6.2$^{a)}$</td><td>0-20</td><td>17.2</td><td>244</td><td>5.29</td><td>NA</td><td>DFOP</td></tr>
<tr><td>Clayey silt (bare)</td><td>DE</td><td>6.2$^{a)}$</td><td>0-20</td><td>7.66</td><td>62</td><td>8.10</td><td>NA</td><td>DFOP</td></tr>
<tr><td>Loamy sand (bare)</td><td>DE</td><td>6.0$^{a)}$</td><td>0-20</td><td>38.2</td><td>127</td><td>6.0</td><td>NA</td><td>SFO</td></tr>
<tr><td>Loamy silt (bare)</td><td>DE</td><td>6.0$^{a)}$</td><td>0-20</td><td>24.1</td><td>80.1</td><td>9.37</td><td>NA</td><td>SFO</td></tr>
</table>

<table>
<tr><th>Method</th><th>Results</th><th>Remarks</th><th>Reference</th></tr>
<tr><td></td><td>
<table>
<tr><td>Sandy silt loam (bare)</td><td>DE</td><td>5.7 $^{a)}$</td><td>0-20</td><td>31.3</td><td>104</td><td>21.1</td><td>NA</td><td>SFO</td></tr>
<tr><td>Silty sand (bare)</td><td>DE</td><td>4.8 $^{a)}$</td><td>0-20</td><td>55.7</td><td>185</td><td>7.37</td><td>NA</td><td>SFO</td></tr>
<tr><td colspan="4">Maximum non-normalized field DT_{50}</td><td>55.7</td><td></td><td></td><td></td><td>SFO</td></tr>
</table>
$^{a)}$ Medium not stated
$^{b)}$ Measured in $CaCl_2$
</td><td></td><td></td></tr>
<tr><td>OECD 316</td><td>Aqueous photochemical degradation
at pH7, sterile buffer:
DT_{50}: 146 days
Natural light, 30° - 50°N; DT_{50} 129 days

<i>Quantum yield of direct phototransformation in water at ≥ 290 nm:</i>
Not relevant
molar absorptivity at wave lengths ≥ 290 nm: $< 10\ L \cdot mol^{-1} \cdot cm^{-1}$

→ direct phototransformation in water is no significant degradation process for S-metolachlor under environmental conditions</td><td>The study is considered acceptable

Reliability 1</td><td>Oddy (2013)</td></tr>
<tr><td>OECD 316</td><td>Aqueous photochemical degradation in sterile natural water
at pH :
DT_{50}:12.1 d, corresponded to 21.5 d natural summer sunlight days at latitudes 30° - 50° N

45 photodegradate fractions (U1 to U45): all fraction < 5.8 %
Metabolites: CGA13656, CGA41638/ CGA40172: each ≤ 0.3%</td><td>The study is considered as additional information</td><td>Berdat, Nicollier (2008)</td></tr>
<tr><td>US-EPA, OPPTS 835-2410 (2008)

OECD guideline for soil photolysis (2002)</td><td>Soil photolysis
<table>
<tr><td>Soil</td><td>$DegT_{50}$ (days)</td><td>$DegT_{90}$ (days)</td><td>Dark control $DegT_{50/90}$ (days)</td><td>Reference</td></tr>
<tr><td>Borstel, Germany, loamy sand, dry soil</td><td>126 / 158* / 169**</td><td>418 / 523* / 559**</td><td>617 / > 1000</td><td>Simmonds, 2012</td></tr>
<tr><td>Borstel, Germany, loamy sand, moist soil</td><td>78.5 / 210*/ 225**</td><td>261 / 698* / 746**</td><td>127 / 421</td><td></td></tr>
</table>
* corrected by subtraction of the dark soil rate constant from the irradiated soil rate constant
** converted to days equivalent summer sunlight 30-50°NField studies

<i>Metabolites</i>
<u>CGA41638:</u> max. 5.4 % (moist soil) and 5.6 % (dry soil) at day 40

<i>Mineralisation after 100 days:</i>
1.4 – 1.5 % after 40 d

<i>Non-extractable residues after 100 days:</i>
8.3 – 10.4 % after 40 d</td><td>The study is considered acceptable

Reliability 1

Photodegradation on soil is no significant route of degradation</td><td>Simmonds (2012)</td></tr>
<tr><td>Theoretical estimation</td><td>Photochemical oxidative degradation in air
DT_{50} of 2.3 hours derived by the Atkinson model (version 1.91). OH-radical concentration assumed = 1.5^6 (12 h).</td><td>long-range transport is not considered to be relevant</td><td>Stamm, (1997)</td></tr>
</table>

11.1.1 Ready biodegradability

Grade, 1996 (study evaluated in DAR, 2000)

Author:	Grade, R.
Title:	Report on the Test for ready biodegradability of S-metolachlor (CGA 77102) tech. in the carbon dioxide evolution test.
Date:	19/12/1996
Doc ID:	Report No. 961567
Guidelines:	OECD guideline 301/B
Deviation:	Only one CO_2 scrubber was used.
GLP:	Yes
Validity:	Yes

Materials and methods:

The aim of the study was the determination of the biodegradability of the test substance S-metolachlor by measurement of the carbon dioxide formation in percent of ThCO2 (theoretical carbon dioxide) calculated from the ThOC (theoretical organic carbon) or TOC (total organic carbon). The test substance S-metolachlor (chemical purity 98.5 %) was mixed with mineral medium in order to obtain 1.5 L of inoculated mineral medium containing 39.3 resp. 38.3 mg/L (16.6 resp. 16.5 mg ThOC/L) as the nominal sole source of organic carbon. The inoculated mineral medium is aerated by the passage of carbon dioxide-free air at the controlled rate in diffuse light. Degradation is followed over 29 days by determining the carbon dioxide produced, which is trapped in sodium hydroxide and which is measured as inorganic carbon by a carbon analyser. The amount of carbon dioxide produced from the test substance (corrected for that derived from blank inoculum) is expressed as a percentage of theoretical carbon dioxide.

The test system is described in the table below:

Table 37: Test system for carbon dioxide evolution test

Test conditions	
pH	7.9 (after collection)
Test system	Activated sludge collected from a sewage treatment plant, CH-4153 Reinach, Switzerland.
Inoculum	24.6 mg sludge/L
Duration:	29 days
Temperature	21 ± 2 °C
Test water:	Distilled water

Results and Discussion:

The mineralization of S-metolachlor under the test conditions was 0 % in 29 days; therefore, the test substance was not biodegradable in this test. S-metolachlor did not inhibit the biodegradation of the reference substance (Sodium benzoate).

Conclusion:

The study was considered acceptable for the first Annex 1 approval of S-metolachlor. After re-evaluation of the study, it was concluded that it is still considered acceptable. Based on the results of this test, S-metolachlor can be classified as “not readily biodegradable”.

11.1.2 BOD$_5$/COD

No data available.

11.1.3 Hydrolysis

Keller, 1996 (study evaluated in DAR, 2000)

Author:	Keller, A.
Title:	Hydrolysis of 14C-labelled metolachlor (CGA 24705) under laboratory conditions
Date:	14/10/1996
Doc ID:	Report No 96AK02
Guidelines:	OECD Guideline No. 111 for testing chemicals, hydrolysis as a function of pH, adopted: 12 May 1981, Paris /France
Deviation:	None
GLP:	Yes
Validity:	Acceptable

Materials and methods:

The hydrolytic stability of ^{14}C-phenyl-labelled metolachlor with a specific radioactivity of 1.50 MBq/mg and a radiochemical purity of 98.6 % was investigated in the laboratory by incubation in aqueous solution at different pH values. The pre-test was conducted in buffer solutions of pH 1, 5, 7 and 9 at 50 °C for 7 days and the final test was conducted at 25 °C in buffer solutions of pH 5, 7 and 9 for 30 days. The concentration of the substance in the buffer solution was 10 mg/L.

Results and Discussion:

No degradation was observed under the conditions of the pre-test and the final test.

Conclusion:

The study was already accepted in the DAR (2000) of S-metolachlor. Under a wide pH range (1-9), metolachlor is hydrolytically stable showing a degradation half-life far above 200 days. No relevant metabolites were found.

11.1.4 Other convincing scientific evidence

No data available.

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

Not relevant for C & L.

11.1.4.2 Inherent and enhanced ready biodegradability tests

No data available.

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

11.1.4.3.1 Aerobic mineralisation in surface water

Crabtree, 2014 (new study)

Author:	Crabtree, G.
Title:	S-Metolachlor - Aerobic mineralization of ^{14}C-S-Metolachlor in surface water
Date:	19/05/2014
Doc ID:	Report No. 3200234, Syngenta file No. CGA077102_11208
Guidelines:	OECD Guidelines for the Testing of Chemicals, 309, Aerobic Mineralization in Surface Water – Simulation Biodegradation Test (13 April 2004)
Deviation:	
GLP:	Yes
Validity:	Acceptable

Materials and methods:

The mineralisation rate and route of degradation of ^{14}C-S-metolachlor was investigated in Calwich Abbey (large perennial lake in Northern Europe) natural water, which had been inoculated with suspended sediment at a concentration of 0.02 g/L under a diffuse non-UV light/dark cycle. Prior use the water was sieved through a 100µm mesh and the sediment was sieved to 2 mm. ^{14}C- S-metolachlor was applied to the water at nominal rates of 10 and 95 µg/L (low and high, respectively). The 95 µg/L rate was also applied to sterilised test system (natural water plus 0.02 g/L suspended sediment). 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. For each system, duplicate samples were taken for analysis at up to seven intervals.

At each sampling time, the quantity of radioactivity in the water was determined by liquid scintillation counting (LSC). Samples were either directly analysed or subjected to solid phase extraction (SPE) and eluted with acidified acetonitrile prior to LSC and chromatographic analysis. Any volatile radioactivity was continuously flushed from the vessels, collected in traps and analysed. A mass balance was determined for each sample.

Separate reference samples (treated with sodium ^{14}C benzoate at 10 µg/L) of natural water plus 0.02 µ/L suspended sediment were prepared to determine whether a viable microbial population was present in the test system.

Separate blank control samples were similarly incubated to allow water quality measurements at each sampling interval and chlorophyll-an assay at the start and end of incubation period.

The half-lives ($DegT_{50}$) of ^{14}C-S-metolachlor (from the HPLC analysis) were determined using a Single First Order (SFO) kinetic model.

Results and Discussion:

The mean mass balance for the low and high-test concentration natural water samples plus sus-pended sediment samples were 96.6 % and 94.0 % of applied radioactivity (AR) with ranges of 95.7 to 97.5 % and 92.3 to 95.2 % respectively. The mean mass balances for the sterilised incubation group was 95.5 % AR.

Over the duration of the study (58 DAT), the mean levels of parent compound decreased to between 54.0 and 62.2 % AR for the water plus suspended sediment. For the sterilised samples, the mean level of parent compound was 92.4 % AR at 58 DAT. The major degradate of S-metolachlor was found to be CGA40172 which reached a maximum of 9.1 % of applied radioactivity after 58 days (10 µg/L rate). In addition, a number of discrete known and unknown degradates were also observed, none exceeding 3.5 % of applied activity. Ultimately, S-metolachlor was mineralised to carbon dioxide (< 5 % AR).

The degradation rates ($DegT_{50}$) of S-metolachlor were determined using non-linear regression and a single first-order kinetic model (SFO, CAKE). The results are summarized below:

System	Test concentration (µg/L)	SFO – kinetic				
		$DegT_{50}$ (days)	k	Chi^2	R^2	Prob > t
Natural water plus suspended sediment	10	74	0.0094	2.41	0.9601	2.2E-17
Natural water plus suspended sediment	95	97	0.0072	1.23	0.9681	3.4E-19

Conclusion:

The extent of mineralisation and the rate and route of degradation of ^{14}C-S-metolachlor were investigated in Calwich Abbey natural water plus 0.02 g/L suspended sediment under a diffuse light/dark light cycle. The mean mass balances for all incubation groups were 94.0 % to 96.6 % AR. For the non-sterilised, viable test systems, the mean levels of parent compound decreased to between 54.0 and 62.2 % AR at the end of the incubation period (58 DAT), 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 % AR (mean) remaining at 58 DAT.

CGA40172 was the only metabolite found at ≥ 5 % AR, reaching a maximum level of 9.1 % AR at 58 DAT.

Ultimately, S-metolachlor was mineralised to carbon dioxide (< 5 % AR).

11.1.4.3.2 Water/sediment studies

Mamouni, 1997 (study evaluated in DAR, 2000)

Author:	Mamouni, A.
Title:	S-Metolachlor (^{14}C-CGA77102): Degradation and Metabolism in Aquatic Systems under various Experimental Conditions
Date:	08.04.97
Doc ID:	Report No. RCC 603551
Guidelines:	BBA Guideline Part IV; 5-1
Deviation:	-
GLP:	Yes
Validity:	Acceptable

Materials and methods:

Analytical grade ^{14}C-labelled S-metolachlor with a specific radioactivity of 1.90 MBq/mg and a radiochemical purity of > 99.0 % was investigated in two different water/sediment systems under various experimental conditions (aerobic 20 °C; anaerobic 20 °C; aerobic 9 °C, anaerobic 9 °C; aerobic sterile 20 °C). The water/sediment characteristics are shown in the table below. Water was sampled down to a depth of 10-30 cm and sediment was sampled from the top 5-10 cm. Prior to the start of the study water was filtered (0.2 mm) and the sediment was sieved (2.0 mm). The sediment was filled into the flasks to a height of about 2 cm and water was added to achieve a layer of about 6 cm. The test system was acclimated in the dark for one month before treatment. During this time the measured pH values, redox potentials, and oxygen concentrations in water and redox potential in sediment had reached constant values. The test substance was applied thereafter in a concentration of 0.676 mg/L corresponding to an application rate of 2.0 kg active ingredient/ha. This concentration was obtained by applying a field rate of 2 kg/ha assuming that the active ingredient is homogeneously distributed in natural water of 30 cm depth. Samples were incubated for up to one year.

Table 38: Water / sediment characteristics of river and pond systems

Sediment	**Rhine river, Mumpf - Zeltplatz, Aargau, Switzerland**	**Pond water, Ormalingen, Rothenfluh, Baselland,**
classification (USDA)	**Sandy loam**	**Silt loam**
sand [%]:	68.7	36.3
silt [%]:	22.5	62.0
clay [%]:	8.8	1.7
pH [H_2O]:	8.3	8.1
pH (KCl) / pH(H2O)	7.7/8.3	7.5/8.1
Redox potential (mV)	-112	-163
N-total (Kjeldahl) (g/kg sediment)	0.8	4.9
P-total (g/kg sediment)	2.04	1.56
organic carbon [%]:	0.22	1.74
CEC [mVal/100g]:	7.8	28.9
Biomass [mg C/100g dry soil]:	63.9	261.8

Results and Discussion:

Results of this study are summarised in Table 39 and in Table 40. The average recoveries ranged from 97.8 % to 98.2 % of the totally applied radioactivity for the river system and from 95.6 % to 98.2 % for the pond system.
Kinetic calculations were based on a pseudo first order kinetic using a non-linear correlation function.

S-metolachlor as well as the different metabolites were distributed to both sediment and water phases. The degradation of S-metolachlor under aerobic and anaerobic conditions showed significant differences. Under aerobic conditions major metabolites identified as CGA41507 (11.6-17.8 %), CGA51202 (OXA) (6.9-21.2 %), CGA354743 (ESA) (4.7-8.5 %) and numerous minor metabolites, each below 5 % (CGA46129, CGA4807, and CGA354743) were detected. For the metabolite CGA217498 the maximum of formation was not yet reached at the end of the study. The maximum amount of CGA217498 after 362 days was 5.6 % in the total system. Under anaerobic conditions, only one major metabolite identified as CGA 41507 (37.1-54.7 %) was observed.

Based these results, oxidation and reduction are two major degradation pathways of S-metolachlor which occur in the water/sediment system. Aerobic microorganisms degrade S-metolachlor by oxidation reactions to hydroxy and acid metabolites. On the oxidative pathway, sulphur-containing metabolites are also formed (CGA354743/CGA380168 (ESA) and CGA217498. Anaerobic microorganisms transformed S-metolachlor by reductive dechlorination to CGA41507. This metabolite can then be further degraded by oxidation reactions when oxygen is available in the water/sediment.

Amounts of bound residue reached levels of 15.8-55.8 % of applied radioactivity after 91-99 days. The amount of bound residues was rather similar for all the incubation scenarios.

Table 39: Recovery and distribution of radioactivity in the river water/sediment system (% applied radioactivity)

Radioactive fraction	**Type of sample**	**Incubation time (days)**									
		0	**3**	**7**	**14**	**28**	**62**	**99**	**175**	**271**	362
S-metolachlor	water	102.7	60.3	57.9	46.3	37.8	22.5	8.9	2.5	<0.1	<0.1
	sediment	1.5	35.4	35.1	37.4	34.2	24.3	21.2	7.1	1.6	1.3
	Total system	104.3	95.7	93.0	83.7	72.0	46.8	30.1	9.6	1.6	1.3
CGA41507	water	<0.1	<0.1	<0.1	<0.1	2.2	3.0	6.1	8.2	3.9	1.0
	sediment	<0.1	<0.1	0.4	2.2	2.8	7.0	6.5	9.6	9.2	5.6
	Total system	<0.1	<0.1	0.4	2.2	5.0	10.0	12.6	17.8	13.1	6.6
CGA51202 (OXA)	water	<0.1	<0.1	<0.1	<0.1	<0.1	4.7	7.3	10.8	13.8	16.8
	sediment	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	1.1	2.3	5.1	4.5
	Total system	<0.1	<0.1	<0.1	<0.1	<0.1	4.7	8.4	13.0	18.8	21.2
CGA46129	water	<0.1	<0.1	<0.1	<0.1	<0.1	1.8	2.5	2.8	1.9	1.8
	sediment	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	1.9	0.7
	Total system	<0.1	<0.1	<0.1	<0.1	<0.1	1.8	2.5	2.8	3.8	2.5
CGA354743 (ESA)	water	<0.1	<0.1	<0.1	<0.1	<0.1	1.8	3.7	3.9	5.5	6.7
	sediment	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	1.7	<0.1	1.8
	Total system	<0.1	<0.1	<0.1	<0.1	<0.1	1.8	3.7	5.6	5.5	8.5
CGA48087	water	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	1.0	1.1	0.9	0.4
	sediment	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	0.7	<0.1	1.0	0.9
	Total system	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	1.7	1.1	1.9	1.3
CGA217498	water	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	0.9	0.8	1.5	2.7
	sediment	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	0.7	1.1	2.9
	Total system	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	0.9	1.5	2.6	5.6
Unknown (up to 7 fractions)	Total system	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	1.5	4.0	7.3	5.0
total (water)	water	102.7	60.3	57.9	46.3	39.9	33.9	31.9	32.8	34.7	33.1
total (extractables)	sediment	1.5	35.4	35.5	39.6	37.0	31.3	29.6	22.5	19.9	19.0
total ^{14}C-CO$_2$	volatiles	not determined	<0.1	<0.1	0.1	0.2	0.5	0.6	1.7	3.0	4.5
Other volatiles			<0.1	<0.1	<0.1	<0.1	<0.1	0.3	0.1	0.1	0.1
total non-extractables	sediment	<0.1	2.7	5.3	13.0	19.8	29.2	34.1	40.3	37.4	39.7
Total average		104.3	98.4	98.8	99.0	97.0	94.9	96.5	97.4	95.1	96.5

Table 40: Recovery and distribution of radioactivity in the pond/sediment system (% applied radioactivity)

Radioactive fraction	**Type of sample**	**Incubation time (days)**									
		0	**3**	**7**	**14**	**28**	**62**	**99**	**175**	**271**	**362**
S-metolachlor	water	104.2	65.6	51.3	37.0	26.4	9.8	3.5	0.6	<0.1	<0.1
	sediment	1.5	31.2	39.5	44.0	44.4	25.3	15.9	3.7	1.9	<0.1
	Total system	105.8	96.8	90.8	81.1	70.9	35.1	19.4	4.3	1.9	<0.1
CGA41507	water	<0.1	<0.1	<0.1	<0.1	<0.1	2.5	2.5	2.5	2.3	0.6
	sediment	<0.1	0.3	<0.1	<0.1	2.2	6.4	6.7	10.0	12.1	4.8
	Total system	<0.1	0.3	<0.1	<0.1	2.2	9.0	9.2	12.6	14.3	5.5
CGA51202 (OXA)	water	<0.1	<0.1	<0.1	<0.1	<0.1	2.1	4.0	7.2	6.4	7.5
	sediment	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	1.3	2.2	4.8
	Total system	<0.1	<0.1	<0.1	<0.1	<0.1	2.1	4.0	8.5	8.6	12.3
CGA46129	water	<0.1	<0.1	<0.1	<0.1	<0.1	0.3	1.0	0.5	0.6	0.7
	sediment	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	1.3	<0.1	0.5
	Total system	<0.1	<0.1	<0.1	<0.1	<0.1	0.3	1.0	1.8	0.6	1.2
CGA354743 (ESA)	water	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	1.4	2.0	1.6	2.0
	sediment	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	1.5	0.7	2.7
	Total system	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	1.4	3.5	2.3	4.7
CGA48087	water	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	0.6	0.8	<0.1	0.5
	sediment	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	2.7	1.3	1.2
	Total system	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	0.6	3.4	1.3	1.6
CGA217498	water	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	0.4	0.6
	sediment	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	0.7	2.1
	Total system	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	1.1	2.7
(up to 8 fractions)	Total system	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	1.6	<0.1	1.8	3.7
total (water)	water	104.2	65.6	51.3	37.0	26.4	14.8	13.2	13.6	13.1	13.8
total (extractables)	sediment	1.5	31.5	39.5	44.0	46.6	31.7	24.0	20.6	19.0	18.0
total ^{14}C- CO_2	volatiles	not deter-mined	<0.1	<0.1	0.1	0.3	0.9	0.7	1.4	1.3	1.8
Other volatiles			<0.1	<0.1	<0.1	<0.1	<0.1	0.1	<0.1	0.1	0.1
total non-extractables		<0.1	2.3	7.5	13.6	22.9	46.2	55.8	58.8	60.2	60.8
Total		105.8	99.4	98.3	94.7	96.3	93.6	93.7	94.3	93.7	94.4

n.d.: not determined

The river and pond sediments of the aerobic incubation part (20 °C) of incubation day 271 were submitted to organic matter fractionation. The results are summarised in *Table 41*, show that the majority of the radioactivity was bound to the insoluble humin fraction, which is immobile in nature.

Table 41: Result of organic matter fractionation of non-extractables of sediment from both systems

Soil Organic Matter Fraction	**aerobic conditions / 20 °C/ day 271 river sediment**		**aerobic conditions / 20°C/ day 271 pond sediment**	
	% of non-extr.	**% applied radioact.**	**% of non-extr.**	**% applied radioact.**
Fulvic acid fraction	32.6	12.2	17.6	10.6
Humic acid fraction	16.7	6.2	37.8	22.8
Humin (immobile) fraction	50.7	18.9	44.6	26.8
Total	100.0	37.4	100.0	60.2

Under aerobic and anaerobic incubation conditions, the same range of $DT_{50/90}$ values of between 42 and 53 days for DT_{50} and 138-176 days for DT_{90} at 20°C were determined. At temperatures below 10 °C the degradation half-life was by a factor of three longer. The results are presented in the table below.

Table 42: S-metolachlor degradation half-life in water/sediment systems under various conditions

study part conditions	**1 aerobic, 20 °C**		**2 anaerobic, 20 °C**		**3 aerobic, 9 °C**		**4 anaerobic, 9 °C**		**5 aerobic sterile 20 °C**	
system	**river**	**pond**	**river**	**pond**	**river**	**pond**	**river**	**pond**	**river**	**pond**
water phase										
$DissT_{50}$ (days)	12	6	32	18	30	23	73	62	101	17
$DissT_{90}$ (days)	99	60	119	82	> 200	176	> 200	> 200	> 200	> 200
R^2	0.999	0.999	0.995	1.00	1.00	1.00	0.996	0.993	0.950	0.997
total system										
$DegT_{50}$ (days)	53	42	53	43	147	150	146	149	>200	193
$DegT_{90}$ (days)	176	138	175	142	>200	> 200	> 200	>200	> 200	>200
R^2	0.999	0.998	0.995	0.999	0.998	0.998	0.996	0.990	0.802	0.945

Conclusion:

The study was considered acceptable for the first Annex 1 approval of S-metolachlor. After re-evaluation of the study, the RMS concluded that it also fulfils the requirements of current guidelines and is thus still considered acceptable.

A new kinetic evaluation of the study results for aerobic conditions at 20 °C has been submitted (Hardy, 2014) for the renewal of the EU approval of S-metolachlor. Therefore, the study $DT_{50/90}$ values were not re-assessed by RMS.

Seyfried, 1997 (study evaluated in DAR, 2000)

Author:	Seyfried, B.
Title:	Metolachlor (CGA24705) (Phenyl-U-^{14}C): Degradation and Metabolism in Aquatic Systems
Date:	14.04.97
Doc ID:	Report No. RCC 603562
Guidelines:	BBA Guideline Part IV; 5-1, December 1990, EC-Directive 95/36/EEC; July 14, 1995
Deviation:	None
GLP:	Yes
Validity:	Acceptable

Materials and methods:

Analytical grade ^{14}C-phenyl-labelled metolachlor with a specific radioactivity of 1.56 MBq/mg. and a radiochemical purity of ≥ 98.0 % was investigated in two different water/sediment systems under aerobic conditions at 20 °C. The water sediment systems from river and pond were the same as those used in the study of Mamouni (1997) discussed above. The water/sediment characteristics are shown in Table 38. Water was sampled down to a depth of 10-30 cm and sediment was sampled from the top 5-10 cm. Prior to the start of the study water was filtered (0.2 mm) and the sediment was sieved (2.0 mm). The sediment was filled into the flasks to a height of about 2 cm and water was added to achieve a layer of about 6 cm. The test system was acclimated to the incubation conditions in the dark for one month before treatment. During this time the measured pH values, redox potentials, and oxygen concentrations in water and re-dox potential in sediment reached constant values. The test substance was applied thereafter in a concentration of 0.680 mg/L corresponding to an application rate of 2.04 kg active ingredient/ha. Samples were incubated for up to 180 days.

Results and Discussion:

The results of this study are summarised in Table 43 and in Table 44. The total recoveries of the radioactivity applied averaged 96.3 ± 1.7 % in the river system and 94.4 ± 2.4 % in the pond system.
Non-extractable radioactivity in the sediments increased and reached a maximum concentration of 35.8 % (day 91) in the river sediment and 56.5 % (day 180) in the pond system. The amount of bound residues in the sediment were thus in the same range when compared to results from the parallel study performed with S-metolachlor at equivalent incubation conditions.

In the water phase, the concentration of metolachlor decreased to 1.4 % and 0.5 % for the river and the pond, respectively, at the end of the study (180 days).

The degradation pattern of metolachlor was the same than for S-metolachlor in the study Mamouni (1997). In the river and pond systems, six metabolites were identified under aerobic condition by co-chromatography with reference standards. Two major metabolites, CGA41507 and CGA51202/CGA351916 (OXA), were detected.

For CGA41507 the maximum concentrations were found after 180 (river) and 92 (pond) days after incubation. No degradation half-life could be calculated due to the limited data set. CGA51202/CGA351916 (OXA) reached its maximum concentration 180 days (river) and 119 (pond) days after incubation. No degradation half-life could be calculated due to the limited data set.

Additional metabolites found were CGA46129, CGA354743/CGA380168 (ESA), CGA48087 and CGA217498. None of these exceeded 5.9 % of the applied dose.

After 180 days, eight unknown radioactive fractions were found none of these exceeded 1.5 %.

Mineralisation was not a significant process and reached 3.1 % and 2.0 % of the applied radioactivity until the end of the study for the river and pond system, respectively.

Table 43: Recovery and distribution of radioactivity in the river/sediment system (% of applied radioactivity)

Radioactive fraction	**Type of sample**	**Incubation time (days)**								
		0	**3**	**7**	**14**	**28**	**62**	**91**	**119**	**180**
metolachlor	water	95.7	70.8	60.1	42.8	31.7	16.6	8.0	5.1	1.4
	sediment	2.7	22.7	32.4	33.1	33.9	20.5	16.7	10.0	5.2
	Total system	98.4	93.5	92.5	75.9	65.6	37.1	24.7	15.1	6.6
CGA41507	water	<0.1	<0.1	<0.1	1.0	2.1	3.6	4.4	3.9	4.0
	sediment	<0.1	<0.1	0.7	1.8	2.7	6.0	6.3	7.0	8.9
	Total system	<0.1	<0.1	0.7	2.8	4.8	9.6	10.7	10.9	12.9
CGA51202 (OXA)	water	<0.1	<0.1	<0.1	1.2	2.8	5.5	7.7	9.6	13.9
	sediment	<0.1	<0.1	<0.1	<0.1	0.6	1.0	1.4	2.0	3.3
	Total system	<0.1	<0.1	<0.1	1.2	3.4	6.5	9.1	11.6	17.2
CGA46129	water	<0.1	<0.1	<0.1	0.4	2.1	2.0	4.2	3.8	3.2
	sediment	<0.1	<0.1	<0.1	<0.1	<0.1	0.6	0.5	0.8	0.7
	Total system	<0.1	<0.1	<0.1	0.4	2.1	2.6	4.7	4.6	3.9
CGA354743 (ESA)	water	<0.1	<0.1	<0.1	0.3	1.4	2.1	2.8	4.8	4.5
	sediment	<0.1	<0.1	<0.1	<0.1	<0.1	0.4	<0.1	1.1	2.0
	Total system	<0.1	<0.1	<0.1	0.3	1.4	2.5	2.8	5.9	6.5
CGA48087	water	<0.1	<0.1	<0.1	<0.1	<0.1	1.0	1.6	1.8	2.2
	sediment	<0.1	<0.1	<0.1	<0.1	<0.1	0.4	1.1	0.7	1.2
	Total system	<0.1	<0.1	<0.1	<0.1	<0.1	1.4	2.7	2.5	3.4
CGA217498	water	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	0.5	0.8	1.4
	sediment	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	0.3	0.7
	Total system	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	0.5	1.1	2.1
(up to 8 fractions)	Total system	0.1	0.8	0.8	1.2	0.4	4.6	4.6	7.3	4.2
total (water)	water	95.7	70.8	60.1	45.9	40.5	31.8	31.3	33.4	32.6
total (extractables)	sediment	2.8	23.5	33.9	35.8	37.2	32.4	28.4	25.6	24.3
total 14C-CO2	volatiles	n.d.	<0.1	<0.1	0.2	0.5	1.0	1.4	2.4	3.1
total volatiles		n.d.	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
total non-extractables	sediment	<0.1	1.4	4.8	11.7	18.4	29.5	35.8	35.2	34.8
Total		**98.5**	**95.7**	**98.8**	**93.7**	**96.6**	**94.8**	**97.0**	**96.5**	**94.8**

n.d.: not determined

Table 44: Recovery and distribution of radioactivity in the pond/sediment system (% of applied radioactivity)

Radioactive fraction	**Type of sample**	**Incubation time (days)**								
		0	**3**	**7**	**14**	**28**	**62**	**91**	**119**	**180**
metolachlor	water	96.9	64.2	56.5	30.4	19.6	6.1	2.9	1.4	0.5
	sediment	2.6	27.6	35.2	40.9	35.3	21.1	12.4	7.3	1.9
	Total system	99.5	91.8	91.7	71.3	54.9	27.2	15.3	8.7	2.4
CGA41507	water	<0.1	<0.1	<0.1	<0.1	0.8	2.0	1.9	2.1	1.5
	sediment	<0.1	<0.1	<0.1	0.9	3.8	7.6	9.9	7.0	8.2
	Total system	<0.1	<0.1	<0.1	0.9	4.6	9.6	11.8	9.1	9.7
CGA51202 (OXA)	water	<0.1	<0.1	<0.1	0.9	1.1	2.1	4.3	5.7	5.4
	sediment	<0.1	<0.1	<0.1	<0.1	0.2	0.8	0.7	2.5	2.6
	Total system	<0.1	<0.1	<0.1	0.9	1.3	2.9	5.0	8.2	8.0
CGA46129	water	<0.1	<0.1	<0.1	<0.1	<0.1	1.1	1.3	1.0	1.0
	sediment	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	0.3	0.3
	Total system	<0.1	<0.1	<0.1	<0.1	<0.1	1.1	1.3	1.3	1.3
CGA354743 (ESA)	water	<0.1	<0.1	<0.1	<0.1	<0.1	0.3	1.1	1.7	1.8
	sediment	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	0.2	1.4
	Total system	<0.1	<0.1	<0.1	<0.1	<0.1	0.3	1.1	1.9	3.2
CGA48087	water	<0.1	<0.1	<0.1	<0.1	<0.1	0.4	0.6	0.5	0.7
	sediment	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	0.8	0.3	1.0
	Total system	<0.1	<0.1	<0.1	<0.1	<0.1	0.4	1.4	0.8	1.7
CGA217498	water	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	0.6
	sediment	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	0.4	1.2
	Total system	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	0.4	1.8
(up to 8 fractions)	Total system	<0.1	1.0	0.5	0.8	0.8	1.5	1.6	6.1	6.1
total (water)	water	96.9	64.2	56.5	31.3	21.5	12.3	12.4	13.5	13.2
total (extractables)	sediment	2.6	28.6	35.7	42.6	40.1	30.7	25.1	22.8	21.0
total ^{14}C-CO2	volatiles	n.d.	<0.1	<0.1	0.1	0.3	0.8	1.1	1.7	2.0
total volatiles		n.d.	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
total non-extractables	sediment	<0.1	2.6	4.4	19.1	32.5	49.4	52.9	55.3	56.5
Total		**99.5**	**95.4**	**96.6**	**93.2**	**94.4**	**93.2**	**91.5**	**93.5**	**92.7**

n.d.: not determined.

Conclusion:

The study was considered acceptable for the first Annex 1 approval of S-metolachlor. After re-evaluation of the study, the RMS concluded that it also fulfils the requirements of current guidelines and is thus still considered acceptable.

The aerobic incubation of metolachlor showed equivalent results in kinetic of degradation and degradation pattern when compared to S-metolachlor. Two major metabolites identified as CGA41507 and CGA51202/CGA351916 (OXA) as well as several minor metabolites were detected. Based on the results shown, oxidation and reduction are two major degradation pathways for metolachlor and S-metolachlor. Besides the two major metabolites, bound residues were the main degradation products in the water / sediment system.

A new kinetic evaluation of the study results for aerobic conditions at 20 °C has been submitted (Hardy, 2014) for the renewal of the EU approval of S-metolachlor. Thus, the DT_{50} and DT_{90} values determined in the study are not presented here.

Hardy, 2014 (new, re-evaluation study)

Author:	Hardy, I.
Title:	Metolachlor/S-Metolachlor – Kinetic Modelling Analysis of Data from Water Sediment Studies to Derive Modeling and Persistence Endpoint DT_{50} values
Date:	2014
Doc ID:	Report No. NC/13/056B
Guidelines:	FOCUS (2006) “Guidance Document on Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration” Report of the FOCUS Work Group on Degradation Kinetics, EC Document Reference Sanco/10058/2005 version 2.0, 434 pp
Deviation:	-
GLP:	not applicable
Validity:	Acceptable

The aim of this evaluation was to conduct a kinetic modelling analysis of the data from aerobic water sediment degradation studies with metolachlor and S-metolachlor in order to derive total system DT_{50} values for use as modelling and trigger endpoints.

For the determination of DT_{50} values for metolachlor and S-metolachlor, all datasets were evaluated according to FOCUS Kinetics guidance using the water sediment Level P-I flowcharts for modelling and trigger endpoints [FOCUS, 2006].

Materials and methods:

The behaviour of metolachlor in water sediment systems has been investigated in two degradation studies conducted on two different water sediment systems under aerobic conditions. ^{14}C-phenyl labels were utilised in the studies for both metolachlor and S-metolachlor. Two sediment systems, River Rhine and Ormalingen Pond, were investigated in both studies.

The metolachlor and S-metolachlor residue data at time zero was set to the total percent recovered radioactivity multiplied by the radiochemical purity. Raw data are available in the original study reports (Mamouni, 1997; Seyfried, 1997). Values <LOQ were set to ½ LOQ (0.05) for the first occurrence.

<u>Kinetic modelling strategy:</u>

All datasets were evaluated using SFO and FOMC kinetics with free optimisation of all parameters.

DT_{50} and DT_{90} values were determined for the degradation of S-metolachlor / metolachlor. The determination of the kinetic values followed the recommendations of FOCUS rules and was aimed at deriving DT_{50} values for use as persistence and model input endpoints according to the FOCUS guidance document on degradation kinetics [FOCUS, 2006]. The kinetic evaluations were performed according to the respective decision flowcharts for the determination of level P-I parent persistence and modelling endpoints.

The kinetic evaluations and the statistical calculations were conducted with CAKE version 1.4 using iteratively re-weighted least-squares (IRLS) optimisation.

Optimisation statistics:

The model fits were evaluated using a chi-square ($\chi 2$) error statistic and visual inspection of residual plots. The kinetic analyses and optimisations were carried out using the residue data.

Results and Discussion:

The kinetic evaluations were performed according to the respective decision flowcharts for the determination of level P-I parent trigger and modelling endpoints. The degradation data for all datasets were entered into CAKE. Optimisations using SFO kinetics showed both visually and statistically acceptable fits (minimum Chi^2 error 1.9 – 3.8 %, t-test > 99 %). Optimisations using FOMC kinetics showed both visually and statistically acceptable (minimum Chi^2 error 4.2 – 7.2 %).

Accordingly, SFO kinetics were applied to all datasets as an initial step and checked for FOCUS acceptability criteria (minimum Chi^2 error <15 %, t-test parameter significance >95 % and visually acceptable). For the total systems degradation of S-metolachlor/metolachlor the FOMC kinetics showed no improvement over SFO kinetics, therefore SFO kinetics were determined to be appropriate for use as modelling and trigger endpoints.

The table below summarises the calculated SFO DT_{50} values for S-metolachlor/metolachlor.

Table 45: SFO DT_{50} values for S-metolachlor/metolachlor

Total System Modelling	**Substance**	**Kinetic**	**DT_{50} (days)**	**DT_{90} (days)**	**Chi^2 (%)**	**t-test (-)**	**Visual**
River	S-Metolachlor	SFO	54.8	182	1.9	8.76E-11	Excellent
Pond	S-Metolachlor	SFO	42.0	140	3.5	7.49E-09	Excellent
River	Metolachlor	SFO	45.4	151	3.1	2.74E-08	Excellent
Pond	Metolachlor	SFO	33.6	112	3.8	1.00E-07	Excellent

Conclusion:

This study was submitted for the renewal of the approval and is considered acceptable.

Kinetic modelling analysis of datasets from aerobic water sediment degradation studies for metolachlor and S-metolachlor showed good model fits when determining modelling endpoints.

11.1.4.3.3 Degradation in soil

11.1.4.3.3.1 Laboratory studies, aerobic

For the initial EU review, the route and degradation on soil of radiolabelled metolachlor (CGA24705) and S-metolachlor (CGA77102) were evaluated in several studies (Clark, 1995; Morgenroth, 1997, Kitschmann, 1997, Keller, 1997). However, these studies were conducted when the trigger for identification and further assessment of metabolites was 10 % of the applied radioactivity. Therefore for the renewal of S-metolachlor two new aerobic soil metabolism studies (Simmonds & Simmonds 2013 and 2014) with S-metolachlor were submitted in order to elucidate whether there were any additional metabolites, which represent > 5 % of applied radioactivity. Additionally, three new soil degradation studies (Lucas, 1996, Phaff, 2001 and Hein, 2007) were newly submitted.

In two studies (Clark, 1995 and Keller, 1997) the route and rate of degradation of S-metolachlor was compared to the behaviour of metolachlor under the same experimental conditions. The results showed that there was no significant difference in the degradation pattern of metolachlor and S-metolachlor. Thus, both the studies performed with metolachlor and S-metolachlor are considered suitable for the environmental fate assessment of S-metolachlor.

For the aerobic route of degradation of S-metolachlor, two principal routes were identified: oxidation and glutathione conjugation. Both routes yield the major soil metabolites ethane sulfonic acid CGA354743 (ESA) with up to 21.3 % applied radioactivity (AR) and oxalic acid CGA51202 (OXA) with up to 21.1 %. The subsequent degradation of CGA354753 (ESA) and CGA51202 (OXA) was found to proceed via NOA436611 (9.2 % AR), CGA368208 (7.6 % AR), CGA50720 (8.2 % AR), CGA37735 (7.1 % AR), CGA40172 (6.5 % AR) and CGA357704 (21.9 %). The mineralization to CO2 ranged to 0.3 – 29 % after 120 days and the non-extractable residue amounted to 4.6 – 44.5 % after 120 days. In the new studies (Simmonds & Simmonds, 2013 & 2014), the same overall profile of metabolism was observed although the final levels of these metabolites were lower than observed in previous studies. Although a number of minor metabolites were observed, none were observed to have exceeded 5 % applied radioactivity.

In addition to aerobic soil metabolism studies on S-metolachlor itself, studies on a number of metabolites were submitted. For the major metabolites CGA51202/CGA351916 (OXA) and CGA354743 (ESA) two soil degradation studies (Kitschmann, 1997b; Mamouni, 1997b) were submitted for the initial EU review and several new studies (Hein, 2004 and 2005; Nicollier, 2003; Nicollier & Glänzel, 2003) were conducted with for the renewal of S-metolachlor.

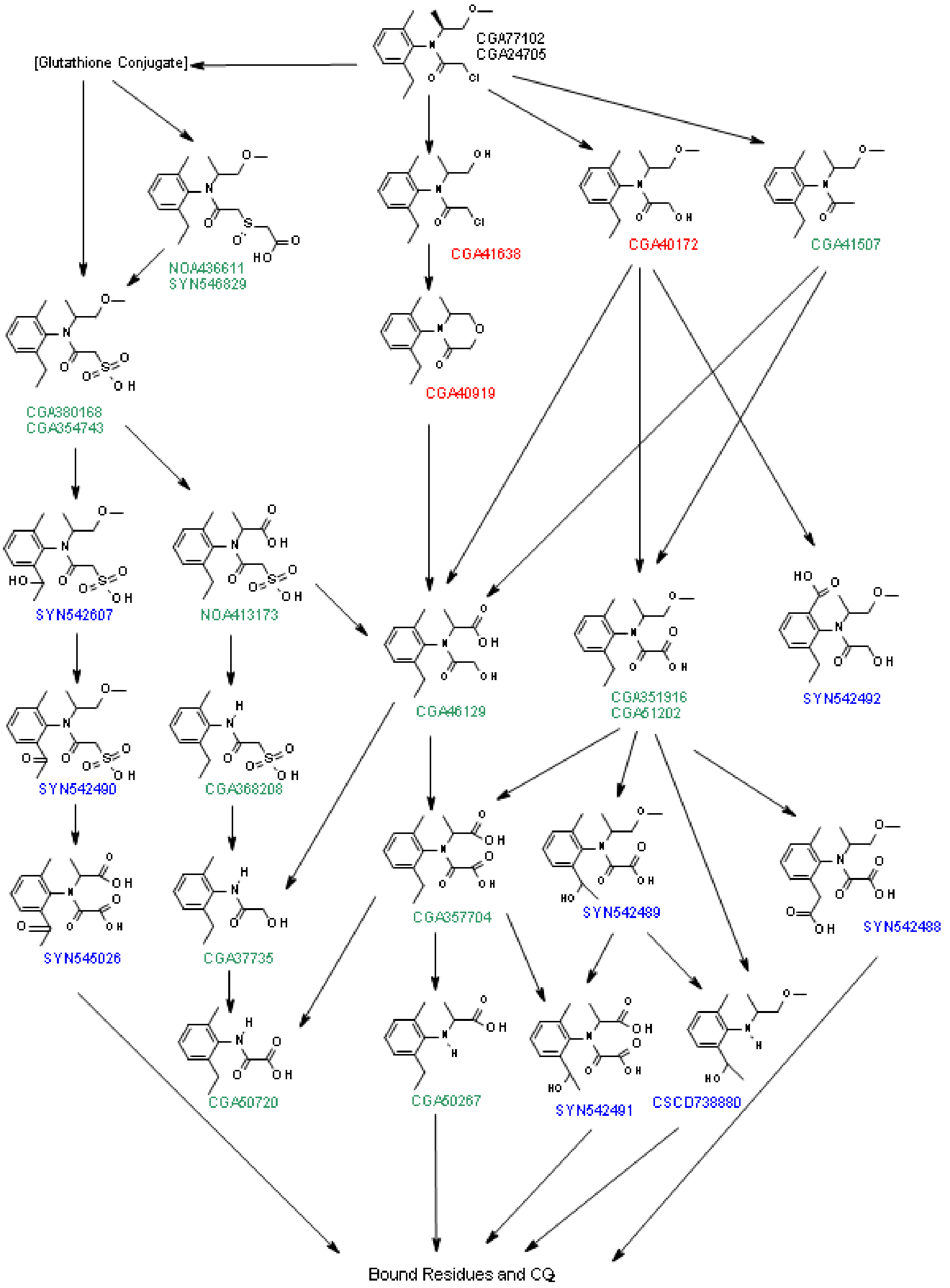
The degradation behaviour were investigated for further metabolites in order to generate degradation rates for leaching assessment, but also to validate steps within the proposed biotransformation pathway in soil. Specifically,

A soil metabolism study was conducted on CGA37735 in order to test whether this metabolite was metabolized to CGA50720.

A soil metabolism study was conducted on NOA436611 (in its racemic form, SYN546829) to test whether this metabolite was metabolized to CGA354743 (ESA).

A soil metabolism study was conducted on SYN542607 to test whether it was metabolized to SYN542490, as anticipated.

In addition to those metabolites observed in the aerobic laboratory degradation studies, analysis of the leachates from outdoor lysimeter studies identified a number of additional metabolites. For completeness, a number of these metabolites have also been considered in the proposed metabolic pathway for S-metolachlor in soil, which is shown in *Figure 11-1*.



KEY :
Bold : S-enantiomer form if two codes are supplied
Red : Only observed in laboratory soil studies
Green : Observed in both laboratory soil studies and lysimeter leachate
Blue : Only observed in lysimeter leachate

Figure 11-1: Proposed metabolic pathway for S-metolachlor in soil

A new kinetic evaluation according to FOCUS Degradation Kinetics 2006 was submitted for S-metolachlor and its metabolites CGA354743 (ESA), CGA51202 (OXA) CGA368208, CGA37735, CGA40172, CGA50720 and NOA436611 for the data from all previous and new studies. For modelling endpoints the recalculated SFO - DT50 values were normalized to reference conditions of 20 °C and pF2. The overall geometric mean modelling endpoint was calculated by firstly calculating the geometric mean DT50 values of the replicate soils.

The non-normalized best fit DT_{50} values for S-metolachlor varied in a wide range between 6.2 d and 257 days and the corresponding DT_{90} values between 33.8 d and > 1000 d following biphasic kinetics.

The normalized recalculated SFO – DT_{50} values for S-metolachlor varied between 11.2 days and 195.5 days. S-metolachlor shows no dissociation in the pH-range 2 – 12 and no clear pH de-pendency is observed. The longest DT_{50} of 195.5 days was determined in a sandy loam soil Borstel, which had a very low biomass in comparison to other soils.

The geometric mean of the DT_{50} values for S-metolachlor normalized to reference conditions of 20 °C and pF2 is 30.5 d.

Under anaerobic conditions, the major metabolite found was CGA41507, the dechlorinated parent compound, with a maximum of 44.2 % RA after 120 days. The degradation of ^{14}C-CGA41507 was studied in one soil Gartenacker under aerobic conditions at 20 °C and 40 % MWHC in the dark for 124 days. CGA41507 was degraded with a DT_{50} value of 51.5 days (SFO)

11.1.4.3.3.2 Field studies

The soil degradation behaviour of S-metolachlor/metolachlor was investigated in field studies conducted on several European sites. For deriving trigger endpoints, a new kinetic re-evaluation of 18 field trials according to FOCUS Degradation Kinetics (2006, 2011, 2014) was performed for the EU renewal. For site Riepsdorf, Germany, no acceptable fit could be obtained. The resulting DT_{50} values of 17 field trials are in a range between 3.55 and 55.7 days.

The maximum dissipation rate of 55.7 d following SFO kinetic can be used as soil degradation trigger endpoint DT_{50}.

11.1.4.4 Photochemical degradation

Oddy, 2013 (new study)

Author:	Oddy, A.
Title:	^{14}C-S-Metolachlor - Aqueous photolysis of 14C-S-Metolachlor. Final report
Date:	07/07/2013
Doc ID:	Syngenta File No CGA077102_11128
Guidelines:	OECD Guidelines for Testing of Chemicals. Test No. 316: Phototransformation of Chemicals in Water- Direct Photolysis (October 2008)
Deviation:	
GLP:	Yes
Validity:	Acceptable

Materials and methods:

The direct photolysis of ^{14}C phenyl-labelled-S-metolachlor was investigated in sterile, pH 7 buffer solution. ^{14}C-S-metolachlor was applied, at a nominal concentration of 1 mg/L, to the buffer solution in individual photolysis vessels. The treated solutions were irradiated using light from a xenon arc lamp, which emitted light that was filtered to give a spectral distribution close to that of natural sunlight at a mean intensity of 22.13 W/m². The samples were attached to a series of trapping solutions to collect any volatile products evolved, maintained at 25°± 2 °C and continuously irradiated for periods up to the equivalent of approx. 32.8 days summer sunlight exposure at latitudes between 30 °N (Florida) and 50 °N, assuming 12 hours of daylight. Conversion of artificial irradiation to equivalent days of natural summer sunlight was performed as recommended in the Draft OECD Guideline: “Phototransformation of Chemicals on Soil Surfaces” (January 2002), based on the intensity of radiation in the 300 – 400 nm range, since this is most relevant to the phototransformation of chemicals in the environment. Treated samples were also incubated under the same conditions but in the dark as controls.

In the irradiated test, duplicate samples were taken for analysis at seven intervals during irradiation. A single dark control sample was taken for analysis at intervals equivalent to that of the irradiation test.

Aqueous samples were radioassayed using LSC and analysed by HPLC to determine the levels of parent and significant photodegrades in each sample. Confirmation analysis by TLC was carried out on representative aqueous extracts.

Structural assignment was initially made by co-chromatography with authenticated reference standards (where available). Confirmation of the presence of any degradation product and the potential identity of unknown degradation products present ≥ 5 % of applied radioactivity was demonstrated by LC-MS-MS. All samples were initially analysed by HPLC within 1 day of sampling.

The half-lives ($DegT_{50}$) of ^{14}C-S-metolachlor in pH 7 buffer (from the HPLC analysis) were determined using a Single First Order (SFO) kinetic model with calculations performed according to the FOCUS guidance document on degradation kinetics.

Results and Discussion:

The mean recovery of radioactivity from the irradiated samples was 98.4 % AR (range 96.30 – 101.27 % AR) and from the dark controls was 97.32 % AR (range 93.82 – 100.53 % AR).

In sterile buffer ^{14}C-S-metolachlor degraded slowly with means of 81.75 % AR and 93.13 % AR (irradiated and dark controls respectively) remaining after 894 hours. Half-lives (DegT50) of 129 days (irradiated) and 624 days (dark control) of summer sunlight using SFO kinetics were determined. The results are presented in the table below.

Table 46: $DegT_{50}$ and $DegT_{90}$ values for S-metolachlor in irradiated and dark control solutions

	SFO		
	$DegT_{50}$ [days]	**$DegT_{90}$ [days]**	χ^2
Irradiated (experimental result)	146	485	1.4
Irradiated (equivalent to summer sunlight, mean for latitude 30°N -50°N)	129	427	
Dark control (experimental result)	624	> 1000	1.1

The major degradate of S-metolachlor was found to be degradate A (MW 265) which reached 7.39 % AR (mean value) at 894 hours. In addition, a number of discrete unknown photodegrades were also observed, none exceeding 3.36 % AR.

Carbon dioxide was a minor product of photolysis reaching a maximum of 0.7 % AR by the end of the irradiation period.

No degradation was apparent in the ‘dark controls’ indicating that the degradation in irradiated samples was due to photodegradation only.

Conclusion:

The study is considered acceptable by the RMS.

Berdat T, Nicollier G, 2008 (new study)

Author:	Berdat T, Nicollier G.
Title:	Amended No.1 to Final Report on Study T017314-04 - CGA24705: Aqueous Photolysis of 14C-Phenylring Labelled CGA24705 (Metolachlor) in Sterile Natural Water under Laboratory Conditions.
Date:	25/01/2008
Doc ID:	No. T017314-04
Guidelines:	JMAFF 12 Nousan No. 8147
Deviation:	
GLP:	Yes
Validity:	Acceptable

Materials and methods:

^{14}C-radiolabelled CGA24705 at the phenylring moiety was applied at a concentration of 1.9 ppm to the sterile natural water and was irradiated with a xenon light source. The mean temperature of the samples was kept at 25 ± 1 °C for a maximum of 25 days of irradiation with artificial light. The 25 days of continuous Suntest irradiation (artificial light) corresponded to 44.4 natural summer sunlight days at latitudes 30 to 50°N according to the lamp irradiation intensity. Duplicate irradiated samples were taken for analysis at evenly spaced intervals over the irradiation period. Corresponding duplicate samples were incubated at 25 ± 1 °C for a maximum of 25 days in the dark.

The DegT$_{50}$ and DegT$_{90}$ of ^{14}C-CGA24705 in natural water (from the HPLC analysis) were determined using Single First Order (SFO) and First Order Two Compartment (FOTC) kinetic models.

Results and Discussion:

The amount of ^{14}C-CGA24705 decreased to 26.9% (mean value) of the applied radioactivity after 25 days of irradiation. The concentration of $^{14}CO_2$ reached a maximum of 20.2% at the end of study. No degradation was observed in the dark controls.

Around 45 photodegradate fractions (U1 to U45) were separated by HPLC and all fractions were below 5.8%. Only the metabolites CGA13656, CGA41638/ CGA40172 could be identified in small amounts of ≤ 0.3% of applied ^{14}C-radioactivity.

The rate of photodegradation of ^{14}C-CGA24705 was described using first order kinetics (SFO) and first order two-compartment (FOTC) kinetics. The results are presented in the table below.

Table 47: DegT50 and DegT90 values for 14C-CGA24705 in irradiated and dark control solutions

Test system	SFO			FOTC		
	DegT50 [days]	DegT90 [days]	r^2	DegT50 [days]	DegT90 [days]	r^2
Irradiated (experimental result)	12.11	40.22	0.94	10.05	69.45	0.98
Summer Sunlight (30-50°N)	21.5	71.4		17.8	123.3	

Conclusion:

RMS considers the study only as additional information, as no harmonized guidance exist until now, how to determine indirect phototransformation in natural water. According to the OECD GD No.316 – Phototransformation in Water, indirect phototransformation of substances in natural water is influenced by many different processes and methods for evaluating the relevance of these processes are not well tested yet.

However, the study results indicate that indirect phototransformation of metolachlor can be occurred in natural waters under influence of sunlight.

11.1.4.4.1 Soil photolysis

The soil photolysis rate of S-metolachlor/metolachlor was investigated in two studies. The first study (Merritt, 1995) was evaluated during the initial EU review (DAR, 2000). No significant difference of the degradation rates was observed between the irradiated and non-irradiated soil samples.

For the renewal a new study (Simmonds, 2012) was submitted to investigate the photodegradation of S-metolachlor in dry and moist soil. Photodegradation of S-metolachlor was slow in both moist and dry soil layers. The major degradation product observed was CGA41638, reaching a maximum level of 5.6 % and 5.4 % (mean values) of the applied dose in dry and moist soil photolysis experiments, respectively. No other single metabolite was observed at > 2.9 % of the applied dose. Low levels of radiolabelled carbon dioxide were produced during incubation for the irradiated samples for both the dry and moist soil experiments. Accumulated levels reached a maximum of 1.5 % of the applied dose in both the dry and moist soil photolysis. Bound residues slowly increased throughout the incubation period to 4.7 % AR and 2.9 % AR at the end of the air dried soil layer experiment (irradiated and dark control series respectively). The bound residues for the moist soil layer experiment increased throughout the incubation period 9.4 % AR and 8.2 % AR at the end of the study (irradiated and dark control series respectively).

The results show that photodegradation on soil is no significant route of degradation under environmental conditions.

11.2 Environmental transformation of metals or inorganic metals compounds

Not relevant for this dossier.

11.3 Environmental fate and other relevant information

11.3.1 Adsorption and desorption in soil

Already during the first Annex 1 approval of S-metolachlor, two studies (Spare, 1995; Ellgehausen, 1997) on the soil adsorption and desorption for the active substance were considered acceptable. Three new studies (Glaenzel, 1999; Nicollier, 2000; Hein, 2004) for the active substance were submitted and considered acceptable for the renewal of S-metolachlor.

Table 48: Freundlich adsorption coefficients and exponents of S-metolachlor

Parent (S-metolachlor, 77102)						
Soil Type	**OC %**	**Soil pH**	**K_F (mL/g)**	**K_{Foc} (mL/g)**	**1/n**	**Reference**
Leland Mississippi (clay)	1.276	7.2$^{a)}$	4.7	368	0.934	Spare, W.C. (1995)
Lime Kiln Maryland (sandy loam)	1.160	8.0$^{a)}$	1.4	121	0.909	
Middletown Maryland (silt loam)	0.986	7.0$^{a)}$	1.1	112	0.914	
Collombey (loamy sand)	0.8	7.3$^{c)}$	1.4	175	0.909	Ellgehausen, H. (1997)
Speyer 2.1 (sand)	0.3	6.8$^{c)}$	1.0	333	0.887	
Gartenacker (silt loam)	2.0	7.1$^{c)}$	4.6	230	0.971	
Vetroz (silt loam)	4.7	7.2$^{c)}$	11.5	245	1.002	
Illarsaz (humic silt loam)	19.8	6.7$^{c)}$	44.8	226	0.926	
Bahus 1 0-10cm (silt loam)	5.91	3.42$^{b)}$	10.82	183	0.927	Glänzel, A. (1999)
Bahus 2 10-20cm (silt loam)	3.02	3.75$^{b)}$	7.63	253	0.925	

Birkenheide, (loamy sand)	0.65	3.42 b)	1.09	168	0.952	Nicollier, G. (2000)
Soil Lorsch Horizon I (sandy loam)	1.63	5.17b)	2.37	145	0.9629	Hein, W. (2004)
Geometric mean (if not pH dependent)			**3.63**	**200.24**	**0.93**	
Arithmetic mean (if not pH dependent)			**7.7**	**213**	**0.935**	
pH dependence, No						

a) Measured in $CaCl_2$

b) Measured in KCl

c) Medium in which the pH measurements were performed is not reported in study

11.3.2 Fate and behaviour in air

S-metolachlor has a vapour pressure of 3.7 x10^{-3} Pa at 25 °C (extrapolated from higher temperatures) and a Henry's Law's constant of 2.20 x 10^{-03} Pa x m^3/mol at 25 °C. These values, especially the relatively high vapour pressure suggest that a volatilisation of S-metolachlor may occur after application.

The experimental data on the fate and behaviour of S-metolachlor in air confirms this view. A wind tunnel experiment (Bourry and Nicollier, 2005) demonstrated that S-metolachlor can enter surface waters by volatilization and subsequent deposition (the maximum concentrations in water bodies 1 m away was 0.75µg/L). In a field experiment (Gish et al, 2011) the cumulative volatilisation losses were measured over an 8-year study period. Volatilization losses were high: ca. 5 – 63 % or 6 – 23 % if one atypical year is excluded. Average of volatilisation losses during the 7-year is about 9 % with a CV = 80 %. The volatilisation losses correlated well with moisture of the soils, with the highest volatilisation value observed in the year of most intense rainfall. Metolachlor volatilisation losses were clearly greater during daytime when compared with the estimated for nighttime. Local effects of S-metolachlor application due to volatilization and subsequent deposition can therefore not be excluded and should be assessed with suitable tools.

The estimated half-life of S-metolachlor in the atmosphere (by hydroxyl radical oxidation) is 2.3 h (calculated with 1.5 x 10^6 OH-radicals/cm^3 and 12 h day). Due to the short persistence in the atmosphere, the PEC$_{air}$ is expected to be negligible and global effects as a result of long-range transport are not expected to be of relevance.

11.4 Bioaccumulation

Table 49: Summary of relevant information on bioaccumulation

Method	Results	Remarks	Reference
OECD (1996). Proposal for Updating Guideline 305	The lipid-corrected steady state BCF for whole fish in the lower concentration (worst-case) is 255.	*Lepomis macrochirus*; 28 d uptake, 14 d depuration; Reliability 1	Anonymous (2001) CGA77102/0580
Partition coefficient n-octanol/water	at 25 °C : log Pow = 3.05	The log Pow is below the cut-off value of ≥ 4	Section 7 of this report (physicochemical properties)

11.4.1 Measured bioaccumulation test data

Anonymous (2001)

Author:	Anonymous
Title:	Accumulation and elimination of [Phenyl-(U)-14C] CGA77102 by bluegill sunfish (Lepomis macrochirus) in a dynamic flow-through system
Date:	2001
Doc ID:	Syngenta File No. CGA77102/0580
Guidelines:	OECD (1996). OECD Guidelines for Testing of Chemicals, Proposal for Updating Guideline 305, Bioconcentration: Flow-through Fish Test. Paris, France.

	EPA 540/09-82-021, Section 165-4 (1982) EPA 540/09-88-051, Addendum 8 on data reporting (1988)
GLP:	Yes
Validity:	Yes
Previous evaluation	In DAR (2018)

Executive Summary

The study was undertaken to determine the bioconcentration and subsequent depuration of [Phenyl-(U)-^{14}C] CGA77102 in bluegill sunfish (*Lepomis macrochirus*). 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 CGA77102/L, and a solvent control.

CGA77102 residues were rapidly concentrated in fish tissues, reaching a steady-state concentration within approximately 7 days.

The measured bioconcentration factor (BCF_{ss}) for the 0.03 mg CGA77102/L treatment, based on ^{14}C-residues, was 169, 17 and 94 in non-edible tissues, edible tissues and whole fish tissues respectively. At the lower concentration (0.003 mg/L) these values were 202, 20 and 112 respectively. Thus, the mean BCF_{ss} for CGA77102 is 103 for whole fish.
The depuration of accumulated residues was rapid, with approximately 91 % depuration after 10 days. The whole fish DT_{90} was 5.4 days at 0.03 mg/L and 7.4 days at 0.003 mg/L.

Validity of the study
The study is considered valid as temperature variations were less than ± 1°C, the dissolved oxygen remained above 60 % 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, and no symptoms of sub-lethal toxicity were observed.

Conclusions

CGA77102 residues were rapidly concentrated in fish tissues, reaching a steady-state concentration within about 7 days.

The whole fish uptake rate constant (K_u) was 40.3/day, and the depuration rate constant (K_d) was 0.42/day.
The depuration of accumulated residues was rapid, with approximately 91 % depuration after 10 days. The whole fish DT_{90} was 5.4 days at 0.03 mg/L and 7.4 days at 0.003 mg/L.
The study has the following shortcomings:

- The study was not performed according to newest guideline OECD 305 of October 2nd, 2012
- It is stated in OECD 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 CGA 77102 or total radioactivity.
- Lipid content for whole fish at day 28 is not reported but needed to express the BCF based on 5 % lipid content as laid out in OECD 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 it can be concluded that S-metolachlor BCF in fish is 255, which is below the CLP criteria of 500. A bioconcentration potential for classification purposes is not indicated. This is supported by the log P_{ow} of 3.05, which is below the cut-off value of 4.

11.5 Acute aquatic hazard

Please note that solely studies for S-metolachlor (CGA-77102) are considered for classification. Studies for metolachlor (CGA 24705) are listed for completeness.

Based on the aquatic toxicity tests with S-metolachlor and its general degradability degradation products are not assumed to cause the observed toxicity. Additionally, degradation products of S-metolachlor are clearly less toxic compared to the parent (please refer to the RAR of S-metolachlor). Degradation products of S-metolachlor do not need to be considered for classification.

Table 50: Summary of relevant information on acute aquatic toxicity

Method	**Species**	**Test material**	**Results**[1]	**Remarks**	**Reference**
U.S. EPA, 1975	*Oncohrynchus mykiss (Salmo gairdneri)*	CGA 24705 (metolachlor)	LC_{50} (96 h) = 3.9 mg a.s./L (nominal)	No analytical verification of test concentrations Reliability 2	Anonymous (1978a)
US Federal Department of the Interior, Fish and Wildlife Services: Procedure for evalu-ation of acute toxicity of Pesticides to fish and wildlife (1964)	*Oncohrynchus mykiss* (Rainbow trout), *Carassius carassius* (Crucian carp), *Ictalurus punctatus* (Channel catfish), *Lepomis macrochirus* (Bluegill), *Poecilia reticulata* (Guppy)	CGA 24705 (metolachlor)	LC_{50} (96 h) = 2 mg a.s./L (nominal) LC_{50} (96 h) = 4.9 mg a.s./L (nominal) LC_{50} (96 h) = 4.9 mg a.s./L (nominal) LC_{50} (96 h) = 15 mg a.s./L (nominal) LC_{50} (96 h) = 8.6 mg a.s./L (nominal)	No analytical verification of test concentrations Reliability 2	Anonymous (1974)
U.S. EPA, 1975	*Lepomis macrochirus*	CGA 24705 (metolachlor)	LC_{50} (96 h) = 10 mg a.s./L (nominal)	No analytical verification of test concentrations Reliability 2	Anonymous (1978b)
EPA guidelines 72-5	Pimephales promelas	CGA 24705 (metolachlor)	LC_{50} (96 h) = 9.2 mg a.s./L (mean measured)	Analytical verification of test concentrations based on data from days 0, 7 and 14 Reliability 2	Anonymous (1993)
American Society for Testing and Materials Committee E-35 on Pesticides, 1980	*Leiostomus xanthurus*	CGA 24705 (metolachlor)	LC_{50} (96 h) = 4.2 mg a.s./L (initial measured)	No analytical verification at test end. Reliability 2	Anonymous (1980a)
ASTM Standard E-35 Standard practice for conducting basic	*Cyprinodon variegatus*	CGA 24705 (metolachlor)	LC_{50} (96 h) = 7.5 mg a.s./L (mean measured)	Study details not fully reported. Reliability 2	Anonymous (1980b)

acute toxicity tests with fishes, macroinvertebrates, and amphibians (1980)					
EPA-660/3-75-009; 1975	*Oncorhynchus mykiss (Salmo gairdneri)*	CGA 77102 (S-metolachlor)	LC_{50} (96 h) = 1.23 mg a.s./L (initial measured)	Key study Minor deviation from validity Reliability 2	Anonymous (1983a)
EPA-660/3-75-009; 1975	*Lepomis macrochirus*	CGA 77102 (S-metolachlor)	LC_{50} (96 h) = 3.16 mg a.s./L (initial measured)	Minor deviation from validity Reliability 2	Anonymous (1983b)
FIFRA Guideline 72-3/72-1	*Cyprinodon variegatus*	CGA 24705 (metolachlor)	LC_{50} (96h) = 9.8 mg a.s./L (mean measured)	Reliability 1	Anonymous (1994a)
FIFRA Guideline 72-1	*Oncohrynchus mykiss*	CGA 77102 (S-metolachlor)	LC_{50} (96 h) = 12 mg a.s./L (	Minor deviation from validity Reliability 2	Anonymous (1995a)
OECD 203	*Cyprinus carpio*	CGA 77102 (S-metolachlor)	LC_{50} (96 h) = 20 mg a.s./L (mean measured)	Reliability 1	Anonymous (2006)
OPPTS 850.1075	*Cyprinodon variegates*	CGA 77102 (S-metolachlor)	LC_{50} (96 h) = 17 mg a.s./L (mean measured)	Reliability 1	Anonymous (2004)
ASTM 1980	*Palaemonetes pugio*	CGA 24705 (metolachlor)	LC_{50} (96 h) = 17 mg/L (initial measured)	Reliability 2	Heitmuller, T. (1980a)
ASTM 1980	*Penaeus duorarum*	CGA 24705 (metolachlor)	LC_{50} (96 h) = 8.3 mg/L (initial measured)	Multiple deviations from the Guideline Reliability 3	Heitmuller, T. (1980b)
US EPA-600/9-78-010	*Acartia tonsa*	CGA 24705 (metolachlor)	LC_{50} (96 h) = 1.5 mg/L (initial measured)	No analytical verification of test concentrations at the end of the test. Reliability 2	Hollister, T.A. and Ward, G.S. (1980a)
ASTM Draft No.7	*Crassostrea virginica*	CGA 24705 (metolachlor)	EC_{50} (96 h) = 18 mg/L (initial measured)	No analytical verification of test concentrations at the end of the test. Reliability 2	Hollister, T.A. and Ward, G.S. (1980b)
ASTM 1981; EPA-660/3-75-009	*Daphnia magna*	CGA 77102 (S-metolachlor)	EC_{50} (48 h) = 11.24 mg/L (initial measured)	No analytical verification of test concentrations at	Spare, W.C. (1983c)

				the end of the test. Reliability 2	
EPA 850.1035, 72-3	*Mysidopsis bahia*	CGA 77102 (S-metolachlor)	LC$_{50}$ (96 h) = 1.4 mg/L (mean measured)	Key study Reliability 1	Spare, W.C. (1983d)
FIFRA Guideline Number 72-3(b)	*Crassostrea virginica*	CGA 24705 (metolachlor)	EC$_{50}$ (96 h) = 1.8 mg/L (mean measured)	Reliability 1	Dionne, E. (1994)
FIFRA Guideline Number 72-3(c)	*Mysidopsis bahia*	CGA 24705 (metolachlor)	LC$_{50}$ (96 h) = 4.9 mg/L (mean measured)	Reliability 1	Machado, M.W. (1994b)
ASTM	*Uca pugilator*	CGA 24705 (metolachlor)	LC$_{50}$ (96 h) > 47 mg/L (initial measured)	Only initial measured concentartions; test system with sand. Reliability 3	Heitmuller, T. (1980c)
FIFRA Guideline Number 72-2(a)	*Daphnia magna*	CGA 77102 (S-metolachlor)	LC$_{50}$ (48 h) = 26 mg/L (mean measured)	Exceedance of the allowed solvent concentration. Reliability 2	Collins, M.K. (1995b)
OPPTS Number 850.1025	*Crassostrea virginica*	CGA 77102 (S-metolachlor)	EC$_{50}$ (96 h) = 4 mg/L (mean measured)	Reliability 1	Palmer, S.J.; Kendall, T.Z. and Krueger, H.O. (2004b)
FIFRA Guideline number 122-2 and 123-2	*Navicula pelliculosa*	CGA 24705 (metolachlor)	E$_r$C$_{50}$ (96 h) = 4.982 mg/L E$_r$C$_{10}$ (96 h) = 0.104 mg/L (mean measured)	Validity criteria not met. Reliability 3	Hoberg, J.R. (1995a)
FIFRA Guideline number 122-2 and 123-2	*Skeletonema costatum*	CGA 24705 (metolachlor)	E$_r$C$_{50}$ (72 h) = 0.423 mg/L E$_r$C$_{10}$ (72 h) = 0.007 mg/L (nominal)	Reliability 1	Hoberg, J. R. (1994)
OECD 201	*Skeletonema costatum*	CGA 77102 (S-metolachlor)	ErC50 (72 h) = 0.340 mg/L E$_r$C$_{10}$ (72 h) = 0.013 mg/L (mean measured)	Minor deviation from validity criteria Reliability 2	Hoberg, J. R. (1995b)
U.S. EPA FIFRA Guideline No. 122-2 and 123-2	*Anabaena flos-aquae*	CGA 24705 (metolachlor)	ErC50 (120 h) = 1.1 mg/L ErC10 = 0.606 mg/L	Several validity criteria not met Reliability 3	Hoberg J.R. (1995c)
FIFRA Guideline number 122-2 and 123-2	*Selenastrum capricornutum*	Metolachlor	ErC50 (96 h) = 0.0278 mg/L NOEC = 0.8 mg/L	Severe violation of validity criteria Reliability 3	Hoberg J.R. (1995d)

FIFRA Guideline number 122-2 and 123-2	*Selenastrum capricornutum*	CGA 77102 (S-metolachlor)	ErC50 (72 h) = 0.024 mg/L ErC10 (72 h) = 0.0036 mg/L	Severe violation of validity criteria Reliability 3	Hoberg J.R. (1995e)
OECD 201	*Desmodesmus subspicatus*	CGA 24705 (metolachlor)	ErC50 (72 h) = 0.247 mg/L (nominal)	Severe violation of validity criteria Reliability 3	Rufli, H. (1985)
US EPA 1974/1978	*Microcystis aeruginosa* *Selenastrum capricornutum* *Chlorella pyrenoidosa* *Dunaliella tertiolecta* *Skeletonema costatum* *Isochrysis galbana* *Porphyridium cruentum*	Metolachlor	ErC50 (72 h): 13.3 mg/L 0.071 mg/L 6.09 mg/L - 0.97 mg/L 0.436 mg/L - All endpoints baded on nominal concentrations	Reliability 4	Hollister, T.A and Ward, G.S. (1980)
OECD 201	*Pseudokirchneriella subcapitata*	CGA 77102 (S-metolachlor)	E_rC_{50} (72 h) = 0.056 mg/L NOEC (growth, 72 h) = 0.012 mg/L (mean measured)	Key study Reliability 1	Memmert, U. (2006)
OECD 201	*Navicula pelliculosa*	CGA 77102 (S-Metolachlor)	E_rC_{50} (72 h) = 31 mg/L NOEC (growth, 72 h) = 9.7 mg/L (mean measured)	Reliability 1	Desjardins, D.; Kendall, T.Z.; Krueger, H.O. (2003)
OECD 201	*Anabaena flos-aquae*	CGA 77102 (S-Metolachlor)	ErC50 (72h) = > 30 mg/L EC10 (72 h) = 13 mg/L	Severe violation of validity criteria Reliability 3	Desjardins, D.; Kendall, T.Z.; Krueger, H.O. (2004)
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 (mean measured	Key study Reliability 2	Teixeira, D. (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 (mean measured)	Supplemental information	Teixeira, D. (2006b)
FIFRA Guideline number 122-2 and 123-2	*Lemna gibba*	CGA 24705 (metolachlor)	E_rC_{50} (14 d) = 0.0367 mg/L NOEC (growth, 14 d)	Minor deviation from validity criteria Reliability 2	Hoberg, J. R. (1995f)

			= 0.0022 mg/L (mean measured)		
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)	Severe violation of validity criteria Reliability 3	Hoberg, J. R. (1995g)
OECD 221	*Lemna gibba*	CGA 77102 (S-metolachlor)	E_rC_{50} (7 d) = 0.133 mg/L NOEC (growth, 7 d) = 0.0021 mg/L (mean measured)	Reliability 1	Eckenstein, H. (2014)
OECD 221	*Lemna gibba*	CGA 77102 (S-metolachlor)	ErC50 (7 d) = 0.149 mg/L NOEC = 0.00384 mg/L (mean measured)	Reliability 1	Kümmrich F. (2019)

11.5.1 Acute (short-term) toxicity to fish

Anonymous (1978a)

Author:	Anonymous
Title:	Acute toxicity of CGA 24705 to rainbow trout (Salmo gairdneri)
Date:	1978
Doc ID:	Report Number BW-78-6-186
Guidelines:	U.S. EPA, 1975
GLP:	No
Validity:	Yes
Previous evaluation:	DAR (2018)

Executive Summary

Rainbow trouts were exposed to the test substance with 10 fish per concentration at nominal concentrations of 0.8, 1.3, 1.9, 2.88, 4.1, 6.0 and 8.8 mg a.s./L, a control and a solvent control for a period of 96 hours in a static test design. No chemical analysis to verify test concentrations was performed.

No mortalities at concentrations up to 2.88 mg a.s/L. Mortality was 70 % in the 4.1 mg a.s./L test concentration and 100 % in the 6.0 and 8.8 mg a.s./L test concentrations.

Conclusions:

LC50 (96 h) = 3.9 mg/L

NOEC (96 h) = 2.8 mg/L

As it was shown in recent studies with comparable static test design that no major decline of test concentrations has to be expected over the test duration the study is considered valid and acceptable to be used for classification even without chemical analysis.

Due to the missing analytical verification at the start and end of the test, the study is considered reliable with restrictions.

Anonymous (1974)

Author:	Anonymous
Title:	Acute toxicity to rainbow trout, crucian carp, channel catfish, bluegill and guppy of technical CGA 24705
Date:	1974
Doc ID:	Report Number SISS-3516
Guidelines:	None
GLP:	No
Validity:	Yes
Previous evaluation:	DAR (2018)

Executive Summary

Five different fish species (rainbow trout, crucian carp, channel catfish, bluegill and guppy) were exposed to the test substance at nominal concentrations of 0.65, 1.0, 6.5 and 10 mg a.s./L and solvent control for a period of 96 hours in a static test design. 12 fish per concentration were used. No chemical analysis to verify test concentrations was performed.

Conclusions

The following endpoints were derived:

Species	**96h-LC$_{50}$ (mg a.s./L)**
rainbow trout	2
crucian carp	4.9
channel catfish	4.9
bluegill	15
guppy	8.6

It was shown in recent studies with comparable static test design that no major decline of test concentrations has to be expected over the test duration. However, as no guideline is reported, the study is not conducted under GLP and more reliable data is available, the study is just considered as supplemental information for the purpose of classification.

Due to the missing analytical verification at the start and end of the test, the study is considered reliable with restrictions.

Anonymous (1978b)

Author:	Anonymous
Title:	Acute toxicity of CGA 24705 to bluegill (Lepomis macrochirus)
Date:	1978
Doc ID:	Report Number BW-78-6-181
Guidelines:	U.S. EPA, 1975
GLP:	No
Validity:	Yes
Previous evaluation:	DAR (2018)

Executive Summary

Bluegill were exposed to the test substance with 10 fish per concentration at nominal concentrations of 1.9, 2.9, 4.1, 6.0, 8.8, 13, 19 and 28 mg a.s./L, a control and a solvent control for a period of 96 hours in a static test design. No chemical analysis to verify test concentrations was performed.
No mortalities at concentrations up to 6.0 mg a.s/L. Mortality was 10 % in the 8.8 mg a.s./L test concentration and 100 % in the 13, 19 and 28 mg a.s./L test concentrations.

Conclusions

LC50 (96 h) = 10 mg a.s./L
NOEC (96 h) = 6.0 mg a.s./L

As it was shown in recent studies with comparable static test design that no major decline of test concentrations has to be expected over the test duration the study is considered valid and acceptable to be used for classification without chemical analysis.

Due to the missing analytical verification at the start and end of the test, the study is considered reliable with restrictions.

Anonymous (1993)

Author:	Anonymous
Title:	Chronic toxicity of CGA 24705 to the Fathead minnow (Pimephales promelas). EG&G Bionomics
Date:	1993
Doc ID:	Unpublished report No. BW-78-11-341
Guidelines:	EPA guidelines 72-5
GLP:	No
Validity:	Cannot be checked due to missing information

Executive summary and methods

A preliminary acute flow-thorugh study with CGA 24705 was conducted sumarised here. For a summary of the chronic study please refer to the respective section.
A preliminary 14-day exposure of fathead minnow juveniles was conducted in a flow-through system using a proportional diluter with a 0.25 dilution factor. Thirty 0.19 g fish were exposed to each of seven unreplicated concentrations of CGA-24705 and a solvent control. The amount of acetone in the solvent control was equal to the 0.028 mg/L acetone in the highest test concentraionts of CGA-24705. Using the mortality of juvenile fish and mean measured concentrations of CGA-24705, a 96-hour LC50 and 95% confidence intervals were

calculated by a moving average method (Stephan, 1978). Juvenile fathead minnows for the acute toxicity tests were obtained from the Newton Fish Toxicology Station, EPA, Cincinnati, Ohio. Newly hatched fry was taken from brood stock at EG & G, Bionomics, Aquatic Toxiciology Laboratory, Warenham, Massachusetts.

Results

Results for 96 h are based on mean measured concentrations derived from water samples taken on days 0, 7 and 14.

Toxicity after 96 h

Mean measured concentration (mg/L)	Juvenile % dead after 96 h
13	70
7.5	40
4.7	10
4.8	7
4.2	23
3.1	0
2.6	3
Solvent control	0

The LC50 96 h and 95% confidence interval were calculated to be 9.2 (7.9 – 11) mg/L

Conclusions

The LC50 96 h is 9.2 (7.9 – 11) mg/L

The study shows the following shortcomings:

- No oxygen concentration is reported. The validity criteria for >60% DO cannot be verified.
- Mean measured concentrations are based on 0, 7 and 14 days instead of considering the for acute effects relevant study duration of 4 d
- Only a solvent control and no negative control was included.
- Detailed information about material and methods is missing
- The study is not conducted under GLP

Despite the shortcomings the study is considered reliable with restrictions. It was conducted under flow-through conditions and the dissolved oxygen concentration is supposed to be above 60%. Also, the mean measured concentrations based on 0, 7 and 14 days should not influence the result. Even though some information is missing, the study can be used for classification purpose.

Anonymous (1980a)

Author: Anonymous
Title: Acute toxicity of metolachlor (CGA 24705) (DUAL7) to spot (Leiostomus xanthurus)
Date: 1980
Doc ID: Report Number BP-80-3-59
Guidelines: American Society for Testing and Materials Committee E-35 on Pesticides, 1980
GLP: No, but complies with sound scientific standards
Validity: Yes
Previous evaluation: DAR (2004, 2018)

Executive Summary

4 replicates of 3 fish per concentration, *Leiostomus xanthurus*, (body length 30-36 mm; body weight 0.44-0.89 g) were exposed to the test substance at concentrations of 1.3, 2.2, 3.6, 6 and 10 mg a.s./L for a period of 96 hours in a static test design per concentration and water control. The concentrations in test media ranged on day 0 from 84 to 104 % of the nominal. The concentrations after 96 hours were not mentioned. Results are based on initial measured concentrations.
No mortalities at the concentrations 1.2, 2.3 and 3.3 ppm. Mortality was 92 % in the 5.4 ppm test concentration and 100 % in the 8.4 ppm test concentration.

Conclusions

LC50 (96 h) = 4.2 mg a.s./L
NOEC (96 h) = 3.3 mg a.s./L

Despite the shortcoming of no analytical verification of test concentrations at the test end the study is considered reliable with restrictions. The study is considered valid and acceptable to be used for classification.

Anonymous (1980b)

Author: Anonymous
Title: Effects of metolachlor (Dual$^{®}$) on survival, growth, and development of sheepshead minnows (*Cyprinodon variegatus*)
Date: 1980
Doc ID:Report Number BP-80-5-80
Guidelines: ASTM Standard E-35 Standard practice for conducting basic acute toxicity tests with fishes, macroinvertebrates, and amphibians (1980)

GLP: No
Validity: Yes

Executive Summary

The main study was a chronic fish study. Some of the initial work included an acute fish study with sheepshead minnow (*Cyprinodon variegatus*). This is reported in this summary. The main study is summarised separately.

The acute toxicity of metolachlor to sheepshead minnow (*Cyprinodon variegatus*) was determined. Fish were exposed to the following range of nominal concentrations of 0.62, 1.2, 2.5, 5.0 and 10 mg metolachlor/L (mean measured concentrations 0.59, 1.0, 2.2., 4.4 and 9.4 mg metolachlor/L), a solvent control and a dilution seawater control. Based on mean measured concentrations, the 96-hour LC_{50} for metolachlor to sheepshead minnow (*Cyprinodon variegatus*) was 7.5 mg/L.

Study Design and Methods

Experimental dates: 11th to 15th March 1980

A flow-through test system was employed. A stock solution consisting of metolachlor in triethylene glycol, with a nominal concentration 80.825 mg metolachlor/L, was delivered to the mixing chamber where it was diluted and made up to a set volume with seawater before being delivered to the test vessels to give the test concentrations. The blank control consisted of seawater only and the solvent control consisted of triethylene glycol and dilution seawater.

At the start of the test 10 fish were placed in each duplicate tank for the test concentrations and each control. Mortality and any abnormal characteristics were recorded at 0, 24, 48, and 96 hours.

Daily measurements of the test solutions were undertaken throughout the 96-hour period for pH, temperature and dissolved oxygen concentration.

The test concentrations were verified by chemical analysis of metolachlor at the beginning and at the end of the test.

The median lethal concentration (LC_{50}) was graphically interpolated (Apha *et al*., 1976).

Results and Discussion

The concentrations of metolachlor technical were determined in the test solutions. The mean measured concentrations ranged from 83 - 95% of nominal concentrations. The mean measured concentrations were used for calculating and reporting the results.

Analytical results

Nominal concentration (mg metolachlor/L)	Measured concentration (mg metolachlor/L) 0 hours	Measured concentration (mg metolachlor/L) 96 hours	% of nominal 96 hours	Mean measured concentration (mg metolachlor/L)
Control	n.d.	n.d.	-	-
Solvent Control	n.d.	n.d.	-	-
0.62	0.56	0.62	95	0.59
1.2	0.95	1.1	83	1.0
2.5	2.0	2.4	88	2.2
5.0	4.4	4.3	88	4.4
10	8.7	10	94	9.4

n.d.: Not Detected

Mortalities were observed at a mean measured concentration of 9.4 mg metolachlor/L. No mortality was observed in the control and solvent control.

The mortality data and estimated LC_{50} values are shown in the table below:

Effects of metolachlor on the survival of *Cyprinodon variegatus*

Mean measured concentration (mg/L)	% Mortality observed (Cumulative number of dead fish)			
	24 hours	48 hours	72 hours	96 hours
Control	0	0	0	0
Solvent Control	0	0	0	0
0.59	0	0	0	0
1.0	0	0	0	0
2.2	0	0	0	0
4.4	0	0	0	0
9.4	0	5	25	70
LC_{50} mg metolachlor/L	-	-	-	7.5

5% confidence limits were not reported

Validity Criteria

- Mortality in the controls was ≤ 10% (0%)
- The dissolved oxygen concentrations were maintained above 60 % (actual recorded was 6.7 to 8.3 mg/L).

Conclusions

Based on mean measured concentrations, the 96-hour LC50 for metolachlor to sheepshead minnow (*Cyprinodon variegatus*) was 7.5 mg/L. The study is reliable with restrictions as details of the study are not full reported, and the study was not performed according to the principles of GLP. The acute phase of the study does meet the validity criteria for acute toxicity testing of fish and is regarded reliable for classification purposes.

Anonymous (1983a)

Author:	Anonymous
Title:	The acute toxicity of S-Metolachlor (CGA 77102 Technical) to rainbow trout (Salmo gairdneri (Oncorhynchus mykiss))
Date:	1983
Doc ID:	Report Number 83-E-168R
Guidelines:	Committee on Methods for Toxicity Test with Aquatic Organisms, 1975, EPA-660/3-75-009
GLP:	No, but complies with sound scientific standards
Validity:	No (minor deviation)
Previous evaluation:	DAR (2004, 2018)

Executive Summary

The acute toxicity of CGA77102 to rainbow trout was determined under static conditions. Fish were exposed to a range of nominal concentrations of CGA77102, 1.3, 2.2, 3.6, 6.0 and 10.0 mg/L, alongside a dilution water control and a solvent control (dilution water plus acetone at the same level as the highest test concentration). Based on measured initial concentrations, the 96 hour LC_{50} for CGA77102 to rainbow trout was 1.23 mg a.s./L (95% confidence intervals 0-5.16 mg a.s./L). The 96 hour no observed effect concentration (NOEC) was <1.08 mg a.s./L.

Study Design and Methods

Experimental dates: 6th to 17th June 1983.

A stock solution was made up at 20 mg/mL in acetone. Treatment solutions were prepared by dilution of appropriate amounts of the stock solution with dilution water to make up to 15 L of test solution in each test vessel. One control vessel consisted of dilution water only and a solvent control vessel contained dilution water plus acetone at the same level as the highest test concentration.

At the start of the test, ten fish were randomly allocated to each of the test concentrations and the controls.

All test vessels were examined at 24, 48, 72 and 96 hours of exposure. Mortalities were recorded and symptoms of abnormal behavioural responses were made.

During the 96 hour test period, daily measurements of the test solutions were undertaken throughout for pH, temperature and dissolved oxygen concentration.

The test concentrations of active ingredient were verified by chemical analysis of CGA77102 at the start of exposure using a residue analysis method.

The initial measured concentrations were used to estimate 24-, 48-, 72- and 96-hour LC_{50} and 95% confidence intervals.

The LC_{50} was determined using the moving average and binomial probability methods. The NOEC was determined by visual inspection of the data.

Results and Discussion

The measured concentrations of active ingredient are shown in the table below in relation to nominal concentrations. Measured concentrations were used for the calculation and reporting of results.

Analytical results for CGA77102

Nominal concentration (mg /L)	Measured concentrations (mg a.s./L)
0 (Dilution water control)	0
0 (Solvent control)	0
1.3	1.08
2.2	1.93
3.6	3.07
6.0	5.16
10.0	9.46

There were no mortalities or sublethal effects in the dilution water or solvent controls. At 96 hours there was 30, 100, 80, 100 and 100% mortality in the 1.08, 1.93, 3.07, 5.16 and 9.46 mg a.s./L groups, respectively. Sublethal effects were present in many of the surviving fish exposed to CGA77102 technical.

The mortality data, LC_{50} values are shown in the table below:

Cumulative mortality of CGA77102 to rainbow trout

Initial measured concentration (mg a.s./L)	Cumulative mortalities (%) (n = 10)			
	24 hours	**48 hours**	**72 hours**	**96 hours**
Dilution water control	0	0	0	0
Solvent control	0	0	0	0
1.08	0	0	0	30
1.93	0	90	100	100
3.07	0	80	80	80
5.16	60	100	100	100
9.46	80[a]	100	100	100
LC_{50} mg a.s./L	5.53	1.64	1.44	1.23
95% confidence interval	4.34 - 7.25	1.23 - 2.02	1.08 - 5.16	0 - 5.16
NOEC	3.07	1.08	<1.08	<1.08

[a] all dead fish had dark pigmentation

Validity Criteria

The following validity criteria, based on current guidance were met:

- Mortality in the negative control and solvent control was ≤ 10 % (0 %).

DO ≥ 60% ASV (60% saturation at 12ºC = 6.5 mg/L; measured range at 0-24 hours was 7.5 to 8.6 mg/L). Values dropped below 60% ASV between 48 and 96 hours in all test vessels (4.9 mg/L). This validity criteria is not fully met.

Conclusions

Based on initial measured concentrations, the 96 hour LC_{50} for CGA77102 to **rainbow trout** was 1.23 mg a.s./L (95% confidence intervals 0-5.16 mg a.s./L). The 96 hour NOEC was < 1.08 mg a.s./L.

The dissolved oxygen concentration after 96 h was below the validity criteria of 60% (45%). Due to the missing analytical measurement at the end of the test and the low oxygen concentration the study is regarded as reliable with restrictions. The study can be considered for classification purposes.

Anonymous (1983b)

Author:	Anonymous
Title:	The acute toxicity of S-Metolachlor (CGA 77102) to Bluegill Sunfish (Lepomis macrochirus)
Date:	1983
Doc ID:	Report Number 83-E-168B
Guidelines:	Committee on Methods for Toxicity Test with Aquatic Organisms, 1975, EPA-660/3-75-009
GLP:	No, but complies with sound scientific standards
Validity:	No (minor deviation)
Previous evaluation:	DAR (2004, 2018)

Executive Summary

10 fish per concentration, *Lepomis macrochirus*, (mean body length 42.3 mm; mean body weight 0.85 g, 7 months old) were exposed to the test substance at concentrations of 1.3, 2.2, 3.6, 6 and 10 mg/L for a period of 96 hours in a static test design per concentration and water control. The concentrations after 96 hours were not mentioned. The concentrations for the LC50 and NOEC calculations were converted according to initial measured concentrations.
No mortality in both controls and the 0.66 and 1.50 ppm group. In the 2.59 ppm group mortality was 10 % after 96 hours. In the 3.29 group mortality was 60 % after 96 hours. There was 100 % mortality in the 8.51 group after 96 hours. The *Lepomis macrochirus* showed a surfacing behaviour in group 3.29 and 8.51 ppm.

Validity criteria

The following validity criterion was met:

> Mortality in the controls was ≤ 10% (observed was 0%)

The following validity criterion was not fulfilled by the study:

> The dissolved oxygen concentration maintained above 60% (actual measured was approximately 46%)

Conclusions

LC50 (96 h) = 3.16 mg a.s./L
NOEC (96 h) = 1.5 mg a.s./L

Due to the missing analytical measurement at the end of the test and the low oxygen concentration the study is regarded as reliable with restrictions. As it was shown in recent studies with comparable static test design that no major decline of test concentrations has to be expected over the test duration the study is considered acceptable to be used for classification purpose.

Anonymous (1994a)

Author:	Anonymous
Title:	Metolachlor technical (CGA 24705) - Acute toxicity to sheepshead minnow (Cyprinodon variegatus) under flow-through conditions
Date:	1994
Doc ID:	Report Number 94-7-5378
Guidelines:	FIFRA Guideline 72-3
GLP:	Yes
Validity:	Yes
Previous evaluation:	DAR (2004, 2018)

Executive Summary

2 replicates of 10 fish/concentration, *Cyprinodon variegatus*, (mean body length 23 mm; mean body weight 0.22 g) were exposed to the test substance at concentrations of 2.6, 4.3, 6.2, 12 and 20 mg/L for a period of 96 hours in a flow-through test design per concentration and water control. The concentrations in the test media ranged on day 0 from 77-108 % of the nominal. The range after 96 hours was from 81-100 %. The concentrations for the LC50 and NOEC calculations were converted according to these analyses.
There were no mortalities at the solvent control and the 3.6 ppm level. There was 5 % mortality in the control 2.8 and 6.2 ppm group. 60 % mortality was observed at the 11 ppm level and 100 % mortality at the 19 ppm level.
Several fish were observed to be lethargic, to be swimming erratically or exhibited partial loss of equilibrium.

Conclusions

LC50 (96 h) = 9.8 mg a.s./L
NOEC (96 h) = 3.6 mg a.s./L

The study is considered reliable without restrictions and acceptable to be used for classification.

Anonymous (1995a)

Author:	Anonymous
Title:	S-Metolachlor (CGA 77102) - Acute toxicity to rainbow trout (Oncohrynchus mykiss) under static conditions
Date:	1995
Doc ID:	Report Number 95-9-6117
Guidelines:	FIFRA Guideline 72-1
GLP:	Yes
Validity:	No (minor deviation)
Previous evaluation:	DAR (2004, 2018)

Executive Summary

Metolachlor (CGA 24705), technical – purity 97.3 %: 10 fish/concentration, *Oncohrynchus mykiss*, (mean body length 42 mm; mean body weight 0.65 g) were exposed to the test substance at concentrations of 3.8, 6.5, 11, 18, 30 and 50 mg/L for a period of 96 hours in a static test design per concentration and water control. The concentrations in the test media ranged on day 0 from 82-90 % of the nominal. The range after 96 hours was from 50-78 %. The concentrations for the LC50 and NOEC calculations were converted according to these analyses.
There were no mortalities after 96 hours in both control groups, the 2.5, 5.3 and 8.3 ppm group. The 15 ppm group showed 90 % mortality after 96 hours while there was 100 % mortality in groups 25 and 42 ppm, also after 96 hours.
Several fish were observed to be lethargic and some surviving fish showed partial or complete loss of equilibrium.

Validity criteria

The following validity criterion was met:

- Mortality in the controls was ≤ 10% (0%)

The following validity criterion was not fulfilled by the study:

- The dissolved oxygen concentration was not maintained above 60% (lowest observed was 35%)

Conclusions

LC_{50} (96 h) = 12 mg a.s./L
NOEC (96 h) = 2.5 mg a.s./L

The study did not meet both of the required validity criteria. However, the control survival was acceptable and the low oxygen concentrations were only observed in highest concentration where measurements were performed (15 mg/L). The study is considered as reliable with restrictions and is used for classification purposes.

Anonymous (2006)

Author:	Anonymous
Title:	S-metolachlor (CGA77102) technical: Acute toxicity to carp (*Cyprinus carpio*) under static conditions
Date:	2006
Doc ID:	Report Number T001970-06-REG
Guidelines:	OECD Guideline for testing of chemicals 203 'Fish Acute Toxicity Test'. Adopted 17 July 1992
GLP:	Yes
Validity:	Yes
Previous evaluation:	DAR (2018)

Executive Summary

The acute toxicity of S-metolachlor (CGA77102) technical to Carp (*Cyprinus carpio*) was determined. Fish were exposed to mean measured concentrations of 1.3, 2.8, 6.2, 14 and 29 mg a.s./L, and a control (dilution water). The measured concentrations at the start of the test ranged from 90 to 93 % of nominal and at the end of the test ranged from 75 to 86 % of nominal. Mean measured concentrations were used for the calculation and reporting of the results.

There was 100 % mortality observed in the highest test concentration of 29 mg/L. In the concentrations below no mortality was observed. Sub-lethal effects were observed at nominal concentrations of 14 mg/L and above. Symptoms of toxicity observed included unusual swimming, increased pigmentation and moribund fish. No mortality or symptoms of toxicity were observed in the control.

Conclusions

The 96 hour LC_{50} for S-metolachlor (CGA77102) technical to carp (*Cyprinus carpio*) is 20 mg a.s./L (95 % confidence interval 14 - 29 mg a.s./L), based on the mean measured concentrations. The study is considered reliable without restrictions and acceptable to be used for classification.

Anonymous (2004a)

Author:	Anonymous
Title:	A 96-hour static-renewal toxicity test with the Sheepshead Minnow (*Cyprinodon variegates*)
Date:	2004
Doc ID:	528-A162
Guidelines:	US EPA Ecological Effects Test Guidelines, OPPTS 850.1075: Fish Acute Toxicity Test, Freshwater and Marine (1996) US EPA, Standard Evaluation Procedure, Acute Toxicity Test for Estuarine and Marine Organisms (Estuarine Fish 96-hour Acute Toxicity Test), EPA-540/9-85-006 (1985) ASTM Standard E729-88a, Standard Guide for Conducting Acute Toxicity Test with Fishes, Macroinvertebrates and Amphibians (1994)
GLP:	Yes
Validity:	Yes
Previous evaluation:	DAR (2018)

Executive Summary

The acute toxicity of CGA77102 to sheepshead minnow, *Cyprinodon variegatus*, was determined under static renewal conditions. Fish were exposed to a range of nominal concentrations of 3.8, 7.5, 15, 30 and 60 mg CGA77102/L, alongside dilution water and solvent controls. The measured concentrations in the freshly prepared medium at the start of the test and after renewal at 48 hours ranged from 70 to 96 % of nominal. After 48 hours before renewal and at the end of the test the measured concentrations ranged from 64 to 81 % of nominal. The mean measured concentrations, calculated from the analysed concentrations, were used for the calculation and reporting of the results.
Mortalities were observed at mean measured concentrations of 23 mg CGA77102/L and above (100 % after 96 hours). Symptoms of toxicity were observed at concentrations ≥ 12 mg CGA77102/L and included discolouration, surfacing and lying on the bottom of the tank. No mortality or symptoms of toxicity were observed in the control.

Conclusions

Based on mean measured concentrations, the 96-hour LC_{50} to sheepshead minnow (*Cyprinodon variegatus*) was 17 mg CGA77102/L with 95 % confidence intervals of 12-23 mg CGA77102/L.
The 96-hour no-mortality concentration was 12 mg CGA77102/L and the NOEC was 6 mg CGA77102/L. The study is reliable without restrictions and considered acceptable to be used for classification.

11.5.2 Acute (short-term) toxicity to aquatic invertebrates

Heitmuller, T. (1980a)

Author:	Heitmuller, T.
Title:	Acute toxicity of metolachlor (CGA 24705) to grass shrimp (*Palaemonetes pugio*)
Date:	1980
Doc ID:	Report Number BP-80-3-62
Guidelines:	ASTM 1980
GLP:	No
Validity:	Yes
Previous evaluation:	DAR (2004, 2018)

Executive Summary

The acute toxicity of CGA 24705 to *Palaemonetes pugio* was determined under static conditions over 96 hours. The following nominal concentrations were tested alongside a dilution water control: 6.7, 11, 18, 30 and 50.0 mg/L. In addition, a control with the solvent (triethylene glycol) was included. The concentrations in the test media ranged from 82-90 % of the nominal at test initiation, however, concentrations in the test media at test end were not determined. The effect concentrations were based on initial concentrations. There were no mortalities after 96 hours in both controls and at 6.9 mg/L (initial). There were 20, 60, 90 and 100 % mortality observed at 11, 17, 33 and 38 mg/L (initial), respectively, after 96 hours. The LC_{50} (96 h) is 17 mg/L, the NOEC (96 h) is 6.9 mg/L based on initial concentrations.

Validity criteria:

This study broadly complies with the current validity criteria for acute toxicity testing with the grass shrimp:

- Mortality in the negative control and solvent control was ≤ 10% (observed was 0%).
- Treatments and organisms were indiscriminately assigned.
- All test vessels were identical.

A surfactant or dispersant was not used in the preparation of the stock/test solution.

Conclusions

LC_{50} (96 h) = 17 mg/L

As it was shown in recent studies with comparable static test design that no major decline of test concentrations has to be expected over the test duration the study is considered valid and acceptable to be used for classification even without chemical analysis at study end.

As solely initial concentrations were measured, the study is considered as reliable with restrictions.

Heitmuller, T. (1980b)

Author:	Heitmuller, T.
Title:	Acute toxicity of metolachlor (CGA 24705) to pink shrimp (*Penaeus duorarum*)
Date:	1980
Doc ID:	Report Number BP-80-4-64
Guidelines:	ASTM 1980
GLP:	No
Validity:	Yes
Previous evaluation:	DAR (2004, 2018)

Executive Summary

The acute toxicity of CGA 24705 to *Penaeus duorarum* was determined under static conditions over 96 hours. The following nominal concentrations were tested alongside a dilution water control: 4, 6.7, 11, 18 and 30.0 mg/L. In addition, a control with the solvent (triethylene glycol) was included. The concentrations in the test media ranged from 97 – 124 % of the nominal at test initiation, however, concentrations in the test media at test end were not determined. The effect concentrations were based on initial concentrations. There were no mortalities after 96 hours in both controls and at 4.4 mg/L (initial). There was 60, 70 and 2 x 100 % mortality observed at 8.3, 12, 20 and 29 mg/L (initial), respectively, after 96 hours.
For the parameter mortality, a dose-response curve was fitted to the data to derive ECx values. The LC_{50} (96 h) is 8.3 mg/L and the NOEC (96 h) is 4.4 mg/L based on initial concentrations.

Validity criteria

This study broadly complies with the current validity criteria for acute toxicity testing with the Penaeid Shrimp. Despite some test conditions not being reported and some deviations, the study is reliable and still valid for use in the risk assessment.

- Mortality in the negative control and solvent control was ≤ 10% (observed was 0%).
- Treatments and organisms were indiscriminately assigned.
- All test vessels were identical.
- A surfactant or dispersant was not used in the preparation of the stock/test solution.

The following deviations were noted:

- 5 shrimp per replicate (20 recommended).
- The maximum concentration of vehicle solvent used was 0.3 mL/L (should not exceed 0.1 mL/L.
- Dissolved oxygen concentration dropped below 60% (36 to 54% ASV at 96 hours).

Test temperature was 21 to 22ºC (23 ± 1ºC recommended).

Conclusions

LC_{50} (96 h) = 8.3 mg a.s./L

Due to the multiple deviations mentioned above the study is not considered as reliable and will not be used for calssification.

Hollister, T.A. and Ward, G.S. (1980a)

Author:	Hollister, T.A. and Ward, G.S.
Title:	Acute toxicity of metolachlor (Dual) to Calanoid copepods (*Acartia tonsa*)
Date:	1980
Doc ID:	Report Number BP-80-6-97
Guidelines:	US EPA-600/9-78-010; ASTM STP 634
GLP:	No
Validity:	Yes
Previous evaluation:	DAR (2004, 2018)

Executive Summary

The acute toxicity of CGA 24705 to *Acartia tonsa* was determined under static conditions over 96 hours. The following nominal concentrations were tested alongside a dilution water control: 0.6, 1.2, 2.5, 5 and 10.0 mg/L. In addition, a control with the solvent (triethylene glycol) was included. The concentrations in the test media ranged from 62 – 74 % of the nominal at test initiation, however, concentrations in the test media at test end were not determined. The effect concentrations were based on initial concentrations. There was 7, 10, 13, 30, 97 and 100 % mortality observed after 96 hours in the control, 0.4, 0.8, 1.7, 3.7 and 6.2 mg/L group, respectively. The LC50 after 96 hours is 1.5 mg/L, the NOEC after 96 hours is below 0.4 mg/L based on initial measured concentrations.

Conclusions

LC50 (96h) = 1.5 mg/L

It was shown in recent studies with comparable static test design that no major decline of test concentrations has to be expected over the test duration. The study is considered reliable with restrictions due to missing analytical verification of test concentrations at the end of the study and can be used for classification.

Hollister, T.A. and Ward, G.S. (1980b)

Author:	Hollister, T.A. and Ward, G.S.
Title:	Acute toxicity of metolachlor (Dual) to embryos-larvae of eastern oysters (*Crassostrea virginica*).
Date:	1980
Doc ID:	Report Number BP-80-6-99
Guidelines:	ASTM Draft No.7
GLP:	No
Validity:	Yes
Previous evaluation:	DAR (2004, 2018)

Executive Summary

The acute toxicity of CGA 24705 to embryos-larvae of *Crassostrea virginica* was determined under static conditions over 96 hours. The following nominal concentrations were tested alongside a dilution water control: 3, 6, 12, 25 and 50 mg/L. In addition, a control with the solvent (triethylene glycol) was included. The concentrations in the test media ranged from 72 – 133 % of the nominal at test initiation, however, concentrations in the test media at test end were not determined. The effect concentrations were based on initial concentrations. Significant reduction of embryo/larvae which developed normally to the straight-hinged veliger larvae stage after 48 hours occurred at concentrations of 26 and 36 mg/L. The 96-h EC_{50} value for embryo/larvae of eastern oyster exposed to metolachlor in static unaerated sea-water was 18 mg/L. The NOEC was 13 mg/L.

Conclusions

EC_{50} (96 h) = 18 mg/L

It was shown in recent studies with comparable static test design that no major decline of test concentrations has to be expected over the test duration. The study is considered reliable with restrictions due to missing analytical verification of test concentrations at the end of the study and can be used for classification.

Spare, W.C. (1983c)

Author:	Spare, W.C.
Title:	The acute toxicity of CGA 77102 (technical) to *Daphnia magna* Straus
Date:	1983
Doc ID:	Report Number 83-E-168D
Guidelines:	ASTM 1981; EPA-660/3-75-009
GLP:	No
Validity:	Yes
Previous evaluation:	DAR (2004, 2018)

Executive Summary

The acute toxicity of CGA 77102 to *Daphnia magna* was determined under static conditions over 48 hours. Daphnids were exposed to a range of nominal concentrations of 4, 6.6, 11, 18 and 30 mg/L alongside a dilution water control. In addition, a control with the solvent (acetone) was included. The concentrations in the test media ranged from 79 - 131% of the nominal at test initiation, however, concentrations in the test media at test end were not determined. The effect concentrations were based on initial measured concentrations. There was 5, 20, 35, 90 and 100 % mortality after 48 hours in the control, 6.44, 11.23, 23.66 and 30.44 mg/L (initial) group, respectively. The EC_{50} (48 h) is 11.24 mg/L and the NOEC (48 h) is 3.15 mg/L based on initial concentrations.

Conclusions

EC_{50} (48 h) = 11.24 mg/L

It was shown in recent studies with comparable static test design that no major decline of test concentrations has to be expected over the test duration. The study is considered valid and acceptable to be used for classification even without chemical analysis at study end.

The study is considered reliable with restrictions.

Spare, W.C. (1983d)

Author:	Spare, W.C.
Title:	The acute toxicity of S-Metolachlor (CGA 77102) (Technical) to *Mysidopsis bahia* (Bay Shrimp)
Date:	1983
Doc ID:	Report Number 83-E-168M
Guidelines:	EPA 850.1035, 72-3
GLP:	No
Validity:	Yes
Previous evaluation:	DAR (2004, 2018)

Executive Summary

The acute toxicity of CGA77102 technical to the bay shrimp *Mysidopsis bahia* was determined under static conditions. Mysid shrimps were exposed to a range of nominal concentrations of CGA77102 mg/L alongside a dilution water control and a solvent control (acetone). Based on mean measured concentrations, the 96-hour LC_{50} was 1.40 mg a.s./L (95% confidence interval 1.16-1.67 mg a.s./L). The 96 hour no observed effect concentration (NOEC) was < 0.51 mg a.s./L.

Study Design and Methods

Experimental dates: 10th to 14th August 1983

A 10 mg/mL stock solution was prepared in acetone. Test solutions were prepared by adding measured volumes of the stock solution to the dilution water and mixing thoroughly. The volume of each replicate per concentration was 200 mL. The controls consisted of dilution water only and solvent controls. Five juvenile mysids (1-5 days old) were randomly added to each test vessel.

Mortalities were recorded after 24 and 48 hours of exposure. Mysids were classed as dead when no movement of appendages was noted upon disturbance of the organism.

Dissolved oxygen and pH were determined initially and at termination and temperature was recorded daily.

The test concentrations were verified by chemical analysis of CGA77102 using a residue analysis method (gas chromatography) at the start of exposure and after 96 hours (50 mL from each of the 4 replicates combined into a single composite sample for residue analysis).

The LC_{50} was calculated for the 24 hour exposure period using the binomial probability method and for the 48, 72 and 96 hour exposure periods using the moving average method. All calculations were based on mean measured concentrations. The NOEC was determined by visual inspection of the data.

Results and Discussion

The mean measured concentrations were 0.51, 0.96, 1.67.3.13 and 4.61 mg a.s./L. Mean measured concentrations were used for the calculation and reporting of results.

Analytical results

Nominal concentrations of AI (mg/L)	Measured concentration at 0 hours (mg ai/L)	Measured concentration at 96 hours (mg ai/L)	Mean measured concentration (mg ai/L)
Dilution water control	< 0.01	< 0.01	< 0.01
Solvent control	< 0.01	< 0.01	< 0.01
0.24	0.468	0.547	0.51
0.40	1.01	0.909	0.96
0.66	1.62	1.72	1.67
1.1	3.08	3.17	3.13
1.8	4.68	4.53	4.61

Effects of CGA77102 on *Mysidopsis bahia* following exposure for 96-hours in a static test

Mean measured concentration(mg a.s./L)	Cumulative percent mortality (n=20)			
	after 24 hours	after 48 hours	after 72 hours	after 96 hours
Dilution water control	0	0	0	0
Solvent control	0	0	0	0
0.51	5	5	5	5
0.96	10	10	10	10
1.67	10	40	70	70
3.13	10	40	75	95
4.61	45	75	75	100
LC_{50} mg a.s./L	>4.61	2.82	1.81	1.40
95% Confidence limits	N/A	2.15-4.12	1.36-2.43	1.16-1.67
NOEC	<0.51	<0.51	<0.51	<0.51

N/A not applicable

Conclusions

Based on mean measured concentrations, the 96-hour LC_{50} was 1.40 mg a.s./L (95% confidence interval 1.16-1.67 mg a.s./L). The 96 hour no observed effect concentration (NOEC) was < 0.51 mg a.s./L.

This study complies with the current validity criteria for acute toxicity testing with the saltwater mysid (US EPA OCSPP 850.1035 (2016)). Despite some test conditions not being reported and minor deviations, the study is reliable without restrictions and can be used for classification.

- Mortality in the negative control and solvent control was ≤ 10% (0%).
- Treatments and organisms were indiscriminately assigned.
- All test vessels were identical.
- A surfactant or dispersant was not used in the preparation of the stock/test solution.

DO ≥ 60%; 4.3 mg/L represents 60% saturation at 25 °C in saltwater with a salinity of 25‰ (4.2 to 7.9 mg/L).

Dionne, E. (1994)

Author:	Dionne, E.
Title:	Metolachlor technical (CGA 24705) - Acute toxicity to eastern oyster (*Crassostrea virginica*) under flow-through conditions
Date:	1994
Doc ID:	Report Number 94-7-5365
Guidelines:	U.S. EPA FIFRA Guideline Number 72-3(b)
GLP:	Yes
Validity:	Yes
Previous evaluation:	DAR (2004, 2018)

Executive Summary

The acute toxicity of CGA 24705 to embryo/larvae of *Crassostrea virginica* was determined under flow-through conditions over 96 hours. The following nominal concentrations were tested alongside a dilution water control: 0.71, 1.2, 2.0, 3.3 and 5.5 mg/L. In addition, a control with the solvent (acetone) was included. The concentrations in the test media ranged from 85-94 % of the nominal at test initiation and from 78-104 % of the nominal after 96 hours. The effect concentrations were based on mean measured concentrations. Significant reduction of shell deposition of eastern oysters 96 hours occurred at 1.1, 1.7, 2.9 and 4.5 mg/L.

The 96-h EC_{50} value for embryo/larvae of eastern oyster exposed to metolachlor was 1.8 mg/L and the NOEC was 0.71 mg/L based on reduction of shell deposition.

Conclusions

EC_{50} (96 h) = 1.8 mg/L

The study is considered reliable without restrictions and acceptable to be used for classification.

Machado, M. W. (1994b)

Author:	Machado, M. W.
Title:	Metolachlor technical (CGA 24705) - Acute toxicity to mysid shrimp (*Mysidopsis bahia*) under flow-through conditions
Date:	1994
Doc ID:	Report Number 94-7-5402
Guidelines:	U.S. EPA FIFRA Guideline Number 72-3(c)
GLP:	Yes
Validity:	Yes
Previous evaluation:	DAR (2004, 2018)

Executive Summary

The acute toxicity of CGA 24705 to *Mysidopsis bahia* was determined under flow-through conditions over 96 hours. The following nominal concentrations were tested alongside a dilution water control: 0.5,1, 2, 4 and 8 mg/L. In addition, a solvent control was included. The concentrations in the test media were measured on day 0 and 4 in all aquaria. The mean range from both analyses was 89 – 120 %. The effect concentrations were based on mean measured concentrations. There were no mortalities after 96 hours in both control groups and the 0.61, 1.0 and 2.3 mg/L group (mean measured). In the 4.0 and 7.1 mg/L group (mean measured), respectively, 35 and 80 % mortality occurred after 96 hours. The LC50 (96 h) is 4.9 mg/L the NOEC (96 h) is 2.3 mg/L based on mean measured concentrations.

Conclusions

LC_{50} (96 h) = 4.9 mg/L

The study is considered reliable without restrictions and acceptable to be used for classification.

Heitmuller, T. (1980c)

Author:	Heitmuller, T.
Title:	Acute toxicity of metolachlor (Dual) to fiddler crabs (*Uca pugilator*)
Date:	1980
Doc ID:	Report Number BP-80-3-61
Guidelines:	ASTM
GLP:	No
Validity:	Yes
Previous evaluation:	DAR (2018)

Executive Summary

The acute toxicity of CGA 24705 to *Uca pugilator* was determined under static conditions over 96 hours. The study was conducted as a limit test with a limit test concentration of 50 mg/L nominal alongside a dilution water control. In addition, a control with the solvent (triethylene glycol) was included. The measured concentration in the test medium was 94 % of the nominal at test initiation; however, the concentration at test end was not determined. The effect concentrations were based on initial concentrations. There were no mortalities after 96 hours at 47 mg/L (initial). The LC_{50} (96h) is above 47 mg/L based on initial measured concentrations.

Validity criteria

This study does not comply with current recognised methods for acute toxicity testing with marine invertebrates. Solely initial measured concentrations are available. The presence of sand in the test system does have unknown effects on the concentration of metolachlor during the course of the study.

Conclusions

In an acute toxicity test in which fiddler crabs (*Uca pugilator*) were exposed to metolachlor for 96 h, the NOEC was determined to be 47 ppm based on the initial measured concentration.

The study is considered as not reliable and is not further considered for classification.

Collins, M.K. (1995b)

Author:	Collins, M.K.
Title:	Acute toxicity to daphnids (*Daphnia magna*) under static conditions
Date:	1995
Doc ID:	Report Number 95-9-6082
Guidelines:	U.S. EPA FIFRA Guideline Number 72-2(a)
GLP:	Yes
Validity:	Yes
Previous evaluation:	DAR (2004, 2018)

Executive Summary

The acute toxicity of CGA 77102 to *Daphnia magna* was determined under static conditions over 48 hours. Daphnids were exposed to a range of nominal concentrations of 3.8, 6.5, 11, 18, 30 and 50 mg/L alongside a dilution water control. In addition, a solvent control was included. The concentrations in the test media were measured at the beginning and the end of the exposure time. The mean range from both analyses was 72-83 %. The effect concentrations were based on mean measured concentrations. There was 10 % mortality in the control and 5 % mortality in the solvent control after 48 hours. There were no mortalities in the 2.9, 4.8, 7.9 and 15 mg/L group (mean measured). The EC_{50} (48 h) is 26 mg/L and the NOEC (48 h) is 15 mg/L based on mean measured concentrations.

It should be noted that the solvent concentration exceeded the allowed limit of 0.1 mL/L five times (0.5 mL/L acetone).

Conclusions

LC_{50} (48 h) = 26 mg/L

The study is considered reliable with restrictions due to exceedance of the allowed solvent concentration. The study can be used for classification.

Palmer S.J., Kendall T.Z. and Krueger H.O. (2004b)

Author:	Palmer, S.J. et al.
Title:	A 96-hour shell deposition test with the Eastern Oyster (Crassostrea virginica).
Date:	2004
Doc ID:	Report Number 528A-127
Guidelines:	U.S. EPA 1996. Series 850 – Ecological Effects Test Guidelines (draft), OPPTS Number 850.1025: Oyster Acute Toxicity Test (Shell Deposition).
GLP:	Yes
Validity:	Yes
Previous evaluation:	DAR (2018)

Executive Summary

The acute toxicity of CGA77102 to the eastern oyster (*Crassostrea virginica*) was determined under flow-through conditions. Oysters were exposed to a range of nominal concentrations of 0.31, 0.63, 1.3, 2.5 and 5.0 mg/L alongside a filtered seawater control and a solvent control. Mean measured concentrations calculated from the average of all samples ranged from 102 to 112 % of nominal concentrations and were used for the reporting of the results. Oysters in the controls appeared normal throughout the test. Based on mean measured concentrations the 96-hour EC_{50} value is 4.0 mg CGA77102 /L with 95 % confidence intervals of 3.5 - 4.1 CGA77102/L.

Conclusions

EC_{50} (96 h) = 4 mg/L

The study is considered reliable without restrictions and acceptable to be used for classification.

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

Hoberg, J. R. (1995a)

Author:	Hoberg, J.R.
Title:	Metolachlor technical (CGA 24705) - 5-day toxicity to the freshwater diatom, *Navicula pelliculosa*, using acetone as a carrier solvent
Date:	1995
Doc ID:	Report Number 94-12-5627
Guidelines:	FIFRA Guideline number 122-2 and 123-2
GLP:	Yes
Validity:	No
Previous evaluation:	DAR (2004, 2018)

Executive Summary

Unicellular diatom inoculum (*Navicula pelliculosa*, strain # 667, class Bacillariophyceae), three days old since previous transfer from Springborn stock culture, was exposed to metolachlor technical (purity 97.3 %), in a static shaken test system for a period of 5 days. Six concentrations ranging from nominal 3.6 - 1500 µg a.s./L were employed in the test with three replicates per treatment level and the controls. At intervals of 24-hours cell counts were made on one sample from each replicate culture. Mean measured concentrations were used for reporting the results. Endpoints are presented in the table below.

Table 51: Endpoints relating to yield and average specific growth rate

Parameter	**After 96 h**	
	Growth rate	**Yield**
EC50 [µg a.s./L] 95 % CL	4982 (3313-8909)	240 (157-384)
EC20 [µg a.s./L] 95 % CL	393 (303-493)	33 (14-58)
EC10 [µg a.s./L] 95 % CL	104 (64-148)	12 (3-25)

CL: Confidence Limits

After 4-day exposure, all validity criteria were met. Therefore, only endpoints derived after 4-day exposure are acceptable.

Validity criteria

The following validity criteria were not met:

- Control biomass did not increase by a factor of at least 16 within 72 hours (factor of 4 observed).
- The mean coefficient of variation for section-by-section specific growth rate exceeded 35%.

The coefficient of variation of average specific growth rates during the whole test period in replicate control cultures must not exceed 7%.

Conclusions

E_rC_{50} (96 h) = 4.982 mg/L

E_rC_{10} (96 h) = 0.104 mg/L

All endpoints are based on mean measured concentrations. The 4-day E_bC_{50} value based on cell density was calculated to be 170 µg a.s./L. The 4-day E_rC_{50} and E_rC_{10} based on average specific growth rate were calculated to be 4982 and 234 µg a.s./L, respectively.

Due to several validity criteria being not met, the study is regarded as not reliable and should not be considered for classification.

Hoberg, J. R. (1994)

Author:	Hoberg, J.R.
Title:	Metolachlor Technical - 5-Day Toxicity to the Marine Diatom, *Skeletonema costatum*
Date:	1994
Doc ID:	Report Number 94-7-5382
Guidelines:	FIFRA Guideline number 122-2 and 123-2
GLP:	Yes
Validity:	Yes, after 3-day exposure
Previous evaluation:	DAR (2018)

Executive Summary

The toxicity of CGA24705 to the marine diatom *Skeletonema costatum* was determined. Algae were exposed to nominal concentrations of 0.0015, 0.0049, 0.016, 0.054, 0.18, 0.60 and 2.0 mg CGA24705/L (mean measured: 0.0017, 0.0048, 0.014, 0.043, 0.15, 0.56 and 1.7 mg CGA24705/L) alongside a culture medium control. Based on mean measured concentrations, the 72-hour EC_{50} based on growth rate was 0.423 mg CGA 247105/L.

Study Design and Methods

Experimental dates: 8th to 13th June 1994.

A primary stock solution with a nominal concentration of 20 mg CGA24705/mL was prepared by dissolving 0.0105 g of CGA24705 in 500 mL of sterile AES medium, stirring overnight with a magnetic stir bar and stirplate. Appropriate volumes of the stock solution were diluted to give the test concentration series. The control consisted of culture medium only.

An aliquot of test solution was placed into each test vessel and the test was started by inoculation of 10,000 algal cells per mL of test medium. Test solutions were constantly shaken and were held in a temperature-controlled chamber under continuous illumination.

Small volumes of all test concentrations and controls were taken from all test flasks after 24, 48, 72, 96 and 120 hours of exposure. The algal cell densities in these samples were determined using a haemocytometer and a compound microscope. Observations of the health of the algal cells were made at each 24-hour interval.

The pH was measured at the start and at the end of the test. The water temperature was measured continuously in a flask incubated under the same conditions as the test flasks.

The test concentrations were verified by chemical analysis of metolachlor at 0 and 120 hours, using GC with nitrogen phosphorus detection.

The algal cell densities were measured at 24, 48, 72, 96 and 120 hours and cell densities calculated. The 120-hour EC_{50} and the 95% confidence intervals were determined by linear regression. For determination of the NOEC values, William's test was used to identify significant differences in the treatments compared to the control data. The data were first checked for normality using Shapiro-Wilks' Test and homogeneity of variance using Bartlett's Test.

Results and Discussion

At the start of the test, the analytically determined concentrations of CGA24705 were in the range 87.5 to 109% of the nominal values and at the end of the test were in the range 70.2 to 129% (see table below). The limit of quantification in this study was 0.388 µg CGA24705/L. Mean measured concentrations were used for the calculation and reporting of results.

Cell density

The cell densities were calculated for each replicate at 24, 48, 72, 96 and 120 hours and the means are shown below, alongside the estimated EC$_{50}$ values.

Mean values at each concentration of CGA24705 for the cell density at 24, 48, 72, 96 and 120 hours for *Skeletonema costatum*

Mean measured concentrations (mg CGA24705/L)	Mean cell density [x 10^4] (s.d.)					Inhibition at 120 hours (%)
	24 h	48 h	72 h	96 h	120 h	
Control	3 (1)	9 (2)	24 (7)	31 (1)	99 (7)	n.a.
0.0017	3 (1)	7 (2)	15 (7)	29 (2)	101 (6)	-2.0
0.0048	3 (1)	7 (3)	19 (11)	23 (12)	79 (3)*	20
0.014	3 (<1)	6 (1)	13 (2)	27 (3)	73 (7)*	26
0.043	1 (<1)	5 (2)	11 (5)	30 (4)	44(4)*	55
0.15	2 (1)	4 (2)	13 (9)	31 (6)	43 (6)*	56
0.56	1 (<1)	2 (1)	7(1)	9 (1)	17 (7)*	83
1.7	1 (<1)	1 (<1)	2 (1)	4 (1)	8 (1)*	92
NOEC	72 h: < 0.0017				0.0017	-

* significantly reduced as compared to the control, based on Williams test
n.a. = not applicable

No cell abnormalities were observed in the controls. At test termination cell fragments and bloated cells were observed in treatment levels ≥ 0.15, mg CGA24705/L.

Effect concentrations relating to yield and average specific growth rate were calculated using the drc package in R (Weibull-model). Calculations are solely conducted for 72 h, as the test is only regarded valid after this period of time. Endpoints based on mean measured concentrations are presented in **Error! Reference source not found.**:

Endpoints relating to yield and average specific growth rate

Parameter	**After 72 h**	
	Growth rate	**Yield**
EC50 [µg a.s./L] 95% CL	423 (145-701)	39 (-17 - 94)
EC20 [µg a.s./L] 95% CL	36 (-18-89)	n.d.
EC10 [µg a.s./L] 95% CL	7 (-9 - 23)	n.d.

n.d.: not determined due to inappropriate data
CL: Confidence Limits

Validity criteria

All validity criteria were met after 72 h

Conclusions

According to validity criteria, endpoints derived after 3-day exposure are acceptable. All endpoints are based on nominal concentrations. The 3-day EyC50 value was calculated to be 39 µg a.s./L. The 3-day ErC50 value was calculated to be 423 µg a.s./L. The study results after 72 h are reliable without restrictions.

Hoberg, J. R. (1995b)

Author:	Hoberg, J.R.
Title:	CGA 77102 - 5-Day toxicity to the marine diatom *Skeletonema costatum*
Date:	1995
Doc ID:	Report Number 95-8-6062
Guidelines:	OECD Guideline 201; US EPA FIFRA Guideline No. 122-2 and 123-2
GLP:	Yes
Validity:	No (minor deviation)
Previous evaluation:	DAR (2004, 2018)

Executive Summary

The marine alga *Skeletonema costatum* (strain CCMP 1332, class Bacillariophyceae) was exposed to S-metolachlor (CGA 77102) in a static test system over 5 days. The following test concentrations plus a control and a solvent control were employed in the test with three replicates each: 2.4, 8.1, 27, 90, 300 and 1000 µg CGA77102 /L (nominal). The cell density in each flask was determined at the beginning of the test and after each 24-hour interval using a haemocytometer.

Effect concentrations relating to yield and average specific growth rate were calculated using ToxRat Professional version 2.10.05. Endpoints based on mean measured concentrations are presented in the Table below:

Table 52: Endpoints relating to yield and average specific growth rate

Parameter	**After 72 h**	
	Growth rate	**Yield**
EC50 [µg a.s./L] 95 % CL	340 (200-710)	53 (35-64)
EC20 [µg a.s./L] 95 % CL	40 (11-76)	34 (15-45)
EC10 [µg a.s./L] 95 % CL	13 (2-32)	27 (10-38)

CL: Confidence Limits

There was a minor deviation from the validity criteria of the current OECD guideline 201 after 72 h exposure (37.7 % section-by-section growth rate instead of 35 %).

Conclusions

E_rC_{50} (72 h) = 0.340 mg/L

E_rC_{10} (72 h) = 0.013 mg/L

The EyC50 (72 h) was calculated to be 53 µg a.s./L. The E_rC_{50} and E_rC_{10} after 72 h were calculated to be 340 and 13 µg a.s./L, respectively. Considering the overall quality of the study, this deviation is considered not to influence the study results. The study is considered reliable with restriction and acceptable to be used for classification.

Memmert, U. (2006)

Author:	Memmert, U.
Title:	S-Metolachlor (CGA77102): Toxicity to *Pseudokirchneriella subcapitata* (formerly *Selenastrum capricornutum*) in a 96-hour algal growth inhibition test, suppl. with testing for algicidal/algistatic effects

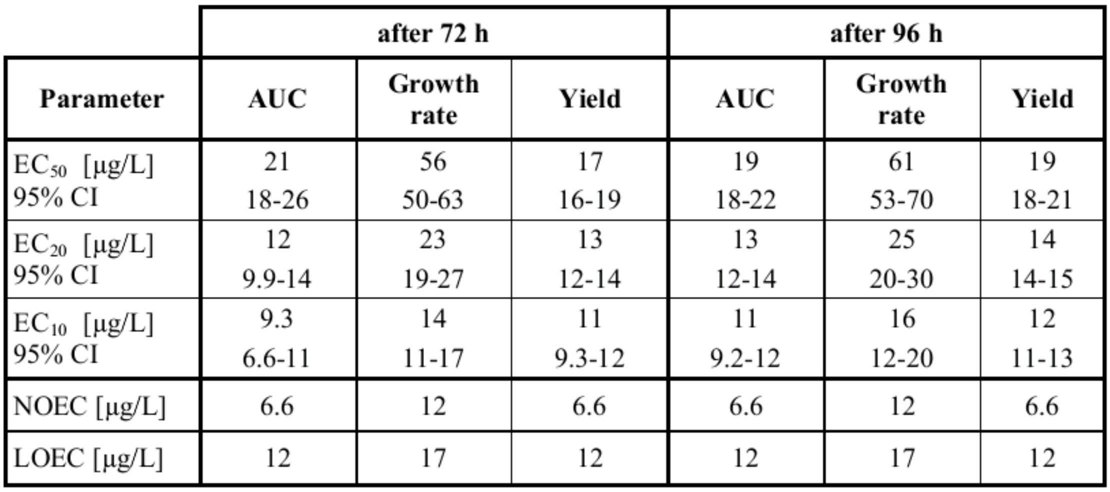
Date: 2006
Doc ID: Report Number 859258
Guidelines: OECD Guideline 201; US EPA OPPTS 850.5400
GLP: Yes
Validity: Yes, only after 3-day exposure
Previous evaluation: DAR (2018)

Executive Summary

The toxicity and of CGA77102 (S-metolachlor) to *Pseudokirchneriella subcapitata* was determined under static conditions. Also, recovery of affected cultures was observed in order to determine whether the effects observed were algicidal or algistatic. Algae were exposed to a range of nominal concentrations of 2, 4, 8, 16, 32, 64 and 128 µg a.s./L alongside a dilution water control. As the mean coefficient of variation for section-by-section specific growth rates in the control was 46.3 % after 96 h exposure, the validity criteria were only fulfilled after 72 h and effect concentrations were based on 72 h exposure. At the start of the test the concentrations of S-metolachlor were in the range 85 to 89 % of the nominal values. Over the 96 h test period the concentration of the test item had decreased to 30-68 % of the nominal concentrations. Therefore, mean measured concentrations were used for the calculation and reporting of results. All statistical determinations were calculated using ToxRat Professional, ToxRat Solutions GmbH, Version 2.10.05.

There were no abnormalities observed in any of the treatment levels or controls after 96 hours of exposure. The effect concentrations derived for yield and average specific growth rate based on mean measured concentrations are presented in the table below.

Table 53: Endpoints relating to biomass and average specific growth rate



	after 72 h			after 96 h		
Parameter	**AUC**	**Growth rate**	**Yield**	**AUC**	**Growth rate**	**Yield**
EC_{50} [µg/L] 95% CI	21 18-26	56 50-63	17 16-19	19 18-22	61 53-70	19 18-21
EC_{20} [µg/L] 95% CI	12 9.9-14	23 19-27	13 12-14	13 12-14	25 20-30	14 14-15
EC_{10} [µg/L] 95% CI	9.3 6.6-11	14 11-17	11 9.3-12	11 9.2-12	16 12-20	12 11-13
NOEC [µg/L]	6.6	12	6.6	6.6	12	6.6
LOEC [µg/L]	12	17	12	12	17	12

95% CI: 95% confidence interval

Based on mean measured concentrations of CGA77102 (6.6, 12, 17, 53 and 126 µg a.s./L), the E_rC_{50} and E_yC_{50} (72 h) were determined to be 56 and 17 µg a.s./L, respectively. The NOEC (72 h) was 12 µg a.s./L and 6.6 µg a.s./L for growth rate and biomass, respectively.

Conclusions

E_rC_{50} (72 h) = 0.056 mg/L
NOEC (growth, 72 h) = 0.012 mg/L

The study is only valid after 3 day-exposure. Based on mean measured concentrations the E_rC_{50} and E_yC_{50} (72 h) of CGA77102 for *Pseudokirchneriella subcapitata* were determined to be 56 and 17 µg a.s./L, respectively. The NOEC (72 h) was 12 µg a.s./L and 6.6 µg a.s./L for growth rate and biomass, respectively. The study results after 3 days are reliable without restriction and considered acceptable for classification.

Hoberg, J. R. (1995c)

Author: Hoberg, J.R.
Title: Metolachlor technical (CGA 24705) - 5-day toxicity to the freshwater green alga, *Anabaena flos-aquae*
Date: 1995
Doc ID:Report Number 94-7-5383
Guidelines: U.S. EPA FIFRA Guideline No. 122-2 and 123-2
GLP: Yes

Validity: No

Executive Summary

The toxicity of CGA24705 to the freshwater blue-green alga *Anabaena flos-aquae* was determined. Algae were exposed to nominal concentrations of 0.024, 0.081, 0.27, 0.90, 3.0 and 10.0 mg CGA24705/L (mean measured concentrations of 0.019, 0.063, 0.19, 0.72, 2.1 and 6.8 mg CGA24705/L), alongside a culture medium control.

Based on growth rate and mean measured concentrations, the 120-hour EC_{50} was 14.8 mg CGA24705/L. Due to several validity criteria being not met, the study should not be considered for classification purposes.

Study Design and Methods

Experimental dates: 9th to 14th June 1994

A stock solution with a nominal concentration of 100 mg CGA24705/L was prepared by dissolving 0.0512 g of the test item in 500 mL of test medium. The stock solution was stirred overnight with a magnetic stir bar and stirplate. Appropriate volumes of the stock solution were diluted with test medium to give the test concentration series. The control consisted of culture medium only.

An aliquot of test solution was placed into each test vessel and the test was started by inoculation of 10,000 algal cells per mL of test medium. Test solutions were constantly shaken at 100 rpm and were held in an environmental chamber under continuous illumination.

Small volumes of all test concentrations and controls were taken from each replicate solution after 24, 48, 72, 96 and 120 hours of exposure. The algal cell densities in these samples were determined using a haemocytometer and a compound microscope. Observations of the health of the algal cells were examined microscopically in these samples.

The pH was measured at the start and at the end of the test. The water temperature was measured continuously in a flask incubated under the same conditions as the test flasks.

The test concentrations were verified by chemical analysis of CGA24705 at 0 and 120 hours, using gas chromatography with nitrogen phosphorus detection. For sampling at the end of the test, the test medium of the treatment replicates was pooled. A sample of the stock solution was also analysed.

The algal cell densities were measured at 24, 48, 72, 96 and 120 hours Effect concentrations relating to yield and average specific growth rate were calculated using ToxRat Professional version 2.10. Probit analysis with linear maximum likelihood regression was used to determine the concentration response function. Chi^2 was used as a goodness of fit measure. For determination of the NOEC value, a William's test was used to identify significant differences in the mean cell density of test item treatments compared to the pooled control.

Results and Discussion

The mean measured concentrations were in the range 68 to 91 % of the nominal values (see table below). The limit of quantitation in this study was based on that obtained for CGA24705 in Hoagland's medium, in a separate method validation study conducted prior to the initiation of this test, which was 0.407 µg CGA24705/L. Mean measured concentrations were used for the calculation and reporting of results.

At 120 hours, cell fragments were observed among algae exposed to concentrations > 0.72 mg CGA24705/L and bloated cells were observed at 6.8 mg CGA24705/L only.

Cell density at 24, 48, 72, 96 and 120 hours was determined for each replicate culture and the means are shown below, alongside the estimated EC values.

Mean values at each concentration of CGA24705 for cell density at 24, 48, 72, 96 and 120 hours for *Anabaena flos-aquae*

Mean measured concentrations (mg CGA24705/L)	Mean cell density (SD) (x 10^4 cells/mL)					Percentage inhibition (%)
	0 – 24 hrs	0 – 48 hrs	0 – 72 hrs	0 – 96 hrs	0 – 120 hrs	0 – 120 hrs
Control	1 (1)	3 (2)	4 (2)	32 (3)	87 (5)	n.a.
0.019	2 (1)	3 (2)	3 (1)	36 (7)	86 (5)	1.2
0.063	1 (< 1)	2 (< 1)	2 (1)	34 (4)	83 (6)	5.3
0.19	2 (< 1)	2 (< 1)	2 (2)	25 (2)	73 (8)*	16
0.72	1 (1)	1 (1)^{b}	3 (1)^{b}	19 (2)^{b}	49 (5)^{b*}	44
2.1	1 (1)^{b}	1 (< 1)^{b}	2 (1)^{b}	9 (2)^{b}	37 (3)^{b*}	56
6.8	< 1 (< 1)^{b}	< 1 (< 1)^{b}	< 1 (< 1)^{b}	7 (2)*	16(3)^{ab*}	82
NOEC	**0.063**					

Mean and standard deviation were calculated from original raw data, not from the rounded values presented in this table

* Statistically significant difference compared to the control (according to Williams' Test, p ≤ 0.05)

[a] Bloated cells observed

[b] Cell fragments were observed

n.a. Not applicable

The effect concentrations (based on mean measured concentrations) are presented in the table below:

Endpoints relating to yield and average specific growth rate

Parameter	After 120 h	
	Growth rate	**Yield**
EC50 [µg a.s./L] 95% CL	14807 (11714-19984)	1148 (943-1404)
EC20 [µg a.s./L] 95% CL	1816 (1531-772)	222 (155-294)
EC10 [µg a.s./L] 95% CL	606 (445-772)	94 (57-137)

n.d.: Not Determined

CL: Confidence Limits

Validity criteria

Compliance with OECD 201 Algal test guideline criteria

Exposure	Cell density (multiplication factor ≥ 16)	Coefficient of variation	
		Section-by-section growth rate (≤ 35 %)	**Average specific growth rate** (≤ 10 %)
72 h	3.67	115.3	46.5
120 h	87	97.9	1.3

Conclusions

The 5-day E_bC_{50} value based on cell density was calculated to be 1100 µg a.s./L. The 5-day ErC50 based on average specific growth rate was calculated to be 14807 µg a.s./L. The NOEC for cell density was found to be 63 µg a.s./L. However, the study is not valid according to the validity criteria of the current OECD guideline 201.

The following validity criteria were not met:

- Control biomass did not increase by a factor of at least 16 within 72 hours (factor of 4 observed).
- The mean coefficient of variation for section-by-section specific growth rate exceeded 35%.
- The coefficient of variation of average specific growth rates during the whole test period in replicate control cultures must not exceed 7%.

Due to several validity criteria being not met, the study should not be considered for classification. The study is not reliable

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Horberg J. R. (1995d)

Author: Hoberg, J.R.
Title: Metolachlor Technical - 5-Day Toxicity to the Freshwater Green Alga, *Selenastrum capricornutum*, using Acetone as a Carrier
Date: 1995
Doc ID:Report Number 94-12-5621
Guidelines: FIFRA Guideline number 122-2 and 123-2
GLP: Yes
Validity: No

Executive Summary

The toxicity of CGA24705 to the green alga *Pseudokirchneriella subcapitata* was determined. Algae were exposed to nominal concentrations of 0.00080, 0.0016, 0.0031, 0.0063, 0.013 and 0.025 mg CGA24705/L (mean measured: 0.00070, 0.0014, 0.0025, 0.0059, 0.014 and 0.023 mg CGA24705/L) alongside culture medium and solvent controls.

The 3-day EyC50 was calculated to be 6.9 µg a.s./L. The 4-day ErC50 value was calculated to be 27.8 µg a.s./L. The NOEC for cell density was found to be 0.8 µg a.s./L. However, the study is not valid according to the validity criteria of the current OECD guideline 201.

Conclusions

The 3-day EyC50 was calculated to be 6.9 µg a.s./L. The 4-day ErC50 value was calculated to be 27.8 µg a.s./L. The NOEC for cell density was found to be 0.8 µg a.s./L. However, the study is not valid according to the validity criteria of the current OECD guideline 201.

This study does not comply with the current validity criteria toxicity testing with algae in that the mean coefficient of variation for section-by-section specific growth rate of 65% at 72 h exceeded clearly the guideline maximum (35%). The study is not reliable and should not be used for classification.

Hoberg J.R. (1995e)

Author: Hoberg, J.R.

Title: CGA 77102 - 5-day toxicity to the freshwater green alga *Selenastrum capricornutum*
Date: 1995
Doc ID:Report Number 95-8-6031
Guidelines: FIFRA Guideline number 122-2 and 123-2
GLP: Yes

Validity: No

Executive Summary

The toxicity of CGA77102 to the green alga *Pseudokirchneriella subcapitata* was determined. Algae were exposed to nominal concentrations of 0.00081, 0.0016, 0.0031, 0.0063, 0.013, 0.025 and 0.050 mg CGA77102/L (mean measured concentrations of 0.00091, 0.0015, 0.0030, 0.0055, 0.011, 0.022 and 0.047 mg CGA77102/L), alongside a culture medium control and a solvent control.

The 3-day EyC50 value based on cell density was calculated to be 5.6 µg a.s./L. The 3-day ErC50 value based on average specific growth rate was calculated to be 24 µg a.s./L. The NOEC for cell density was found to be 3.0 µg a.s./L. However, the study is not valid according to the validity criteria of the current OECD guideline 201.

Conclusions

The 3-day EyC50 value based on cell density was calculated to be 5.6 µg a.s./L. The 3-day ErC50 value based on average specific growth rate was calculated to be 24 µg a.s./L. The NOEC for cell density was found to be 3.0 µg a.s./L. However, the study is not valid according to the validity criteria of the current OECD guideline 201.

This study does not comply with the current validity criteria toxicity testing with algae in that the mean coefficient of variation for section-by-section specific growth rate of 52% at 72 h exceeded the guideline maximum (35%). Therefore, the study is not reliable and cannot be used for classification.

Rufli, H. (1985)

Author: Rufli, H.
Title: Acute toxicity of CGA 24705 technical to algae
Date: 1985
Doc ID:Report Number 84 01 99
Guidelines: OECD Guideline 201
GLP: No
Validity: No

Executive Summary

The toxicity of CGA24705 to the alga *Desmodesmus subspicatus* was determined. Algae were exposed to nominal concentrations of 0.1, 0.3, 0.9, 2.7 and 8.1 mg CGA24705/L alongside a water control. Based on nominal concentrations, the estimated 72-hour E_rC_{50} of CGA24705 to *Desmodesmus subspicatus subspicatus* was 0.247 mg a.s./L. The study is not valid and should not be used for classification.

Validity criteria

The section-by-section growth rate after 72 h was 56.8% instead of the required < 35%. All other validity criteria were met.

Conclusions

Based on nominal concentrations, the estimated 72-hour E_rC_{50} of CGA24705 to *Desmodesmus subspicatus subspicatus* was 0.247 mg a.s./L.

This study does not comply with the current validity criteria toxicity testing with algae in that the mean coefficient of variation for section-by-section specific growth rate of 57% at 72 h exceeded the guideline maximum (35%). Therefore, the study should not be used for classification.

Hollister, T.A and Ward, G.S. (1980)

Author: Hollister, T.A and Ward, G.S.
Title: Effects of metolachlor (Dual) on two freshwater and five marine algae
Date: 1980
Doc ID:Report Number BP-80-4-73
Guidelines: US EPA 1974/1978
GLP: No
Validity: Validity according to OECD 201 could not be checked

Executive Summary

The toxicity of metolachlor to seven algal species (two freshwater and five marine) was tested. Algae were exposed to the test concentrations and a dilution water control over 5 days and a minimum algistatic concentration (MAC-5) was estimated. After a 9 day recovery period, the growth of cultures previously exposed to 250 and 500 ppb was significantly less than growth of the control. Due to missing information validity criteria could not be examined.

Results

The endpoints are listed below:

Algal species	**E_yC_{50} (mg/L)**		**E_rC_{50} (mg/L)**	
	72 hr	**120 hr**	**72 hr**	**120 hr**
Freshwater				
Microcystis aeruginosa	-	8.35	13.3	11.4
*Selenastrum capricornutum**	-	0.065	0.071	-
Marine				
Chlorella pyrenoidosa	4.72	-	6.09	4.47
Dunaliella tertiolecta	-	3.62	-	6.11
Skeletonema costatum	0.500	0.364	0.970	0.714
Isochrysis galbana	0.220	0.241	0.436	0.591
Porphyridium cruentum	-	3.15	-	4.29

Conclusion

Cell numbers were not reported for each day for any of the species, hence it is not possible to calculate the coefficient of variation for section-by-section specific growth rate. Furthermore, only stock solutions were analysed and there is no verification of test concentrations. The study is not regarded valid and will not be used for classification. Due to missing information the reliability of the study is not assignable.

Desjardins, D., Kendall, T.Z., Krueger, H.O. (2003)

Author:	Desjardins, D.; Kendall, T.Z.; Krueger, H.O.
Title:	CGA77102: A 96-hour toxicity test with the freshwater diatom (*Navicula pelliculosa*)
Date:	2003
Doc ID:	Report Number 528A-129
Guidelines:	OECD Guideline 201; US EPA OPPTS 850.5400
GLP:	Yes
Validity:	Yes, only after 3-day exposure
Previous evaluation:	DAR (2018)

Executive Summary

The toxicity of CGA77102 to the freshwater diatom *Navicula pelliculosa* was determined. Algae were exposed to nominal concentrations of 2.5, 5.0, 10, 20 and 40 mg a.s./L (2.3, 4.0, 9.7, 19 and 33 mg a.s./L, mean measured) alongside a culture medium control and a solvent control. Mean measured (2.3, 4.0, 9.7, 19 and 33 mg a.s./L) concentrations were used for the calculation and reporting of results. As the validity criteria set in OECD 201 were only met after 72 h exposure, as the mean coefficient of variation for section-by-section specific growth rates in the control was 63 % after 96 h exposure (required < 35 %), effect concentrations are related to 72 h exposure. Based on mean measured concentrations, the 72-hour E_rC_{50} for CGA77102 for *Navicula pelliculosa* was 31 mg a.s./L and the E_yC_{50} was 16 mg a.s./L. The 72- hour NOEC for growth rate and yield was 9.7 mg a.s./L.

Conclusions

E_rC_{50} (72 h) = 31 mg/L

NOEC (growth, 72 h) = 9.7 mg/L

The study is only valid after 72 h of exposure. Based on mean measured concentrations, the 72-hour E_rC_{50} for *Navicula pelliculosa* was 31 mg a.s./L and the E_yC_{50} was 16 mg a.s./L. The 72-hour NOEC for growth rate and yield was 9.7 mg a.s./L. The study results after 3 d are reliable without restriction and considered acceptable for classification.

Desjardins, D., Kendall, T.Z., Krueger, H.O. (2004)

Author:	Desjardins, D., Kendall, T.Z., Krueger, H.O.
Title:	CGA77102: A 96-hour toxicity test with the freshwater alga (*Anabaena flos-aquae*)
Date:	2004
Doc ID:	Report Number 528A-128A
Guidelines:	OECD Guideline 201; US EPA OPPTS 850.5400
GLP:	Yes
Validity:	No

Executive Summary

The toxicity of CGA77102 to the freshwater alga *Anabaena flos-aquae* was determined. Algae were exposed to nominal concentrations of 2.5, 5.0, 10, 20 and 40 mg a.s./L (2.4, 4.8, 9.6, 19 and 30 mg a.s./L, mean measured) alongside culture medium and solvent controls. Based on mean measured concentrations, the 96-hour ErC50 for CGA77102 for *Anabaena flos-aquae* was > 30 mg a.s./L and the EbC50 was 24 mg a.s./L. The 96- hour NOEC for growth rate and biomass was 9.6 mg a.s./L. However, the study is not valid according to the validity criteria of OECD guideline 201 and should not be used for classification.

Results and Discussion

At the start of the test, the analytically determined concentrations of CGA77102 were in the range 76.6 to 98.9% of the nominal values and at the end of the test were in the range 74.9 to 95.7%. Mean measured concentrations were used for the calculation and reporting of results.

The validity criteria set in OECD guideline 201 were not met, as the mean coefficient of variation for section-by-section specific growth rates in the control was 43 % after 96 h exposure and 53 % after 72 h (required < 35 %). Therefore, the study is not valid.

There were no abnormalities observed in any of the treatment levels or controls after 96 hours of exposure.

Conclusions

Based on mean measured concentrations, the 96-hour ErC50 of CGA77102 for *Anabaena flos-aquae* was > 30 mg a.s./L and the EbC50 was 24 mg a.s./L. The 96- hour NOEC for growth rate and biomass was 9.6 mg a.s./L. Due to not meeting the validity criteria the study is not reliable and should not be used for classification.

Teixeira, D. (2006a)

Author:	Teixeira, D.
Title:	The Toxicity of S-Metolachlor to *Elodea canadensis* during a 7-day Exposure Followed by a 14-day Recovery Period.
Date:	2006
Doc ID:	Report Number 1781.6638
Guidelines:	US EPA Ecological Effects Test Guidelines, OPPTS 850.4450; EPA 712-C-96-157
GLP:	Yes
Validity:	Not applicable
Previous evaluation:	DAR (2018)

Executive Summary

The toxicity of CGA 77102 to the aquatic plant *Elodea canadensis* was determined in a 7-day semi-static test, with medium renewal on day 3. The *Elodea* were exposed to nominal concentrations of 0.0081, 0.027, 0.090, 0.30 and 1.0 mg CGA 77102/L (0.0089, 0.029, 0.11, 0.36 and 1.1 mg CGA 77102/L, mean measured), alongside a solvent control. Recovery, whereby *Elodea* was transferred to culture medium without the test material present after 7 days of exposure, was assessed at 21 days from test commencement, for all treatment levels. For the purpose of classification, just the 7-day exposure phase is of interest.
For shoot length, the 7-day E_yC_{50} for CGA 77102 to *E. canadensis* was 0.049 mg CGA 77102/L and 0.1 mg CGA 77102/L for plant biomass (wet weight), based on mean measured concentrations. The 7-day E_rC_{50} for CGA 77102 was 0.062 mg CGA 77102/L for shoot length and 0.12 mg CGA 77102/L for plant biomass (wet weight), based on mean measured concentrations. The NOEC based on growth rate and the E_rC_{10} after 7 days were 0.029 and 0.0049, respectively.

Study Design and Methods

Experimental dates: 7th to 28th June 2005

A stock solution with a nominal concentration of 30 mg CGA 77102/L was prepared by dissolving1.5164 g of the test item in 50 mL acetone. Secondary stock solutions were prepared from dilutions of the primary stock solution and refrigerated (4 °C) when not in use. Individual exposure solutions were prepared at test initiation (day 0) and on day 3. Following application of the stock solution to each aquarium, the test solutions were mixed gently for one minute to avoid disturbing or uprooting the plants. The control consisted of culture medium containing acetone (1 mL/30L dilution water).
Twenty-four 37.5 L glass aquaria were placed on a bench in a greenhouse and filled with 30 L (23 cm depth) of water. Twelve pots each containing one plant were placed in each replicate aquarium. The sides of the aquaria were covered with black plastic to limit light penetration to the water surface.
Following 7 days of exposure, all plants were carefully rinsed to remove epiphytic algal growth. Maximum length and wet weight were then determined for each plant before being transferred to clean dilution water for the recovery phase. Following 14 days of recovery in clean water, maximum length and wet weight of plants were determined for each remaining plant. An inspection of their appearance (e,g., necrosis, chlorosis, damage) and mortality was made throughout the testing period.
Temperature was continually monitored in the solvent control and replicate 2 with a minimum-maximum thermometer. Whenever natural light intensity in the greenhouse fell below 8600 lux, sodium vapour lights automatically turned on until natural light intensity increased or until the end of the light period. The pH, measured in composite samples from all replicates of each test solution concentration and the solvent control was measured at the start and end of each medium renewal period. The test concentrations were verified by chemical analysis of CGA 77102 on freshly prepared and aged test media of all test concentrations and from the solvent control on days 0, 3 and 7 of exposure and in the 1.0 mg/L nominal concentration on Day 1 of recovery using HPLC/UV.
Data for shoot length and wet weight biomass were used to calculate growth rates for the solvent control and each exposure concentration. The 7-days EC10, EC20 and EC50 values for the inhibition of yield and average growth rate and their 95% confidence intervals for the exposure period were calculated by Probit Analysis using linear maximum likelihood regression. All statistical determinations were calculated using ToxRat Professional (version 2.10.05). For the NOEC and the LOEC, a Dunnett's Test (one sided, $\alpha = 0.05$) was used to determine values significantly different from the solvent control.

Results and Discussion

Mean measured concentrations ranged from 110-120% of nominal concentrations. Day 1 recovery samples from the 1.0 mg CGA 77102/L (nominal) treatment level ranged from 0.053-0.067 mg CGA 77102/L. Mean measured concentrations were used for the calculation and reporting of results. All test media were clear throughout the test period. There are no validity criteria set in OPPTS 850.4450. However, the validity criteria of the current OECD guideline 239 for doubling of the mean total shoot length and mean total shoot fresh weight in control plants during the exposure phase was met. No mortalities were observed during this study. Several plants exposed to ≥ 0.30 mg CGA 77102/L were observed to be chlorotic during the exposure period. Several plants exposed to ≥ 0.30 mg CGA 77102/L were also observed to have insect damage
Endpoints based on mean measured concentrations are presented in the following tables:

Table 54: Endpoints relating to shoot length for exposure and recovery period

	Shoot length			
Parameter	**Exposure to CGA 77102**		**Recovery**	
	yield	**growth rate**	**yield**	**growth rate**
EC_{50} [mg a.s./L] 95% CL	0.049 0.029-0.078	0.062 0.029-0.093	n.a.	0.066 0.026-0.074
EC_{20} [mg a.s./L] 95% CL	0.0083 (0.0026-0.016)	0.013 (0.0048-0.023)	n.a.	n.a.
EC_{10} [mg a.s./L] 95% CL	0.0033 (0.0007-0.0076)	0.0049 (0.0013-0.011)	n.a.	n.a.
NOEC	0.0089	0.029	n.a.	0.029

CL: Confidence Limits
n.a. not applicable

Table 55: Endpoints relating to wet weight for exposure and recovery period

	Wet weight			
Parameter	**Exposure to CGA 77102**		**Recovery**	
	yield	**growth rate**	**yield**	**growth rate**
EC_{50} [mg a.s./L] 95% CL	0.1 0.067-0.15	0.12 0.027 – 0.19	n.a.	0.092 0.021 – 0.53
EC_{20} [mg a.s./L] 95% CL	0.12 (0.0048-0.022)	0.029 (0.0069-0.060)	n.a.	n.a.
EC_{10} [mg a.s./L] 95% CL	0.0041 (0.0011-0.0089)	0.0081 (0.0009-0.023)	n.a.	n.a.
NOEC	0.0089	0.0089	n.a.	0.0089

Conclusions

E_rC_{50} (7 d) = 0.062 mg/L

E_rC_{10} (7 d) = 0.0049 mg/L

The duration chosen for the exposure phase (7 days) was considerably shorter than recommended in OECD guideline 239 (14 days), whose test design was particularly developed to investigate effects on higher aquatic plants. Therefore, additional effects may have been overlooked due to the short exposure phase. The study reliable with restrictions and considered acceptable for the purpose of classification.

Teixeira, D. (2006b)

Author:	Teixeira, D.
Title:	The toxicity of S-Metolachlor to *Myriophyllum heterophyllum* during a 7-day exposure followed by a 14-day recovery period
Date:	2006
Doc ID:	Report Number 1781.6639
Guidelines:	US EPA Ecological Effects Test Guidelines, OPPTS 850.4450; EPA 712-C-96-157
GLP:	Yes
Validity:	No
Previous evaluation:	DAR (2018)

Executive Summary

The toxicity of CGA 77102 to the aquatic plant *Myriophyllum heterophyllum* was determined in a 7-day semi-static test, with medium renewal on day 3. The *Myriophyllum* plants were exposed to nominal concentrations of 0.0081, 0.027, 0.090, 0.30 and 1.0 mg CGA 77102/L (0.010, 0.029, 0.080, 0.30 and 1.0 mg CGA 77102/L, mean measured), alongside a solvent control. Recovery, whereby *Myriophyllum* was transferred to culture medium without the test material present after 7 days of exposure, was assessed at 21 days from test commencement, for all treatment levels. For the purpose of classification, just the 7-day exposure phase is of interest.

For shoot length, the 7-day E_rC_{50} for CGA 77102 to *M. heterophyllum* was >1.0 mg CGA 77102/L and 0.065 mg CGA 77102/L for plant biomass (wet weight), based on mean measured concentrations. Due to the low biomass growth in the control (no doubling of biomass parameters during the exposure phase) and several other deficiencies of the study (such as no clear dose/response and the short exposure phase) the reliability of the results has to be questioned.

Conclusions

E_rC_{50} (7 d) = 0.065 mg/L

NOEC (7 d) = 0.01 mg/L

Due to the low biomass growth in the control and several other deficiencies of the study (such as no clear dose/response and the short exposure phase), the study is regarded as supplementary information for the purpose of classification.

Hoberg, J. R. (1995f)

Author:	Hoberg, J.R.
Title:	Metolachlor technical - Toxicity to duckweed *Lemna gibba*
Date:	1995
Doc ID:	Report Number 94-8-5404
Guidelines:	FIFRA Guideline number 122-2 and 123-2
GLP:	Yes
Validity:	No (minor deviation)
Previous evaluation:	DAR (2018)

Executive Summary

The freshwater aquatic plant *Lemna gibba* was exposed to metolachlor technical (CGA 24705) in a static test system over 14 days. The test design consisted of seven concentrations of the test substance with nominal concentrations of 0.0016, 0.0031, 0.0063, 0.013, 0.025, 0.050 and 0.10 mg alongside a control treatment. Mean measured concentrations (0.0005; 0.001; 0.0016; 0.0022; 0.0036; 0.0071; 0.0187) were used for the calculation and reporting of results as measured concentrations at the end of the test were in the range of only 5.7 to 20.5 % of initially measured concentrations. The frond production was determined at the beginning of the test and after on Day 3, 6, 9, 12 and 14. The frond biomass (dry weight) was determined at the end of the test. The 14-day EC_{10}, EC_{20} and EC_{50} values for the inhibition of the frond numbers (growth rate and yield) and the end dry weights and their 95 % confidence limits were calculated by Probit Analysis using linear maximum likelihood regression. According to the current validity criteria set in OECD GL 221 the study is not valid after the test period of 14 d as the doubling time for front number is 2.86 (required < 2.5) over the test period of 14 d. However, the deviation from the validity criteria is only minor and additionally, the study fulfils the validity criteria for doubling time after 7 d.

Based on mean measured concentrations the 14-day E_yC_{50} (fronds) value was determined to be 0.0148 mg a.s./L and the EC50 (dry weight) was 0.0132 mg a.s./L. The 14-day ErC50 value (fronds) was calculated to be 0.0367 mg a.s./L. The 14-day NOEC (fronds yield and growth rate) was found to be 0.0022 mg a.s./L and the 14-day NOEC (dry weight) was 0.0019 mg a.s./L.

Conclusions

E_rC_{50} (14 d) = 0.0367 mg/L

NOEC (growth, 14 d) = 0.0022 mg/L

The study is reliable with restrictions and should be considered for classification.

Hoberg, J. R. (1995g)

Author: Hoberg, J.R.
Title: Toxicity to duckweed *Lemna gibba*
Date: 1995
Doc ID: Report Number 95-8-6068
Guidelines: FIFRA Guideline number 122-2 and 123-2
GLP: Yes
Validity: No
Previous evaluation: DAR (2004, 2018)

Executive Summary

The freshwater aquatic plant *Lemna gibba* was exposed to S-metolachlor technical (CGA 77102) in a semi-static test system over 14 days with solution renewal on day 6. The test design consisted of seven concentrations of the test substance (nominally 0.0016, 0.0031, 0.063, 0.013, 0.025, 0.050 and 0.10 mg CGA77102 /L) and a control as well as a solvent control. The frond production was determined at the beginning of the test and after on Day 3, 6, 9, 12 and 14. The frond biomass (dry weight) was determined at the end of the test. The 14-day EC10, EC20 and EC50 values for the inhibition of the frond numbers (growth rate and yield) and the end dry weights and their 95 % confidence limits were calculated by Probit Analysis using linear maximum likelihood regression. Endpoints are based on mean measured concentrations. All statistical calculations were performed using ToxRat Professional (version 2.10.05).

Table 56: Endpoints relating to fronds (yield/ growth rate) and dry weight after 14 d

Parameter	fronds growth rate	fronds yield	dry weight
EC50 [mg a.s./L] **95 % CL**	0.039 0.034-0.045	0.016 0.014-0.018	0.021 0.010 – 0.044
EC20 [mg a.s./L] **95 % CL**	0.014 0.011-0.017	0.0099 0.0074-0.012	0.01 0.00055 – 0.017
EC10 [mg a.s./L] **95 % CL**	0.0081 0.0058-0.010	0.0077 0.0052-0.0096	0.0068 0.00009 – 0.013
NOEC	0.0076	0.0076	0.0076

CL: Confidence Limits

The 14-day EC_{50} values for inhibition of the growth rate and yield based on frond numbers were calculated to be 0.039 and 0.016 mg a.s./L, respectively. The 14-day EC_{50} for inhibition of frond biomass (dry weight) was calculated to be 0.021 mg a.s./L. The 14-day NOEC was 0.0076 mg a.s./L for all parameters.

The validity criterium of OECD 221 is clearly not met. The doubling time is shown below

Average specific growth rates and doubling times fronds

Day	Average specific growth rate fronds (1/d) Required > 0.275	Doubling time d Required < 2.5 d
3	0.23	3
6	0.12	3.51
9	0.22	3.18
12	0.22	3.15
14	0.21	3.29

Conclusions

E_rC_{50} (14 d) = 0.039 mg/L
NOEC (growth, 14 d) = 0.0076 mg/L

The study is not reliable and should not be considered acceptable for the purpose of classification.

Eckenstein, H. (2014)

Author:	Eckenstein H.
Title:	S-metolachlor - Toxicity to the Aquatic Higher Plant *Lemna gibba* in a 7-Day Growth inhibition Test Supplemented with Testing for Recovery of Growth.
Date:	2014
Doc ID:	Report Number D67101
Guidelines:	OECD 221; US EPA OPPTS 850.4450;
GLP:	Yes
Validity:	Yes
Previous evaluation:	DAR (2018)

Executive Summary

The toxicity of S-metolachlor to the aquatic plant *Lemna gibba* was determined in a 7-day semi-static test followed by testing for recovery of growth. The *Lemna* were exposed to concentrations of 2.2, 10, 22, 100, 220, 340, 730 and 1000 µg ai/L for 7 days alongside a dilution water control. In the freshly prepared and aged test solutions the test item was found to be in the range 82 and 109 % of the nominal values. Endpoints are based on mean measured concentrations. For frond number, the 7-day EC_{50} for yield (E_yC_{50}) and growth rate (E_rC_{50}) for S-metolachlor to *Lemna gibba* were 37 and 133 µg ai/L respectively. For dry weight, the 7-day EC_{50} for yield (E_yC_{50}) and growth rate (E_rC_{50}) were 75 and > 916 µg ai/L respectively. The NOEC based on dry weight after 7 days for growth rate is 0.0021 mg/L.

Table 57: Effect of S-metolachlor on growth rate and yield (frond number) of Lemna gibba

Mean Measured concentration (µg/L)	**Mean No. fronds/replicate (day 7)**	**Based on Frond Number (0-7 days)**			
		Growth Rate	**Inhibition of Growth Rate (%)**	**Yield**	**Inhibition of Yield (%)**
Control	158.0	0.368	.0.	146.0	0.0
2.1	163.0	0.373	-1.2	151.0	-3.4
9.8	122.0	0.331*	10.0	110.0*	24.7
22	97.7	0.299*	18.8	85.7*	41.3
96	44.3	0.187*	49.3	32.3*	77.9
204	33.7	0.147*	60.0	21.7*	85.2

Mean Measured concentration (µg/L)	**Mean No. fronds/replicate (day 7)**	**Based on Frond Number (0-7 days)**			
		Growth Rate	**Inhibition of Growth Rate (%)**	**Yield**	**Inhibition of Yield (%)**
322	31.0	0.135*	63.2	19.0*	87.0
683	29.3	0.127*	65.4	17.3*	88.1
916	27.3	0.118*	68.1	15.3*	89.5
EC_{50} µg/L		133		37	
95 % confidence limits		154 – 113		44 – 31	

*: mean value significantly lower than in the control (Dunnett's t-test, one sided smaller, α = 0.05)

Conclusions

E_rC_{50} (7 d) = 0.133 mg/L

NOEC (growth, 7 d) = 0.0021 mg/L

Not all colonies per replicate were included for final dry weight determination at test concentration levels up to 22 µg ai/L as 12 fronds were removed from these test vessels for use in the following recovery phase before dry weight determination. It remains unclear whether also in the control fronds were removed for the recovery phase. Due to these deviations, the results for dry weight may not be reliable and should be regarded with caution.
The study results based on dry weight are regarded as reliable without restrictions and the relevant endpoints are considered acceptable for classification.

Kümmrich (2019)

Author: Kümmich F.
Title: Toxicity to the Duckweed *Lemna gibba* in a 7-day Semi-Static Test under Laboratory Conditions
Date: 2019
Doc ID:Report Number S18-00204
Guidelines: OECD 221
GLP: Yes
Validity: Yes

Executive Summary

The toxicity of CGA77102 to the aquatic plant *Lemna gibba* was determined in a 7-day semi-static test. *Lemna* were exposed to nominal concentrations of 1.00, 3.20, 10.3, 32.8, 105 and 336 µg CGA77102/L alongside a dilution water control. Based on mean measured concentrations, the 7-day EC_{50} values for yield (EyC_{50}) were 29.6 and 36.6 µg CGA77102/L based on frond number and dry weight, respectively. The 7-day EC_{50} values for growth rate (ErC_{50}) were 149 and 250 µg CGA77102/L based on frond number and dry weight, respectively.

Study Design and Methods

Experimental dates: 21st May to 29th October 2018

At the start of the test, a 33600 µg CGA77102/L stock solution was prepared by placing 33.6 mg CGA77102 in a volumetric flask and bringing it to a volume of 1000 mL with test medium. The solution was homogenised by intense shaking and afterwards the solution was clear and transparent. Test concentrations were prepared by serial dilution of appropriate solutions with test medium. The control consisted of test medium only.

150 mL of the test solutions were transferred into 250 mL glass flasks and inoculated with *Lemna* plants. Cultures were maintained under the conditions indicated above.

Assessments of frond number were made on days 0, 2, 4 and 7. Fronds were harvested for measurement of dry weight after 7 days, and the initial dry weight was determined using six representative batches of plants with in total 12 fronds from the culture used in the test. Test plants were moved to fresh test solutions on days 2 and 4.

Temperature was measured continuously in a separate vessel and recorded on days 0, 2, 4 and 7. pH was measured on days 0 (fresh solutions), 2 (aged and fresh solutions), 4 (aged and fresh solutions) and 7 (aged solutions) and light intensity was measured at test start.

The test concentrations were verified by chemical analysis of CGA77102 at days 0, 2, 4 and 7, using high performance liquid chromatography (HPLC) with MS-MS detection.

Data for frond number and dry weight were used to calculate growth rates and yield for the control and each exposure concentration. A test for normality was performed by calculating the Shapiro-Wilk's statistic, a test for homogeneity of the data was performed according to Levene. The NOEC and LOEC were determined by using a multiple comparison method (Dunnett's-t-test, left sided). The $EC_{10, 20, 50}$-values were determined by probit analysis following logistic distribution (yield and growth rate of frond numbers and yield of dry weight) and normal distribution (growth rate of dry weight), which resulted in the best fit of the data.

Results and Discussion

At the start of the test at each media renewal, the analytically determined concentrations of CGA77102 were in the range 83 to 197% of the nominal values and at the end of each media renewal were in the range 82 to 196% (see table below). The limit of quantification in this study was 0.1 µg CGA77102/L. Since not all measured concentrations in the test solutions were between 80 – 120% of nominal, mean measured concentrations of the test item were used for the calculation and reporting of results.

Summary of biological results for toxicity of CGA77102 to Lemna gibba

Parameter	**Frond number (µg CGA77102/L)**		**Dry weight (µg CGA77102/L)**	
	Growth rate	**Yield**	**Growth rate**	**Yield**
EC_{10} **95% CI**	11.6 1.89 – 26.1	4.80 0.965 – 10.0	9.87 5.81 – 14.7	2.85 0.635 – 6.30
EC_{20} **95% CI**	29.8 9.41 – 58.1	9.39 2.96 – 17.4	29.9 20.8 – 41.1	7.32 2.56 – 13.7
EC_{50} **95% CI**	149 76.6 – 433	29.6 15.7 – 57.2	250 170 – 415	36.6 20.7 – 68.5
NOEC	3.84	3.84	3.84	3.84
LOEC	11.7	11.7	11.7	11.7

Validity criteria

The test was considered valid:

- The doubling time of frond number in the control was 35.6 hours (must be <2.5 days)

Conclusions

For frond number, the 7-day EC_{50} for yield (EyC_{50}) and growth rate (ErC_{50}) for CGA77102 to *Lemna gibba* were 29.6 and 149 µg CGA77102/L, respectively, based on mean measured concentrations.

For dry weight, the 7-day EC_{50} for yield (EyC_{50}) and growth rate (ErC_{50}) for were 36.6 and 250 µg CGA77102/L, respectively, based on mean measured concentrations.

The 7-day NOEC was determined to be 3.84 µg CGA77102/L and the 7-day LOEC was determined to be 11.7 µg CGA77102/L.
The study is reliable without restrictions and shoud be considered for classification.

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

No information available. All the information on acute toxicity is taken from the RAR and list of endpoints for S-metolachlor, January 2018.

11.6 Long-term aquatic hazard

Please note that solely studies for S-metolachlor (CGA-77102) are considered for classification. Studies for metolachlor (CGA 24705) are listed for completeness.

Based on the aquatic toxicity tests with S-metolachlor and its general degradability degradation products are not assumed to cause the observed toxicity. Additionally, degradation products of S-metolachlor are clearly less toxic compared to the parent (please refer to the RAR of S-metolachlor). Degradation products of S-metolachlor do not need to be considered for classification.

Table 58: Summary of relevant information on chronic aquatic toxicity

Method	Species	Test material	Results[1]	Remarks	Reference
FIFRA Guideline 72-4	*Pimephales promelas*	CGA 77102 (S-metolachlor)	NOEC (35 d) = 0.03 mg/L (mean measured)	Key study Reliability 1	Anonymous (1999)
ASTM, Draft No. 3	*Cyprinodon variegatus*	CGA 24705	NOEC (26 d) = 2.2 mg/L (mean measured)	Reliability 3	Anonymous (1980)
OPPTS 850.1400	*Pimephales promelas*	CGA 24705 (metolachlor)	NOEC (35 d) = 1.3 mg/L (mean measured)	Reliability 2	Anonymous (2006)
FIFRA Guideline Reference No. 72-4	*Cyprinodon variegatus*	CGA 77102 (S-metolachlor)	NOEC (34 d) = 1.3 mg/L (mean measured)	Reliability 1	Anonymous (2000)
EPA guidelines 72-5	Pimephales promelas	CGA 24705 (metolachlor)	NOEC (35 d) = 0.78 mg/L EC10 (64 d) = 0.934 mg/L (mean measured)	Reliability 1	Anonymous (1993)
OECD 204	*Oncorhynchus mykiss*	CGA 77102 (S-metolachlor)	NOEC (28 d) = 0.89 mg/L (mean measured)	Supplemental information	Anonymous (1997)
OECD 204	*Oncorhynchus mykiss*	CGA 77102 (S-metolachlor)	NOEC (28 d) = 1.9 mg/L (nominal)	Supplemental information	Anonymous (2001)
OECD 204	*Oncorhynchus mykiss*	CGA 24705 (metolachlor)	NOEC (21 d) = 0.25 mg/L (nominal)	Supplemental information	Anonymous (1990)
OECD 202	*Daphnia magna*	CGA 24705 (metolachlor)	NOEC (21 d) = 0.6 mg/L EC_{10} (21 d) = 0.56 mg/L (nominal)	Minor deviation from validity Reliability 2	Rufli, H. (1989)
EPA 850.1300, 72-4(b)	*Daphnia magna*	CGA 24705 (metolachlor)	NOEC (21 d) = 5.9 mg/L EC_{10} (21 d) = 6 mg/L (mean measured)	Reliability 1	Putt, A.E. (1995)
OECD 202	*Daphnia magna*	CGA 24705 (metolachlor)	NOEC (21 d) = 2.5 mg/L (nominal)	Reliability 2	Müllerschön H. (1990)

OECD 211	*Daphnia magna*	CGA 77102 (S-metolachlor)	NOEC (21 d) = 5.2 mg/L EC_{10} (21 d) = 1.29 mg/L (mean measured)	Reliability 1	Palmer, S.J.; Kendell, T.Z; Krueger, H.O. (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, W. (1999)
BBA Guideline Proposal 1995	*Chironomus riparius*	CGA 77102 (S-metolachlor)	NOEC (28 d) = 2.38 mg/L EC10 (28 d) = 5.4 mg/L (mean measured)	Reliability 1	Grade, R. (1998)
FIFRA Guideline number 122-2 and 123-2	*Navicula pelliculosa*	CGA 24705 (metolachlor)	E_rC_{50} (96 h) = 4.982 mg/L E_rC_{10} (96 h) = 0.104 mg/L (mean measured)	Reliability 3	Hoberg, J.R. (1995a)
FIFRA Guideline number 122-2 and 123-2	*Skeletonema costatum*	CGA 24705 (metolachlor)	E_rC_{50} (72 h) = 0.423 mg/L E_rC_{10} (72 h) = 0.007 mg/L (nominal)	Reliability 1	Hoberg, J. R. (1994)
OECD 201	*Skeletonema costatum*	CGA 77102 (S-metolachlor)	ErC50 (72 h) = 0.340 mg/L E_rC_{10} (72 h) = 0.013 mg/L (mean measured)	Minor deviation from validity criteria Reliability 2	Hoberg, J. R. (1995b)
U.S. EPA FIFRA Guideline No. 122-2 and 123-2	*Anabaena flos-aquae*	CGA 24705 (metolachlor)	ErC50 (120 h) = 1.1 mg/L ErC10 = 0.606 mg/L	Several validity criteria not met Reliability 3	Hoberg J.R. (1995c)
FIFRA Guideline number 122-2 and 123-2	*Selenastrum capricornutum*	Metolachlor	ErC50 (96 h) = 0.0278 mg/L NOEC = 0.8 mg/L	Severe violation of validity criteria Reliability 3	Hoberg J.R. (1995d)
FIFRA Guideline number 122-2 and 123-2	*Selenastrum capricornutum*	CGA 77102 (S-metolachlor)	ErC50 (72 h) = 0.024 mg/L ErC10 (72 h) = 0.0036 mg/L	Severe violation of validity criteria Reliability 3	Hoberg J.R. (1995e)
OECD 201	*Desmodesmus subspicatus*	CGA 24705 (metolachlor)	ErC50 (72 h) = 0.247 mg/L (nominal)	Severe violation of validity criteria Reliability 3	Rufli, H. (1985)
US EPA 1974/1978	*Microcystis aeruginosa* *Selenastrum capricornutum* *Chlorella pyrenoidosa*	Metolachlor	ErC50 (72 h): 13.3 mg/L 0.071 mg/L 6.09 mg/L - 0.97 mg/L 0.436 mg/L -	Reliability 4	Hollister, T.A and Ward, G.S. (1980)

	Dunaliella tertiolecta *Skeletonema costatum* *Isochrysis galbana* *Porphyridium cruentum*		All endpoints baded on nominal concentrations		
OECD 201	*Pseudokirchneriella subcapitata*	CGA 77102 (S-metolachlor)	E_rC_{50} (72 h) = 0.056 mg/L NOEC (growth, 72 h) = 0.012 mg/L (mean measured)	Key study Reliability 1	Memmert, U. (2006)
OECD 201	*Navicula pelliculosa*	CGA 77102 (S-Metolachlor)	E_rC_{50} (72 h) = 31 mg/L NOEC (growth, 72 h) = 9.7 mg/L (mean measured)	Reliability 1	Desjardins, D.; Kendall, T.Z.; Krueger, H.O. (2003)
OECD 201	*Anabaena flos-aquae*	CGA 77102 (S-Metolachlor)	ErC50 (72h) = > 30 mg/L EC10 (72 h) = 13 mg/L	Severe violation of validity criteria Reliability 3	Desjardins, D.; Kendall, T.Z.; Krueger, H.O. (2004)
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 (mean measured	Reliability 2	Teixeira, D. (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 (mean measured)	Supplemental information	Teixeira, D. (2006b)
FIFRA Guideline number 122-2 and 123-2	*Lemna gibba*	CGA 24705 (metolachlor)	E_rC_{50} (14 d) = 0.0367 mg/L NOEC (growth, 14 d) = 0.0022 mg/L (mean measured)	Minor deviation from validity criteria Reliability 2	Hoberg, J. R. (1995f)
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)	Severe violation of validity criteria Reliability 3	Hoberg, J. R. (1995g)
OECD 221	*Lemna gibba*	CGA 77102 (S-metolachlor)	E_rC_{50} (7 d) = 0.133 mg/L NOEC (growth, 7 d) = 0.0021 mg/L (mean measured)	Key study Reliability 1	Eckenstein, H. (2014)
OECD 221	*Lemna gibba*	CGA 77102 (S-metolachlor)	ErC50 (7 d) = 0.149 mg/L NOEC = 0.00384 mg/L (mean measured)	Reliability 1	Kümmrich F. (2019)

11.6.1 Chronic toxicity to fish

Anonymous (1999)

Author:	Anonymous
Title:	S-metolachlor (CGA77102) - Early Life-Stage Toxicity Test with Fathead Minnow (Pimephales promelas), Report Number 1781.6576, Springborn Laboratories Inc., 790 Main St., Wareham, Massachusetts, 02571-1075, USA. (Syngenta File No. CGA77102/0516)
Date:	1999
Doc ID:	Report Number 1781.6576
Guidelines:	FIFRA Guideline 72-4
GLP:	Yes
Validity:	Yes
Previous evaluation:	DAR (2018)

Executive Summary

The toxicity of CGA77102 to early-life stages of fathead minnow (*Pimephales promelas*) was determined in a flow-through test system. Fish were exposed to a range of nominal concentrations of 31, 63, 130, 250, 500 and 1000 µg a.s./L, and a dilution water control. The mean measured concentrations ranged from 84 to 96 % of their nominal concentrations (mean measured 30, 56, 110, 220, 450 and 870 µg a.s./L). Endpoints are based on mean measured concentrations.

Based on the results of this study, statistical effects on larval growth (wet weight and dry weight) were evident in the 5 highest treatment levels. Therefore, the NOEC for S-metolachlor (CGA77102) and fathead minnow (*P. promelas*) was determined to be 30 µg a.s./L. Hatchability, post-hatch survival and fish length in the treated groups were significantly different to the control, however, as there was no meaningful dose response for any of these parameters, EC_{10} and EC_{20} values could not be calculated.

Results for survival at hatch, larval survival and growth (total length, wet weight and dry weight are presented in the table below.

Table 59: Effects of CGA77102 on the growth of Pimephales promelas

Mean measured concentration (µg a.s./L)	Hatching success (%) [a]	Fry survival day 5 to test end (%) [b]	Total length (mm) (SD)	Wet weight (mg) (SD)	Dry weight (mg) (SD)
Control	85	93	33.4 (1.9)	399 (77)	101 (20)
30	90	95	33.1 (1.9)	396 (77)	99.1 (20)
56	88	99	32.9 (1.7)	375 (72)[c]	94.4 (18)[c]
110	89	100	32.9 (1.6)	373 (69)[c]	93.6 (18)[c]
220	87	95	32.7 (1.6) [c]	355 (64)[c]	90.7 (17)[c]
450	85	96	32.2 (1.8)[c]	343 (67)[c]	86.4 (17)[c]
870	85	98	31.6 (2.1)[c]	334 (71)[c]	83.4 (18)[c]

a The number of live larvae on the day they are transferred from the egg cups to the test vessels (day 5), expressed as a percentage of the number of eggs added at the start of the test (day 0).

b The number of surviving larvae at the end of the test (day 35), expressed as a percentage of the number of live larvae on day 5.

c Significantly reduced when compared to the control (Williams test)

There were significant differences between the wet weights and dry weights of control and treated groups, suggesting the effects were treatment related. The calculated EC_{10} and EC_{20} values for larval weights and larval lengths are shown below:

Parameter	EC_{10} (95 % CL) µg/L	EC_{20} (95 % CL) µg/L
Wet Weight	264 (139 – 404)	1435 (840 – 4621)
Dry Weight	220 (145 – 298)	1284 (877 – 2384)

CL: Confidence Limits

Conclusions

NOEC 0.03 (35 d) mg/L

The 35-day No-Observed Effect Concentration (NOEC) for S-metolachlor (CGA77102) and fathead minnow (*P. promelas*) was determined to be 30 µg a.s./L.

The study is reliable without restrictions and considered acceptable for classification.

Anonymous (1980)

Author: Anonymous
Title: Effects of metolachlor (CGA 24705, Dual) on survival, growth and development of sheepshead minnows (*Cyprinodon variegatus*)
Date: 1980
Doc ID:Report Number BP-80-5-80
Guidelines: Standard Practice for Conducting Toxicity Tests with the Early Life Stages of Fishes (ASTM, Draft No. 3)

GLP: No
Validity: No

Executive Summary

The toxicity of metolachlor to early-life stages of sheepshead minnow (*Cyprinodon variegatus*) was determined. Fish were exposed to the following range of nominal concentrations of 0.62, 1.2, 2.5, 5 and 10 mg metolachlor/L (mean measured concentrations 0.55, 1.0, 2.2, 4.1, and 8.6 mg metolachlor/L), a solvent control and a dilution seawater control.

Based on the significantly reduced survival and length of juveniles at concentrations ≥ 4.1 mg metolachlor/L, the MATC was estimated to be > 2.2 and < 4.1 mg metolachlor/L.

Study Design and Methods

Experimental dates: 19th March to 21st April 1980

A flow-through test system was employed. 4 hours after visual confirmation of fertilization, embryos were randomly allocated to incubation cups. Each treatment received four groups of 25 embryos. Embryo mortality and time to hatch were recorded. After hatch, juveniles were transferred to growth chambers. Observations of survival, time to hatch, and any behavioural or physical changes of juveniles were made daily. At the end of the test, lengths and wet weights of the surviving juveniles were measured.

Embryos and fish were exposed to measured concentrations of 0.55, 1.0, 2.2, 4.1, and 8.6 mg metolachlor/L, a solvent control and a dilution water control.

A stock solution consisting of metolachlor in triethylene glycol, with a nominal concentration 80.825 mg metolachlor/L, was delivered to the mixing chamber where it was diluted and made up to a set volume with seawater before being delivered to the test vessels to give the test concentrations. The blank control consisted of seawater only and the solvent control consisted of triethylene glycol and dilution seawater.

Salinity, temperature, pH and dissolved oxygen concentrations were measured in all treatment replicates at the beginning of the test, and then daily in one duplicate set of test containers.

The MATC was estimated from the data obtained as follows:

Quantal responses

Hatching success: ratio between the number of embryos which hatched and the number of embryos per replicate (n=25), or the number of embryos per treatment (n=4)

Survival: ratio between the number of juveniles that died throughout the test and the number that hatched, examined on test day 22

Non-quantal responses

Length: mean length of surviving juveniles per replicate was measured on test day 26.

Wet weight: mean wet weight of juveniles per replicate was measured on test day 26.

Statistical analysis

Data for hatching success, juvenile survival and growth were subjected to analysis of variance ($p = 0.05$). The Williams's test was used to identify significant differences between each treatment and solvent control.

Results and Discussion

Analytical data

The concentrations of metolachlor were determined in the test solutions. The mean measured concentrations ranged from 82 - 89% of nominal concentrations. The mean measured stock concentration was 99% of nominal throughout the study. The mean measured concentrations were used for calculating and reporting the results.

Biological data

Exposure to mean measured concentrations ≤ 8.6 mg metolachlor/L had no significant effect on the hatching success of fish embryos. No delay in hatch was observed in any treatment.

Exposure to concentrations ≥ 4.1 mg metolachor/L significantly increased the mortality of juvenile fish after 8, 15, and 22 days post-hatch. By day 8 post-hatch, 94% of the fish exposed to 8.6 mg metolachlor/L had died and 100% were dead at day 15.

There was a significant effect of metolachlor on growth of juvenile fish based on length, but no significant effect based on weight.

The MATC was estimated to be > 2.2 < 4.1 mg metolachlor/L.

Validity Criteria

Although this study broadly complies with the current validity criteria for early life-stage testing with fish (OECD 210; 2013), an error in salt addition after day 22 resulting in mortality invalidates the study. Therefore, the study is regarded as not reliable and should not be used for classification. Also, measured values for dissolved O_2 (DO) were 19 to 108% of ASV (guideline states that DO concentration should be >60% of ASV throughout the test.

Conclusions

The toxicity of metolachlor to early-life stages of sheepshead minnow (*Cyprinodon variegatus*) was determined. Based on the significantly reduced survival and length of juveniles to concentrations ≥ 4.1 mg metolachlor/L, the MATC was estimated to be > 2.2 and < 4.1 mg metolachlor/L. The study is not reliable and should not be used for classification.

Anonymous (2006)

Author:	Anonymous
Title:	Metolachlor (CGA24705) – The Toxicity to Fathead Minnow (*Pimephales promelas*) during an Early Life-Stage Exposure, Report Number 1781.6631, Springborn Laboratories Inc., 790 Main St., Wareham, Massachusetts, 02571-1037, USA. (Syngenta File No. CGA24705/2840)
Date:	2006
Doc ID:	Report Number 1781.6631
Guidelines:	Ecological Effects Test Guidelines OPPTS 850.1400 “Fish, Early-life Stage Toxicity Test”, Public Draft, (April 1996)
GLP:	Yes
Validity:	Yes
Previous evaluation:	(DAR 2018)

Executive Summary

The toxicity of metolachlor to early-life stages of fathead minnow (*Pimephales promelas*) was determined. Fish were exposed in a flow-through test-system to the following range of nominal concentrations: 0.094, 0.19, 0.38, 0.75 and 1.5 mg a.s./L, and a dilution water control. The mean measured concentrations ranged from 80 to 87 % of their nominal concentrations. Endpoints were related to measured concentrations (0.083, 0.15, 0.31, 0.67 and 1.3 mg a.s./L).

There were significant effects on the survival fathead minnow larvae for at 0.15 mg metolachlor/L (m.m.).

However, effects did not follow a dose response relationship as they were not observed in higher concentrations. Therefore, the 35-day NOEC for larval survival, total length, wet and dry weights was determined to be 1.3 mg metolachlor/L (m.m.)~~.~~

. There was no statistical difference in egg viability between the control and any of the test treatments. As statistical analysis revealed no significant dose response for any of the parameters and effects observed were <10 %, EC_{10} and EC_{20} values could not be derived.

Study Design and Methods

Experimental dates: 17th December 2001 to 21st January 2002.

A stock solution (90 µg a.s./mL) was prepared daily by diluting 0.6482 g metolachlor in 7000 mL of reagent grade water. The diluter was used for introduction of test solution (test item and dilution water) into the test vessels. A set volume of the stock solution was delivered to the mixing chamber and made up to a set volume with dilution water to give a nominal concentration of 1.5 mg a.s./L. Appropriate volumes of the mixing chamber solution were then dispensed into the test solution chambers and appropriate volumes of dilution water added to achieve the required test concentrations. These then emptied into the test vessels and this cycle was repeated such that the daily replacement rate of medium in the test aquaria was 6.8 aquarium volumes.

A flow-through test system was employed. At the start of the test 60 eggs were randomly allocated to egg cups and one egg cup suspended in each of two replicate test vessels at each test and control treatment. Hence, 120 eggs were exposed at each treatment. The test was undertaken in a temperature controlled water-bath.

Eggs and fry were exposed to mean measured concentrations of 0.083, 0.15, 0.31, 0.67 and 1.3 mg a.s./L, and a dilution water control.

The concentrations of metolachlor in the test solutions were measured at 0, 4, 10, 17, 24, 31 and 35 days using a gas chromatography method.

Observations for time to hatch, hatching success, larval mortality and other symptoms of toxicity were made daily during the pre and post-hatch phases, as appropriate. At the end of the test, lengths, and wet and dry weights of the surviving fry were measured.

Statistical analysis

At test termination the survival at hatch, larval survival and growth (total length, wet weight and dry weight) were analysed to identify significant differences between treatment and control organisms. Analyses were performed using the mean organism response in each treatment group. The data were arcsine square-root percentage transformed in order to check for homogeneity of variance (Bartlett's Test), and to confirm they were normally distributed (Shapiro-Wilk's Test). The NOECs were estimated from the data obtained by comparing the response for the test item treatments with the control using a Williams' test with a 95% level of certainty. The mean total length, mean wet weight and mean dry weight of surviving fish at 35 days were analysed separately.

EC_x calculations were carried out in ToxRat Professional version 2.10 (ToxRat Solutions GmbH, 2001-2010). The effective concentrations for hatching and post-hatch survival were assessed. Probit analysis with linear maximum likelihood regression was used to determine the concentration response function. Chi^2 was used as a goodness of fit measure. The effective concentrations for weight and length were assessed. Probit analysis with linear maximum likelihood regression was used to determine the concentration response function. Chi^2 was used as a goodness of fit measure, and the proportion of variance explained by the dose/response function was determined. Where no meaningful concentration/response was found ($p(F) > 0.05$) the calculated EC_x's were not valid.

Results and Discussion

The concentrations of metolachlor were determined in the test solutions. The mean measured concentrations ranged from 80 to 87 % of their nominal concentrations. The limit of quantification was 0.407 µg/L. The mean measured concentrations were used for calculating and reporting the results.

Table 60: Analytical results

Nominal concentration mg a.s./L)	Measured concentration (mg a.s./L)			% of nominal
	Day 0	Day 35	Mean	
0	<0.03	<0.026	0	NA
0.094	0.087	0.069	0.083	86
0.19	0.16	0.15	0.15	80
0.38	0.33	0.31	0.31	81
0.75	0.65	0.59	0.67	87
1.5	1.4	1.2	1.3	87

Embryo survival - There was no statistical difference in egg viability between the control and any of the test treatments.

There were significant effects on larval survival at 0.15 mg metolachlor/L (m.m.). Therefore, the 35-day NOEC for larval survival, total length, wet and dry weights was determined to be 0.083 mg metolachlor/L (m.m.), and the LOEC is determined to be 0.15 mg metolachlor/L (m.m.). As statistical analysis revealed no significant dose response for any of the parameters and effects observed were <10 %, EC_{10} and EC_{20} values could not be derived.

Table 61: Effects of metolachlor on the growth of fathead minnow

Mean measured concentration (mg a.s./L)	Hatching success (%) [1]	Larval survival (day 35) (%) [2]	Mean length (mm) ± SD[3]	Mean wet weight (mg) ± SD[3]	Mean dry weight (mg) ± SD[3]
0.0 (control)	84	98	30.1 (2.6)	269 (67)	66.0 (17)
0.083	84	94	30.2 (2.0)	270 (59)	67.0 (15)
0.15	86	88*	31.1 (2.1)	296 (61)	73.0 (14)
0.31	86	90*	31.2 (2.0)	302 (60)	70.0 (14)
0.67	88	99*	30.0 (2.2)	267 (57)	65.9 (14)
1.3	86	91*	30.0 (2.8)	272 (68)	67.1 (16)

[1] The number of live larvae on the day they are transferred from the egg cups to the test vessels (day 5), expressed as a percentage of the number of eggs added at the start of the test (day 0), mean of two replicates.

[2] The number of surviving larvae at the end of the test (day 35), expressed as a percentage of the number of eggs added on day 0, mean of two replicates.

[3] Mean of two replicates

* Statistically significant, based on William's test

As there was no significant dose response for any of the parameters and effects observed were <10 % for all parameters, EC_{10} and EC_{20} values could not be calculated.
Considering the minimum acceptable post-hatch survival criteria for this type of test (75 %, OECD TG210) as well as the non-monotonous response, the NOEC is determined to be >1.3 mg a.s./L

Conclusions

There were significant effects on the survival fathead minnow larvae for at 0.15 mg metolachlor/L (m.m.). However, effects on survival did not follow a dose-response relationship and the underlying assumptions of the statistical method applied (William's test) are not fulfilled. Using the more appropriate Dunnett's test instead only the 0.15 and 0.31 mg/L treatments were significantly different from the control. Therefore, the 35-day NOEC for larval survival, total length, wet and dry weights was determined to be >1.3 mg metolachlor/L (m.m.). As statistical analysis revealed no significant dose response for any of the parameters and effects observed were <10%, EC_{10} and EC_{20} values could not be derived. Due to the missing dose-response relationship and the significant effects observed in lower concentrations, the study is considered reliable with restrictions and acceptable for classification.

Anonymous (2000)

Author:	Anonymous
Title:	S-Metolachlor (CGA 77102) – Early Life-Stage Toxicity Test with Sheepshead Minnow (*Cyprinodon variegatus*)
Date:	2000
Doc ID:	Report Number 1781.6613
Guidelines:	FIFRA Guideline Reference No. 72-4 US EPA. 1986. Office of Pesticide Programs. Standard evaluation procedure for fish early life-stage. EPA540/9-86. July 1986. U. S. Environmental Protection Agency, Washington, DC. ASTM. 1995. Standard Guideline for Conducting Early-Stage Toxicity Tests with Fishes. ASTM designated E 1241-92.
GLP:	Yes
Validity:	Yes
Previous evaluation:	DAR (2018)

Executive Summary

The effects of CGA77102 to early-life stages of sheepshead minnow (*Cyprinodon variegatus*) embryos and larvae were determined under flow-through conditions. Fish were exposed to nominal concentrations of 94, 190, 370, 750 and 1500 µg CGA77102/L and a dilution water control. Results were based on the mean measured concentrations of 87, 180, 330, 710 and 1300 µg CGA77102/L.
No statistically significant adverse effects, as compared to the control, were observed in any of the treatment levels for any of the monitored end points (survival at hatching and at 28 days post-hatch, larvae total length, dry and wet weight at test termination).
Based on the above data, the 34-day NOEC for CGA77102 was determined to be 1300 µg CGA77102/L, and the 34-day LOEC was determined to be > 1300 µg CGA77102/L, the highest concentration tested. As statistical analysis revealed no significant dose response for any of the parameters and effects observed were below 10 %, EC_{10} and EC_{20} values could not be derived.

Conclusions

NOEC = 1.3 mg/L

The study is reliable without restriction and considered acceptable for classification.

Anonymous (1993)

Author:	Anonymous
Title:	Chronic toxicity of CGA 24705 to the Fathead minnow (Pimephales promelas). EG&G Bionomics
Date:	1993
Doc ID:	Unpublished report No. BW-78-11-341
Guidelines:	EPA guidelines 72-5
GLP:	No
Validity:	Yes
Previous evaluation:	DAR (2004, 2018)

Executive summary

Fathead minnows (*Pimephales* promelas) were continuously exposed to five concentrations of CGA-24705 throughout a complete life cycle. Data was compiled on the survival, growth and reproduction success of first generation (F_0) fish and on the hatching success, survival and growth of their progeny (F_1). The study is summarized in the original monograph and still considered valid and acceptable. EC_{10} and EC_{20} for the response variables of embryo hatch success, survival, total length and total weight in both the F_0 and F_1 generations, and reproductive success of the F_0 generation have been re-analysed for the DAR 2018 in order to estimate these values. Results were based on the mean measured concentrations of 0.2, 0.37, 0.78, 1.6 and 3.4 mg/L.

F_0 Generation
Hatchability, length at days 35 and 64, survival at day 181, male and female lengths and weights on day 266, eggs per female and eggs per spawn in the treated groups were not significantly different to the control groups, therefore no EC_{10} and EC_{20} values could be calculated.
There were statistically significant differences in fry survival at days 35 (p(F) = 0.000) and 64 (p(F) = 0.000) between control and treated groups. The calculated EC_{10} and EC_{20} values for F_0 survival are shown below:

Parameter	EC_{10} (95 % CL) mg/L	EC_{20} (95 % CL) mg/L
F_0 survival day 35	0.967 (0.827 – 1.074)	1.108 (0.981 – 1.210)
F_0 survival day 64	0.934 (0.804 – 1.034)	1.057 (0.939 – 1.151)

cl: confidence limits

F_1 Generation
Hatchability, length and weight on day 34 were not significantly different to the control groups, therefore no EC_{10} and EC_{20} values could be calculated.
There was a statistically significant difference in fry survival on days 34 (p(F) = 0.003) between control and treated groups. The calculated EC_{10} and EC_{20} values for F_1 survival are shown below:

Parameter	EC_{10} (95 % CL) mg/L	EC_{20} (95 % CL) mg/L
F_1 survival day 34	1.47 (1.05 – 1.77)	1.78 (1.38 – 2.06)

CL: confidence limits

Conclusions

NOEC (35 d) = 0.78 mg metolachlor/L (mean measured)
EC_{10} (64 d) = 0.934 mg metolachlor/L (mean measured)

The study is reliable without restrictions and considered acceptable for classification.

Anonymous (1997)

Author: Anonymous
Title: Prolonged toxicity test of CGA 77102 tech. to Rainbow Trout (*Oncorhynchus mykiss*) in the flow-through system
Date: 1997
Doc ID:Report Number 971605
Guidelines: OECD Guidelines for Testing Chemicals. Section 2: Effects on Biotic Systems Method. 204, Fish, Prolonged Toxicity Test (1984).
GLP: Yes
Validity: Yes

Executive Summary

The prolonged toxicity of CGA77102 to rainbow trout *Oncorhynchus mykiss* was determined under flow-through conditions. Fish were exposed to a range of nominal concentrations of 0.10, 0.17, 0.30, 0.56, 1.0 and 1.8 mg CGA77102/L (0.12, 0.13, 0.28, 0.49, 0.89 and 1.7 mg CGA77102/L mean measured), alongside a dilution water control.

Based on mean measured concentrations, the 28-day NOEC was 0.89 mg CGA77102 /L, the highest concentration tested.

Validity criteria

The validity criteria for the study were met:
Control fish mortality ≤ 10 % (0 % observed)
Oxygen concentration in the test media should not drop below 60 % of air saturation during test (75 - 105 % saturation observed)

Conclusions

At the highest concentration one fish died and another fish showed persistent sublethal signs of toxicity that did not recover at the end of the test. Even though not statistically significant, these effects are considered to be biologically relevant. Therefore, based on mean measured concentrations, the 28-day NOEC was 0.89 mg CGA77102 /L. The fish prolonged toxicity test is considered as supplemental information for the purpose of classification.

Anonymous (2001)

Author: Anonymous
Title: Prolonged Toxicity test of CGA77102 tech. to Rainbow Trout (*Oncorhynchus mykiss*) under Flow-Through Conditions, Report Number 2011771, Syngenta Crop Protection

AG, Ecological Sciences, CH-4002, Basel, Switzerland. (Syngenta File No. CGA77102/0594)

Date:	2001
Doc ID:	Report Number 2011771
Guidelines:	OECD Guidelines for Testing of Chemicals 215, Fish Juvenile Growth Test. OECD Guidelines for Testing of Chemicals 204, Fish Prolonged Toxicity Test.
GLP:	Yes
Validity:	Yes
Previous evaluation:	DAR (2018)

Executive Summary

The effects of CGA77102 on mortality and growth of rainbow trout (*Oncorhynchus mykiss*) were determined over a 28-day study period under flow-through conditions. Fish were exposed to a range of nominal concentrations of 1.2, 1.9, 3.0, 4.8 and 7.7 mg a.s./L and a dilution water control. The mean measured concentrations were 1.2, 2, 3.2, 4.9 and 8.1 mg CGA77102/L.
Endpoints are related to nominal concentrations. The 28-day NOEC was estimated to be 1.9 mg CGA77102/L since there was no statistically significant difference compared to the control considering mortality, growth and sublethal effects. The LC_{50} was calculated to be 4.6 mg CGA77102/L.

Conclusions

NOEC (28 d) = 1.9 mg/L

The study is reliable without restrictions. The fish prolonged toxicity test is considered as supplemental information for the purpose of classification.

Anonymous (1990)

Author:	Anonymous
Title:	Metolachlor: 21-day prolonged toxicity study in the rainbow trout under flow-through conditions
Date:	1990
Doc ID:	Report Number 234652
	Guidelines: OECD Guidelines for Testing of Chemicals 204, Fish Prolonged Toxicity Test.
GLP:	Yes
Validity:	Yes
Previous evaluation:	DAR (2018)

Executive Summary

The prolonged toxicity of CGA24705 to rainbow trout *Oncorhynchus mykiss* was determined under flow-through conditions. Fish were exposed to a range of nominal concentrations of 0.016, 0.063, 0.25, 1 and 4 mg/L, alongside a dilution water control. Mean measured concentrations were in the range of 87 – 98.5 % of nominal. Mortalities were observed at nominal concentrations of 1.0 mg/L and above. Symptoms of toxicity observed included lethargy and were observed in treatments of 1 mg/L and above. No mortality or symptoms of toxicity were observed in the control. Based on nominal concentrations, the 21 day NOEC was 0.25 mg /L and the LOEC was determined to be 1 mg /L. The LC_{50} was determined to be 1.23 mg /L.

Conclusions

NOEC (21 d) = 0.25 mg/L

The study is reliable without restrictions. The fish prolonged toxicity test is considered as supplemental information for the purpose of classification.

11.6.2 Chronic toxicity to aquatic invertebrates

Rufli, H. (1989)

Author:	Rufli, H.
Title:	Report on the Daphnia, reproduction test with CGA 24705 technical
Date:	1989
Doc ID:	Report Number 891103
Guidelines:	OECD-Guideline No. 202, Part 2, Paris 1984, modified according to EEC/OECD ring test 1985/86
GLP:	No, but complies with sound scientific standards
Validity:	No (minor deviation)
Previous evaluation:	DAR (2004, 2018)

Executive Summary

Chronic toxicity of metolachlor to *Daphnia magna* has been evaluated in a 21-day reproduction test. Daphnids were exposed to nominal concentrations of 0.024, 0.12, 0.60, 3.0 and 15 mg/L, alongside a solvent control in a semi-static test design. The mean measured concentrations were 0.023, 0.12, 0.54, 2.78 and 13.9 mg/L and the results are based on nominal concentrations. Ten daphnia per concentration and control with one daphnia each per test vessel were employed in the study. Observations made during the test included immobilization, cumulative number of young per female, fraction of dead young and length of time for appearance of first brood. At the highest concentration tested the number of immobilized daphnia was statistically significantly increased and the cumulative number of offspring produced per female was statistically significantly reduced after 21 days. At 3.0 mg/l the fraction of dead young per female was significantly increased and the number of mobile adults and the cumulative number of young were affected although these effects were not statistically significant. The EC_{50} after 21 days was 6.8 mg ai/l. A NOEC of 0.60 mg/L was determined for the fraction of dead young per female. The same NOEC could be assumed based on the number of immobilized adults and the decreased number of offspring. The EC_{10} and EC_{20} values for living young per female have been calculated to be 0.56 and 1.21 mg/L, respectively.

Validity criteria

The study does not meet all the current validity criteria for chronic toxicity testing with *Daphnia magna* (OECD 211; 2012):

- Mortality of the parent female *Daphnia* should not exceed 20% at the end of the test (0% in the control).

- DO concentrations were >3 mg/L throughout the study (93 to 128% ASV).

- Mean number of living offspring produced per parent animal surviving at the end of the test should be > 60 (54 observed).

Conclusions

All results are based on nominal concentrations. It was concluded that the 21-day EC_{50}, EC_{20} and EC_{10} reproduction for CGA24705 to *Daphnia magna* was > 3.0 mg, 1.21 mg and 0.56 mg CGA24705/L, respectively. The 21-day NOEC values were 3.0 mg CGA24705/L based on total cumulative number of young and length of time for appearance of first brood, and 0.60 mg CGA24705/L based on fraction of dead young.

This study is considered to be reliable with restrictions and to provide valid and useful data for classification.

NOEC (21 d) = 0.6 mg/L
EC_{10} (21 d) = 0.56 mg/L

Putt, A.E. (1995)

Author:	Putt. A.E.
Title:	Metolachlor technical - the chronic toxicity to Daphnia magna under flow-through conditions.
Date:	1995
Doc ID:	Report Number 95-8-6061
Guidelines:	EPA 850.1300, 72-4(b)
GLP:	Yes
Validity:	Yes
Previous evaluation:	DAR (2004, 2018)

Executive Summary

Chronic toxicity of metolachlor to *Daphnia magna* has been evaluated in a 21-day reproduction test under flow-through conditions. Daphnids were exposed to nominal concentrations of 2.5, 5, 10, 20, 40 mg/L alongside a control and a solvent control. Mean measured concentrations of metolachlor were 0.87, 1.8, 2.9, 5.9 and 12 mg/L and results are based on mean measured values. Forty daphnia per concentration and control with ten daphnia each per test vessel were employed in the study. Observations made during the test included immobilization, cumulative number of young per female, and length of time for appearance of first brood. Furthermore, the length and the dry weight of the parental daphnids were measured at the end of the test. No significant test substance dependent mortality was found during the test period among adult and offspring. At the highest concentration tested (12 mg/l), however, the cumulative number of young per female and the physical constitution of the parental daphnids were impaired after 21 days. None of these effects were found at concentration levels ≤ 5.9 mg/l mm. The EC_{50} after 21 days was > 12 mg ai/l and the LOEC with regard to the number of offspring and the physical constitution of the adults was 12 mg. The NOEC of this study was found to be 5.9 mg/l mm. The derived EC_{10} for reproduction is 6 mg/L.

Conclusions

NOEC (21 d) = 5.9 mg/L
EC_{10} (21 d) = 6 mg/L

The study is reliable without restrictions and considered acceptable for classification.

Müllerschön H. (1990)

Author: Müllerschön, H.
Title: Influence of Metolachlor on the reproduction of *Daphnia magna*
Date: 1990
Doc ID:Report Number 164204
Guidelines: OECD Guidelines for Testing of Chemicals, No. 202. *Daphnia magna* Reproduction test. Adopted 21 September 1998
GLP: Yes
Validity: No, after 6 day of explosition half oft the 20 daphnia were separated. That means, that half of the animals were removed.

Executive Summary

The effect of CGA24705 on the survival and reproduction of *Daphnia magna* was determined over 21 days under semi-static conditions. The study was run with a culture medium control, a solvent control and nominal concentrations of 0.25, 0.625, 1.25, 2.5 and 5.0 mg ai/L. Based on nominal concentrations, the 21-day NOEC for reproduction was 2.5 mg ai/L.

Study Design and Methods

Experimental dates: 14th February to 7th March 1990
The test medium was treated with the test article before the introduction of *Daphnia*. The test concentrations were based on the 48 hour EC_{50} value. The final stock was prepared freshly immediately prior to the preparation of the test concentrations. At the respective test days, dilutions of the test article stock were performed. The final concentrations of the test article were: 0.25, 0.625, 1.25, 2.5 and 5.0 mg ai/L. The concentration of Acetone in all test samples amounted to 0.01%. The test medium was renewed at day 3, 6, 8, 10, 13, 15, 17, and 20 of the exposure period.
The *Daphnia* were fed with the same time intervals as test medium renewal on the green alga (*Scenedesmus subspicatus*).
The mortality of adults and the number of young was controlled three times per week before renewal of the test media. Dead animals and offsprings were removed at the observation dates.
The concentration of the test article was determined at the first and the last treatment period directly after treatment and at the end of the respective period. Analyses were performed in duplicate with low, medium and high test concentrations. Test concentrations were determined by Nitrogen-phosphate detection.
The pH and concentration of dissolved oxygen were measured in one replicate at the start and end of the test and in the new and old solutions at each medium renewal. At the same time the temperature was measured in one of the control replicates. The room temperature was continually monitored. The appearance of the test medium was visually recorded for the old and new media at the beginning and end of each medium renewal.

Results and Discussion

The measured concentration of the test item in the new test media were in the range 96.4 to 117.8% of the nominal values and the measured concentrations in the old media were in the range 92.0 to 112.0 % (see table below). Therefore, the test item was stable in the test medium over the renewal periods of 48 hours. Nominal concentrations were used for the calculation and reporting of the results.

Survival of the parent animals was 100 % in the solvent control and in the water control and in all test concentrations up to and including 0.250 mg ai/L. At the highest concentration (5.00 mg ai/L) all parent daphnids survived until the end of the test.
The first brood juveniles were observed on day10 in the controls and all test concentrations up to and including 2.50 mg ai/L. Hence, time to first brood was unaffected at these concentrations.

The NOEC (No Observed Effect Concentration) is defined as the highest tested concentration at which there was no observed effect on the reproduction of the parent *Daphnia* within the period of the test and was determined directly from the data.

Conclusions

It was concluded that the 21-day EC_{50} reproduction for CGA24705 to *Daphnia magna* was > 5.000 mg ai/L, based on the nominal concentrations. The 21-day NOEC was 2.5 mg ai/L. Estimation of EC_{10} and EC_{20} values was not conducted.

The study is reliable with restrictions and should be considered for classification.

Palmer S.J., Kendell T.Z, Krueger H.O. (2004)

Author:	Palmer S.J.; Kendall T.Z.; Krueger H.O.
Title:	A Flow-Through Life-Cycle Toxicity Test with the Cladoceran (*Daphnia magna*)
Date:	2004

Doc ID:	Report Number 528A-130
Guidelines:	OECD (1984). OECD Guidelines for Testing of Chemicals, No. 211. *Daphnia magna* Reproduction test. Adopted 21 September 1998
GLP:	Yes
Validity:	Yes
Previous evaluation:	DAR (2018)

Executive Summary

The effect of CGA77102 on the survival, growth and reproduction of *Daphnia magna* was determined over 21 days. The study was run with a dilution water control, a solvent control and nominal concentrations of 2.5, 5.0, 10, 20 and 40 mg a.s./L. Mean measured concentrations at the start and the end of the study were 90.6 – 104 % of nominal. Based on mean measured concentrations, the 21-day NOEC and EC_{10} for first generation growth (lowest endpoint obtained) was 5.2 mg a.s./L and 1.29 mg/L, respectively.

Conclusions

NOEC (21 d) = 5.2 mg/L
EC_{10} (21 d) = 1.29 mg/L

The study is reliable without restrictions and considered acceptable for classification.

Lima, W., (1999)

Author:	Lima, W.
Title:	S-metolachlor (CGA 77102) – Life-cycle toxicity test with mysid (Mysidopsis bahia) Novartis Crop Protection AG, Basel
Date:	1999
Doc ID:	Report N° 1781.6575
Guidelines:	EPA 850.1300, 72-4
GLP:	Yes
Validity:	Yes
Previous evaluation:	DAR (2018)

Executive summary

The chronic toxicity of CGA77102 to the mysid (*Mysidopsis bahia*) was determined under flow-through conditions. Mysids were exposed to nominal concentrations of 19, 38, 75, 150, 300 and 600 µg CGA77102/L (18, 37, 62, 130, 250 and 510 µg CGA77102/L, mean measured), together with a dilution water control. Based on statistical analysis of female mysid length (determined to be the most sensitive performance criteria), the 28-day LOEC was 300 µg CGA77102/L. The results are reported using nominal concentrations as the mean measured concentrations are in the range 82 – 97 % of nominal values.

Study Design and Methods

The life-cycle toxicity test was conducted under flow-through conditions. An intermittent-flow proportional diluter was used to deliver the test substance at a rate of approximately 7.7 aquarium volume additions per day to provide a 90% test solution replacement rate of 7 hours. Each day a 90 µg CGA77102/L stock solution was prepared by diluting 0.73 g of test material in NANOpure® water to a total volume of 8L. This was pumped into the mixing chamber at 13.2 mL/cycle together with 1.975 L of dilution water per cycle. The solution in the mixing chamber constituted the highest nominal test concentration (600 µg/L) and was diluted (50%) to provide the remaining nominal test concentrations (300, 150, 75, 38 and 19 µg CGA77102/L). The test chambers were impartially positioned within a water bath to maintain temperature. Two replicate tanks were prepared for the controls and each test solution. After 4 days of test system equilibration, 15 mysids were randomly allocated to each retention chamber. When a sufficient number of mysids reached sexual maturity (day 14) one mature male and one mature female were randomly assigned to each of the pairing chambers. Observations were made daily for mortality and clinical symptoms of toxicity throughout the test. The number of offspring produced per female per reproductive day was recorded after pairing. At test termination total body length (to the nearest 0.1mm) using a dissecting microscope with calibrated stage micrometer, and total dry body weight for each mysid was determined (to the nearest 0.01 mg). Temperature, dissolved oxygen concentration, pH and salinity were measured daily in each replicate of each treatment level and the control solutions. The concentrations of test material in the dilution water control and the high, middle and low test concentrations before test initiation and test solutions from alternating replicates of each treatment level and control were measured at test day 0, 7, 14, 21 and 28 using HPLC/UV analysis. Effects on survival, reproduction and growth were analysed using William's Test. The percentage survival data underwent angular (arcsine square-root percentage) transformation before significant differences were determined. The Bartlett's test was used to analyse the homogeneity of variance.

Results and Discussion

All validity criteria set out in the guideline were met. The analytically determined mean measured concentrations of CGA77102 ranged from 82 to 97% of nominal values (see table below). The limit of quantification in this study was 0.00223 mg CGA77102/L. Mean measured concentrations were used for the calculation and reporting of results

Table 62: Effects on reproduction, growth and survival of the adult generation

Nominal concentrations (μg CGA77102/L)	Mean measured concentrations (μg CGA77102/L)	% Number of surviving adults[a]	Offspring/ female/ reproductive day[a]	Mean dry weight (mg)[a]		Mean body length (mm)[a]	
				Male	Female	Male	Female
Control	Control	73	0.98	0.94	1.4	7.7	8.1
19	18	75	0.85	0.99	1.3	8.0	8.0
38	37	73	1.1	0.94	1.2	7.5	8.0
75	62	80	1.0	1.0	1.3	7.9	8.0
150	130	90	1.1	1.0	1.4	7.9	7.9
300	250	85	0.59	0.93	1.2	7.7	7.7[b]
600	510	83	0.17[b]	0.88	1.1[b]	7.4	7.5[b]

a Values presented have been rounded to two significant figures.
b Significantly different (p ≤0.05) from the control (Williams' Test)

Survival of the parent animals was 73 % in the control and the survival rate was equal to or higher than this in all test concentrations. A statistically significant inhibitory effect on the reproductive success of mysids over 28 days, together with decrease in mean dry weight, was observed at 600 μg CGA-77201/L (see table below). However, at 300 μg CGA-77201/L a 40 % reduction of reproductive success was observed but not statistically significant. A statistically significant decrease in mean dry weight and mean body length of female mysids was observed at concentrations of 600 μg CGA77102/L and 300 μg CGA77102/L, respectively. The 28-day NOEC based on female body length was determined to be 150 μg CGA77102/L. The derived EC_{10} value for reproduction results in 182 μg CGA77102/L.

Conclusions

NOEC (28 d) = 0.15 mg/L

EC_{10} (28 d) = 0.182 mg/L

The study is reliable without restrictions and considered acceptable for classification.

Grade, R., (1989)

Author:	Grade, R.
Title:	Acute toxicity test of CGA 77102 tech. on sediment-dwelling Chironomus riparius (syn. Chironomus thummi) under static conditions
Date:	1998
Doc ID:	Report N° 971562
Guidelines:	BBA Guideline Proposal 1995; Guideline for toxicity test with Chironomidae was proposed in November 1997
GLP:	Yes
Validity:	Yes
Previous evaluation:	DAR (2018)

Executive summary

The purpose of these tests is to determine the effects of CGA 77102 tech. on the day of test, first emergence, the time distribution (peak) of emergence of male and female midges, and the total number of fully emerged male and female midges (Chironomus riparius larvae (1st instar, 1-3 days old).
For the assessment of possible effects of such substances on sediment dwelling organisms an OECD Guideline for toxicity test with Chironomidae was proposed in November 1997. Two exposure scenarios are included in the Test Guideline. Therefore, the study was extended to cover both types of exposure, i.e. CGA 77102 was tested with exposure scenario A and exposure scenario B in separate test vessels. The test was performed with:
Exposure scenario A: By applying a range of concentrations of CGA 77102 to the water column ofsediment-water systems containing 25 first instar larvae of Chironomus riparius each under static conditions. 24 hours after addition of the test organisms, 10 mL of test substance (in stock solu-tions) was introduced by pipette below the surface into the water column of the test system.
Exposure scenario B: By mixing CGA 77102 directly into aged artificial sediment at a range of concentrations prior to introduction of Chironomus larvae. Spiked sediment and water were added to the test vessels 23 hours prior to test initiation.
The studies were conducted in 1 L glass beakers containing about 1.5 cm artificial sediment and a water column of a height of approximately 8 cm at the beginning of the lest and about 6 cm at the end of the test (samples for chemical analysis were taken during the test). The tests were performed at a constant temperature of 20 ± 2° C with a photoperiod of 16 hours light (intensity 800 ± 200 lux) and 8 hours dark (twice/day ca. 30 minutes transition period). The biological assessment was based on impacts on full maturation of the larvae to adult midge. Main parameters examined were the rate and time of emergence and the total number of fully emerged male and female midges.

Study Design and Methods

Water spiked:

1 L glass beakers (tall form. 9 cm diameter) were filled with a layer of 1-2 cm of artifical sediment (corresponding to 86 g sediment (moist weight); for composition of the sediment see Table below).
The sediment was overlaid with reconstituted water of a height of approximately 8 cm. The water level was marked outside on the test beaker. The test beakers were then covered with parafilm to reduce evaporation throughout the test and to allow collection of emerged midges. Gentle aeration was provided through a glass pasteur pipette situated about 2 to 3 cm above the sediment.
The test beakers were prepared 15 days before the start of the definitive test (test substance application) to allow stabilization of the systems under test conditions.
One day before treatment, 25 larvae of the first larval stage were allocated randomly to each test vessel with a blunt pipette. After addition of the larvae aeration was stopped for the following 24 hours.

The application of the test substance was carried out one day later.
The stock solutions (see 2.4.1) were added to the water column of the test vessels below the water surface by using a pipette and gently mixing the upper water Jayer to ensure homogeneous distribution without disturbing the sediment.
Foreach test concentration and for the control three replicates were carried out. The test system was kept in a temperature controlled room at 20 °C, a relative air humidity of > 70% under a light:dark rhythm (16-8h) and a light intensity of 800 to 1000 Iux.

Results and Discussion

The nominal test concentrations added to the water column were 0.5, 1, 2, 4, 8, 16 and 32 mg/L. The actual measured test concentrations in the water phase were 0.57, 1.14, 2.16, 4.25, 8.59, 12.8 and 13.9 mg/L at test day 0 (1-3 hours after application). At the end of the test (test day 28) water concentrations had decreased to average values of 0.06, 0.12, 0.25, 0.49, 0.66, 2.82 and 8 mg/L, respectively. The substance concentrations in sediment were analyzed from samples with the highest administration rates, i.e. 32 mg/L. The measured test substance concentrations in sediment (incl. interstitial water) were 28.8, 26.2 and 21.5 mg/kg fresh weight at day 0, 7 and 28, respectively.
Calculations of effect concnetrations in the study report for the rate of emergence, the delevopment time and the rate of development (reciprocal of the development time) were based on nominal concentrations in the water phase. Recalculated effect concentrations for emergence rate and development rate are based on mean measured concentrations using the drc package 3.0.1 and R version 3.5.1.

As the test substance was disappearing from the test system over time (37% in the highest concentration), results should be preferably based on mean measured concentrations.

Based on mean measured concentrations the effect concentrations are as follows:

- Emergence rate (log-normal model):
  EC_{10}: 5.4 (CI: 3.7 – 7.1)
  EC_{20}: 5.7 (CI: 4.8 – 6.6)
  EC_{50}: 6.3 (CI: 5.4 – 7.2)
- Development rate (log-logistic model):
  EC_{10} 5.8 (CI: 4.6 – 7)
  EC_{20} 6.1 (CI: 5.3 – 6.9)
  EC_{50} 6.8 (CI: 2.3 – 11.3)

Based on mean measured concentrations the NOEC is determined as 2.38 mg/L and 6.01 mg/L for emergence rate and development rate, respectively.

Conclusions

The EC-50 values for emergence rate and development rate of Chironomus riparus were 6.3 and 6.8 mg/L respectively, for organisms exposed to CGA 77102 via spiking of the water column based on mean measured concentrations in the water phase. The corresponding NOEC values based on mean measured concentrations in the water phase were 2.38 and 6.01 mg/L for emergence rate and development rate, respectively.
The study is reliable without restrictions and can be used for classification.

11.6.3 Chronic toxicity to algae or other aquatic plants

Please refer to section 11.5.3. Endpoints used for acute and chronic classification regarding algae and other aquatic plants do not differ and are not repeatedly listed in this section.

11.6.4 Chronic toxicity to other aquatic organisms

No information available. All the information on chronic toxicity is taken from the RAR and list of endpoints for S-metolachlor, January 2018.

11.7 Comparison with the CLP criteria

Please note that solely studies for S-metolachlor (CGA-77102) are considered for classification. Studies for metolachlor (CGA 24705) are listed in this CLH-report for completeness.

Based on the aquatic toxicity tests with S-Metolachlor and its general degradability degradation products are not assumed to cause the observed toxicity. Additionally, degradation products of S-metolachlor are clearly less toxic compared to the parent (please refer to the RAR of S-metolachlor). Degradation products of S-metolachlor do not need to be considered for classification.

11.7.1 Acute aquatic hazard

Suitable data is available for all three trophic levels. S-metolachlor fulfils the classification criteria for Aquatic Acute 1. The acute toxicity to algae and aquatic plants is pivotal with E_rC_{50} values of 0.056 mg/L (*P. subcapitata*) and 0.062 mg/L (*E. canadensis*), respectively. 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).

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

S-metolachlor fulfils the criteria for classification as Aquatic Chronic 1 since its chronic toxicity to aquatic species from two out of three trophic levels is below 0.1 mg/L and the substance is not rapidly biodegradable. 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 the experimentally determined BCF in fish of 255, S-metolachlor is not considered to have a potential to bioconcentrate for classification purposes.

11.8 CONCLUSION ON CLASSIFICATION AND LABELLING FOR ENVIRONMENTAL HAZARDS

S-metolachlor can be classified as Aquatic Acute 1 with an M-factor of 10 (0.01 mg/L $< L(E)C_{50} \leq$ 0.1 mg/L) based on the acute toxicity to algae.

S-metolachlor can be classified as Aquatic Chronic 1 with an M-factor of 10 (0.001< NOEC ≤ 0.01 mg/L) based on the long-term toxicity to aquatic plants and the substance being not rapidly biodegradable.

12 EVALUATION OF ADDITIONAL HAZARDS

12.1 Hazardous to the ozone layer

This endpoint is not addressed in the CLH report.

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