Committee for Risk Assessment

RAC

Opinion

proposing harmonised classification and labelling
at EU level of

mesotrione (ISO) 2-[4-(methylsulfonyl)-2-nitrobenzoyl]-1,3-cyclohexanedione

EC Number: -
CAS Number: 104206-82-8

CLH-O-0000001412-86-232/F

Adopted

14 September 2018

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14 September 2018

CLH-O-0000001412-86-232/F

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

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

Chemical name: **mesotrione (ISO) 2-[4-(methylsulfonyl)-2-nitrobenzoyl]-1,3-cyclohexanedione**

EC Number: **-**

CAS Number: **104206-82-8**

The proposal was submitted by the **United Kingdom** and received by RAC on **12 September 2017.**

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

PROCESS FOR ADOPTION OF THE OPINION

The United Kingdom has submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/harmonised-classification-and-labelling-consultation/*** on **17 October 2017**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **1 December 2017**.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Riitta Leinonen**

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

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

The RAC opinion on the proposed harmonised classification and labelling was adopted on **14 September 2018** by **consensus**.

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

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATE	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	609-064-00-X	mesotrione (ISO) 2-[4-(methylsulfonyl)-2-nitrobenzoyl]-1,3-cyclohexanedione	-	104206-82-8	Aquatic Acute 1 Aquatic Chronic 1	H400 H410	GHS09 Wng	H410			
Dossier submitters proposal	609-064-00-X	mesotrione (ISO) 2-[4-(methylsulfonyl)-2-nitrobenzoyl]-1,3-cyclohexanedione	-	104206-82-8	**Retain** Aquatic Acute 1 Aquatic Chronic 1 **Add** Repr. 2 STOT RE 2	**Retain** H400 H410 **Add** H361d H373 (kidney)	**Retain** GHS09 Wng **Add** GHS08	**Retain** H410 **Add** H361d H373		**Add** M=10 M=10	
RAC opinion	609-064-00-X	mesotrione (ISO) 2-[4-(methylsulfonyl)-2-nitrobenzoyl]-1,3-cyclohexanedione	-	104206-82-8	**Retain** Aquatic Acute 1 Aquatic Chronic 1 **Add** Repr. 2 STOT RE 2	**Retain** H400 H410 **Add** H361d H373 (eyes, nervous system)	**Retain** GHS09 Wng **Add** GHS08	**Retain** H410 **Add** H361d H373		**Add** M=10 M=10	
Resulting Annex VI entry if agreed by COM	609-064-00-X	mesotrione (ISO) 2-[4-(methylsulfonyl)-2-nitrobenzoyl]-1,3-cyclohexanedione	-	104206-82-8	Repr. 2 STOT RE 2 Aquatic Acute 1 Aquatic Chronic 1	H361d H373 (eyes, nervous system) H400 H410	GHS08 GHS09 Wng	H361d H373 H410		 M=10 M=10	

GROUNDS FOR ADOPTION OF THE OPINION

RAC general comment

Mesotrione (ISO) is a pesticide active substance used as an herbicide. There is an existing entry in Annex VI of CLP regulation for environmental hazards only. The proposal from the Dossier Submitter (DS) addressed only the following endpoints: STOT RE, mutagenicity, carcinogenicity, toxicity to reproduction and hazardous to the aquatic environment including M-factors for both acute and chronic aquatic toxicity.

Mesotrione acts *via* inhibition of 4-hydroxyphenylpyruvate dioxygenase (HPPD) in plants, a key enzyme of the tyrosine catabolism pathway. HPPD occurs both in plants and animals. The consequence of HPPD inhibition in mammals is tyrosinaemia which is species dependant. This mode of action (MoA) is common to the herbicides of the triketone family already evaluated by RAC, such as sulcotrione (RAC, 2011) and tembotrione (RAC, 2013) and to the pharmaceutical drug 2-[2-nitro-4-(trifluoromethyl)benzoyl]cyclohexane-1,3-dione (NTBC), used in the treatment of human hereditary tyrosinaemia type 1.

HUMAN HEALTH HAZARD EVALUATION

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

Summary of the Dossier Submitter's proposal

Oral route

The DS based the evaluation of STOT RE on 12 repeated-dose toxicity studies in rats, 3 studies in mice, 2 studies in dogs and one human volunteer study. Additionally, relevant results from reproductive toxicity studies were taken into account. In rats, eyes and kidneys were identified as target organs by the DS whereas only the eyes where affected in mice and dogs.

Eye effects were consistently seen in rats, mice and dogs, with male rats being the most sensitive. Eye effects consisting of ocular opacity, as a result of corneal keratitis, epithelial disruption and corneal vascularisation, were seen from a dose value of 0.71 mg/kg bw/day in males (90-day toxicity studies). These effects were reversible after cessation of exposure. Ocular lesions seen in mice and dogs were observed at top doses only, not relevant for STOT RE classification. The mode of action (MoA) reported by the DS suggests that the eye effects observed in rats and other species are due to hypertyrosinaemia. Mechanistic studies investigating tyrosine administration in rats have shown that tyrosine exposure produces the same ocular lesions as in rats exposed to mesotrione. Data on other HPPD inhibitors have demonstrated that plasma tyrosine levels maintained at above 1 000 nmol/mL will result in corneal lesions in rats. Studies from several HPPD inhibitors showed that species sensitivity is due to the degree of hypertyrosinaemia and species differences in tyrosine aminotransferase (TAT) activity. TAT activity in human is similar to that of mice but significantly higher in rats. The DS considered that there is an intrinsic possibility that mesotrione could cause similar effects in humans as observed with the HPPD inhibitor NTBC. Nevertheless, a human volunteer study using mesotrione demonstrated that mesotrione is 400 times less potent than NTBC for effects in the eyes. Therefore, the DS concluded that eye effects are not expected to occur at doses relevant for classification.

Kidney findings observed in rats were considered inconsistent by the DS, without clear dose-response relationship but overall, of sufficient concern for STOT RE 2. The proposal was mainly based on renal pelvic dilatation and chronic progressive glomerulonephropathy observed at the top dose of 14.5 mg/kg bw/d in one of the 90-day toxicity study in rats. Renal effects observed in the 2-year and 2-generation reproductive toxicity study are considered by the DS as difficult to interpret due to variation in the nature and severity of the effects. According to the DS, it is plausible that the effects are linked to hypertyrosinaemia but there is no definitive evidence. Moreover, there is no information on human relevance and relative potency of effects in kidneys. Therefore, the DS proposed to classify mesotrione as STOT RE 2; H373 indicating that kidneys are the target organs and should be identified in the hazard statement.

Dermal route

One 28-day dermal study in rabbits was used by the DS for the evaluation of STOT RE after dermal exposure. No classification is proposed as no effects were observed in this study.

Inhalation route

No studies were located regarding effects in humans or animals after inhalation exposure.

Comments received during public consultation

One Member State Competent Authority (MS) agreed with the DS's proposal to classify mesotrione as STOT RE 2; H373 for effects observed on kidney and no classification for the eyes.

Industry submitted a position paper on the MoA of mesotrione. Industry claimed that the mouse is the most appropriate model for human health risk assessment and also for classification and labelling. In their position paper, they provided information on different HPPD inhibitors (bicyclopyrone, tisoxaflutole, mesotrione, sulcotrione, tembotrione, tolpyralate, topramezone) and a mode of action/adverse outcome pathway (MoA/AOP) analysis as proposed by the US Environmental Protection Agency (US EPA, 2015) (see also Table below).

***Table**: Evidence of Key Events proposed by US EPA for HPPD inhibitors registered/submitted for registration (selection from industry's position paper)*

Key Event	Mesotrione	Sulcotrione	Tembotrione
Decreased HPPD enzyme activity	x		x
Increased plasma tyrosine concentration	x	x	x
Clearance of tyrosine by alternative pathway	x	↑ ketones rats	x
Tyrosine-mediated effects (rats)			
Ocular opacity	Y	Y	Y
Liver effects	Y	Y	Y
Kidney effects	Y	Y	Y
Developmental changes in pattern of ossification	Y	Y	Y
↓ Pup survival	Y	Y	Y
↑ pelvic dilatation	Y	Y	Y

Industry disagreed with the classification proposal for STOT RE 2 on kidneys. Industry provided a position paper with the following three main arguments in favour of no classification.

First, there is no evidence of treatment-related effect on pelvic dilatation/hydronephrosis in repeated-dose toxicity studies. The effects observed in the 90-day and carcinogenicity studies are within historical control data (HCD) of the testing laboratory. Secondly, evidence of treatment related effects in rat multigenerational studies in F1 animals and subsequent generations are attributable to hypertyrosinaemia. According to the position paper, this is suggested by a better correlation of the effects with plasma tyrosinaemia than dose of mesotrione administered to rats and similarities with other HPPD inhibitors (e.g. kidney findings observed in rats but not in mice multigeneration studies).
Finally, increased severity of chronic progressive nephropathy in male rats in the carcinogenicity study 07is considered secondary to hypertyrosinaemia and is not expected to occur in humans following exposure to mesotrione at dose relevant for classification.

The DS replied to these comments that the available data are supportive of the proposed MoA (i.e. tyrosinaemia) and that humans are likely to be less sensitive to these effects than the rat. However, DS highlighted the uncertainties regarding the overall relevance of certain findings (i.e. kidneys, developmental toxicity) to humans.

Assessment and comparison with the classification criteria

In the repeated-dose oral toxicity studies reported in the CLH dossier, mesotrione caused effects on the eyes in rats, mice and dogs. Additionally, liver, kidney and sciatic nerves were also identified as target organs in rats.

Eye effects

Ophtalmoscopy and gross pathology were not performed in the oral 28-day toxicity study in rats (mesotrione tested up to 2 464 mg/kg bw/day).

In the two 90-day toxicity studies conducted according to OECD test guideline TG 408 (Anonymous, 1994b, 1997a), ophthalmoscopy revealed moderate to marked corneal opacity and vascularisation in males at ≥ 0.71 mg/kg bw/day. After microscopic examination, slight to moderate corneal keratitis waswere observed in 40 % of males dosed with 0.71 mg/kg bw and in 70 %-100 % of males rats at ≥ 11 mg/kg bw/day. A plateau of incidence was observed in males at ≥ 11 mg/kg bw/day as few animals did not respond at the top doses of 112 and 1 111 mg/kg bw/day. In females, incidence and severity of corneal keratitis was lesser than in males. Corneal keratitis was observed at ≥ 12.5 mg/kg bw/day. Consistently, similar ocular findings at ophthalmoscopy were observed in two non-guideline 90-day dose-response studies in male and female rats (Anonymous, 1997d, 1997e) and in a 90-day range-finding study investigating non-ocular endpoints (Anonymous, 1995a).

Two non-guideline 90-day studies investigated reversibility of ocular effects in male rats (Anonymous, 1997b) at 0, 0.37, 7.5 mg/kg bw/day in the first study and 0, 192 mg/kg bw/day in the second study. In these studies a dose-related increase in incidence and severity of corneal opacity and vascularisation was observed at ophthalmoscopy at ≥ 0.37 mg/kg bw/day. Marked corneal effects appeared at ≥ 7.8 mg/kg bw/day. No microscopic examination was performed in the eyes. The findings decreased during the recovery period and corneal opacity was not apparent following recovery period of 8 weeks. Corneal vascularisation resolved to ghost vascularisation. In this study a dose-related, statistically significant, increase in tyrosineamia was observed at ≥ 0.37 mg/kg bw/day (> 1 000 nmol/mL) with a plateau at ≥ 7.5 mg/kg bw/day.

In the two-year toxicity study in rats, dose-related corneal opacity was increased at ≥ 0.16 mg/kg bw/day in males and ≥ 7.7 mg/kg bw/day in females and was frequently accompanied by corneal vascularisation.

In a 5-week non-guideline ocular developmental toxicity and reversibility study in male rats (Anonymous, 1997c), corneal effects were observed at 272 mg/kg bw (single dose tested in the study) just after 1 week consisting of corneal opacity and vascularisation. After 5-week exposure, corneal keratitis was reversible after the end of the recovery period (8 weeks).

Three additional non-guideline mechanistic (exacerbation) studies were performed to investigate repeated effects of mesotrione and/or tyrosine after repeated exposure in rats. In a 21-day study looking at the effects of dietary tyrosine on ocular lesions in male rats (Anonymous, 1995b), corneal keratitis was noted in the animals exposed to 2.5 % and 5 % tyrosine as expected. In a 28-day variable dose study of mesotrione in male rats (0.025 to 13 mg/kg bw/day), no ocular effects were observed (Anonymous, 2000a). In a 28-day toxicity study with tyrosine and/or mesotrione (100 ppm: 0 %, 0.5 %, 1.0 %, 2.5 %) in female rats only, corneal opacity was noted in groups treated with mesotrione but these effects were more pronounced with 100 ppm dietary tyrosine concentration as expected (Anonymous, 1997g).

Ocular lesions were also observed in the multigeneration study in rat at dietary dose levels ≥ 0.9-2.3 mg/kg bw/day (Anonymous, 1997p).

In mice and dogs, ocular effects were observed at top doses only, not relevant for STOT RE classification.

In comparison to classification criteria, STOT RE is reserved to substances showing significant health effects that can impair function or morphology of a tissue/organ, both reversible and irreversible, immediate and/or delayed and that are relevant for human health. The guidance value (GV) for STOT RE is 100 mg/kg bw/day for category 2 and 10 mg/kg bw/day for category 1 obtained in a 90-day rat study. Adjusting this GV from 90-day to two years duration gives a value of 12.5 mg/kg bw/day for STOT RE 2 and 1.25 mg/kg bw/day for STOT RE 1.

At doses below the GVs for STOT RE 1, severe ocular toxicity was observed in male rats at ophthalmoscopy confirmed by microscopic examinations in seven 90-day studies and in the 2-year study. In females, ocular toxicity was observed at dose levels relevant for classification STOT RE 2 (90-day toxicity studies, carcinogenicity study). Reversibility of the ocular effects were observed in male rats.

Human relevance needs to be taken into account in deciding upon classification STOT RE. According to the data provided by industry on other HPPD inhibitors, ocular effects are not seen at plasma tyrosine levels below 1 000 nmol/mL in rats. Monkeys seem to be recalcitrant to the ocular toxicity induced by HPPD inhibitors like sulcotrione (RAC, 2011). In humans, ocular effects have been reported in children and adults at dose below this threshold but there is no exact relationship between plasma tyrosine concentration and ocular toxicity. US FDA (FDA, 2018) recommended to keep serum tyrosine below 500 nmol/mL in order to avoid toxic effects in the eyes using NTBC (potent HPPD inhibitor). In a human volunteer study (Hall *et al*., 2001) performed with a single dose of 0.1, 0.5 and 4 mg/kg, mesotrione was shown to be 400 time less potent that the medicinal drug NTBC given at 1 mg/kg. Mesotrione has a shorter plasma half-life than NTBC. Mean plasma tyrosine concentration was around 300 nmol/mL (individual data not detailed). Effects returned to predose levels by 48 h at 4 mg/kg. No explanation on dose levels were provided in the study and no repeated exposure was performed. No plateau in plasma tyrosine concentration was shown in the study. Therefore, there is uncertainty whether higher tyrosine concentration in plasma could be higher after repeated exposure. RAC considers corneal toxicity as potentially relevant to human. Nevertheless, as the rat is more sensitive than human subjects for this type of effects, classification STOT RE 2 (H373) "May cause damage to organs (eyes) through prolonged exposure" is considered more appropriate than STOT RE 1

Kidney effects

Kidney toxicity was only observed in rats. One 28-day toxicity study, two 90-day toxicity studies, one carcinogenicity study and a 2-generation study investigated kidney effects including microscopic or histopathological examination. Two 90-day mechanistic studies investigated kidney weight but histopathology was either not performed or done in a limited number of animals. In the repeated dose toxicity studies, two findings were highlighted by the dossier submitter; renal pelvic dilatation/hydronephrosis and chronic progressive nephropathy. Other findings such as hyaline droplet formation or transitional epithelial hyperplasia are not further considered by RAC as these effects were not consistently seen among the studies.

According to the position paper provided by industry during public consultation (PC), pelvic dilatation is the term usually reserved for macroscopic examination whereas hydronephrosis is the histopathological diagnosis term applied to this finding. Unilateral lesion is a common finding in rat and is considered spontaneous whilst the bilateral lesion is much less common and can reflect exposure to a chemical. Chronic progressive nephropathy is a common finding in rats particularly in animals older than one year.

In the 28-day toxicity study (Anonymous, 1994a), pelvic dilatation/hydronephrosis was only seen in males and females at top doses (2 646/2 424 mg/kg) not relevant for STOT RE. In the two 90-day guideline studies (Anonymous, 1994b, 1997a), unilateral hydronephrosis was observed in males within HCD (historical controls of the laboratory, years 1999-2000, provided during PC by industry) and are therefore not considered treatment-related. Chronic progressive glomerulopathy (CPG) was observed in these studies only in males, at low incidences, without dose-response (no historical control data (HCD) provided) and therefore could be spontaneous (see table below).

***Table**: Incidence of kidney findings in males observed in 90-day studies (incidences from industry's position paper submitted during PC)*

Males (n=12)	**Dose Level** (mg/kg bw/day)						
	0	*0*	0.9	11	*14.5*	112	1 111
Kidney relative weight (g)	3.0	*3.2*	3.2	3.3*	*3.4*	3.5**	3.4*
Hydronephrosis	4	*1*	2	2	*3*	1	3
CPG	1	*0*	2	3	*2*	0	0

*p<0.05 ; **p<0.01; in italic: Anonymous 1997a, interim doses in Anonymous 1997a not examined; In black, Anonymous 1994b;

In the chronic/carcinogenicity study performed in rats (Anonymous, 1997f), incidences of unilateral hydronephrosis were seen without dose-response in males and females. Increased incidence of bilateral hydronephrosis was observed only at top doses in males and females (160/190 mg/kg bw/day) not relevant for STOT RE. With regards to chronic progressive glomerulopathy, the severity of the lesions was increased in males and females at ≥ 7.5 ppm (0.48 mg/kg bw/day) as shown in the table below.

***Table**: Incidence of total kidney findings after interim or terminal kill in the 2-year rat study (Data from industry's position paper submitted during public consultation)*

	Males				**Females**			
Doses (mg/kg bw/day)*	**0**	**0.48**	**6.5**	**160**	**0**	**0.57**	**7.7**	**190**
Unilateral hydronephrosis	5	7	5	3	3	3	5	4
Bilateral hydronephrosis	1	0	0	3	0	1	1	2

CPG (total)	62	63	64	63	56	56	56	56
Minimal	8	3	4	5	21	34	21	29
Slight	16	18	12	17	29	17	24	18
Moderate	14	9	14	10	6	4	6	7
marked	24	33	34	31	0	1	5	2

CPG: chronic progressive glomerulopathy; * Not investigated at 1 and 2.5 ppm;

In the 2-generation study in rats (Anonymous, 1997p), in F0 parental animal only unilateral hydronephrosis was observed without dose-response. A clear dose-related increase in the incidence of bilateral hydronephrosis was seen in F1 and subsequent generations in both sexes. Severity was increased in pups. Pelvic dilatation was increase at ≥ 10 ppm (equivalent to 0.9-2.3 mg/kg bw/day) in F1 and F2 animals, depending on sex, generation and period of study (pre-mate, gestation or lactation. These results indicated that longer duration of exposure resulted in renal changes. These results are further discussed in the reproductive toxicity section.

Overall, RAC is of the opinion that in repeated dose-toxicity studies, bilateral hydronephrosis was not observed below the cut-off value for STOR RE 2 (100 mg/kg for 90-day and 12.5 mg/kg for 2-years). The increase in severity of chronic progressive glomerulopathy, observed in the 2-year study in male rats only, is not considered sufficient to trigger a classification as STOT RE. Overall, no classification is proposed for kidney effects.

Liver effects

Liver findings in the 28-day and 90 days toxicity studies at doses relevant for classification STOT RE 2 were confined to changes in weight and low incidences of histopathological findings without consistency between the studies. In the chronic/carcinogenicity study in rats, relative liver weight was increased at interim and final kill (15-18 %) and incidences of macroscopic findings (pale liver, enlarged liver) were observed at terminal kill. Microscopic findings consisted of hepatocyte fatty vacuolation at terminal kill at ≥ 0.48 mg/kg bw/day in males and at ≥ 7.7 mg/kg bw/day in females (see table below).

Table*: Incidence and severity of non-neoplastic hepatological findings in male rats in liver*

	Males				**Females**			
Doses (mg/kg bw/day)	**0**	**0.48**	**6.5**	**160**	**0**	**0.57**	**7.7**	**190**
Hepatocyte fat vacuolation	17	36	39	39	8	9	16	14

Liver effects (weight, hepatocyte fatty vacuolation) occurring in male rats ≥ 0.48 mg/kg bw/day are consistent with a classification as STOT RE 1 (GV = 1.25 mg/kg bw/day). No dose-response was observed, but as these effects are likely related to hypertyrosinaemia, a plateau might have been attained (not investigated in the study). As no severity in hepatocyte fatty vacuolation is reported in the dossier, RAC proposed no classification for liver findings.

Sciatic nerve effects

Sciatic nerve toxicity was observed in the 2-year study in male rats in males and to a lesser extent in females. Effects consisted of increased severity in sciatic nerve demyelination (See table 5). These effects were not observed in lower duration studies in rats, mice or dogs.

***Table 5**: Incidence and severity of non-neoplastic findings in male rats in sciatic nerves*

Findings	Males				Females			
Dietary level (ppm)	**0**	**0.48**	**6.5**	**160**	**0**	**0.57**	**7.7**	**190**
Demyelination	63	60	60	59	61	58	57	58
Minimal	26	15	13	14	32	23	20	15
Slight	29	29	20	28	28	33	30	31
Moderate	8	16	23	13	1	2	7	12
marked	0	3	4	4	-	-	-	-

Effects on sciatic nerves (demyelination) occurring in male and female rats at ≥ 0.48 mg/kg are severe enough to consider classification for STOT RE. Moreover, impaired cognitive functioning has been observed within some patients treated with NTBC.
RAC noted that the threshold plasma tyrosine level recommended by US FDA (500 µmol/L) aimed to protect eyes effects but also to avoid toxic effects on the nervous system (various degrees of mental retardation and developmental delay). These effects are likely secondary to tyrosinaemia and rat is more sensitive than human. Nevertheless, there is no information on relative potency of effects in nevous system. Therefore, RAC concluded that the classification STOT RE 2; H373 for the nervous system is warranted for mesotrione.

RAC agrees with DS that no classification for STOT RE by dermal route is warranted.

No classification by inhalation is warranted based on lack of data.

In conclusion, RAC proposes to classify mesotrione (ISO) as **STOT-RE 2; H373 (eyes, nervous system).**

RAC evaluation of germ cell mutagenicity

Summary of the Dossier Submitter's proposal

The outcomes of one *in vitro* bacterial gene mutation test (OCED 471) and one *in vitro* gene mutation assay in L5178Y mouse lymphoma cells (OECD 476) were both negative, according to the DS. An *in vitro* chromosomal aberration test (OECD 473) resulted in equivocal results without metabolic activation (positive results in one out of two donors). *In vivo*, a micronucleus test (OECD 474) from mice was negative. Overall, no classification was proposed by the DS for germ cell mutagenicity.

Comments received during public consultation

No comments were submitted.

Assessment and comparison with the classification criteria

In vitro tests

In the renewal assessment report (RAR) of mesotrione, several Ames assays were available with mesotrione at doses up to 5 000 µg/plate. Some positive results were observed due to the presence of a mutagenic impurity. A negative result was obtained and reported in the CLH dossier with and without metabolic activation using a batch representative of mesotrione current specification.

A gene mutation assay on mouse lymphoma cells resulted in a negative result. In an *in vitro* chromosome aberration assay, a statistically significant increase in the number of aberrant cells was observed without metabolic activation only. The positive results were dose related but only observed in one of the two human donors. The study is thus considered equivocal by RAC.

In vivo tests

There is one *in vivo* micronucleus test in mice available with mesotrione. The assay was negative at 500 mg/kg bw. One male was found dead following exposure and no further clinical signs were observed during the study period. No specific evidence of bone marrow exposure was provided but mortality was observed at 800 mg/kg in a preliminary study. Thus, RAC considers the dose levels used in the study as appropriate.

Altogether, RAC agrees with the DS that **classification for germ cell mutagenicity is not warranted.**

RAC evaluation of carcinogenicity

Summary of the Dossier Submitter's proposal

Two oral studies, one in mice and one in rats (TG 453, GLP compliant) were summarised in the CLH report by the DS.

In mice, mesotrione was administered during 80 weeks at up to 898/1 103 mg/kg bw in males/females (Anonymous, 1997o). There was no increase in neoplastic lesions. Therefore, the substance did not show carcinogenic potential in mice.

In rats study (Anonymous, 1997n, OECD TG 453, GLP statement), mesotrione was administered up to 104 weeks at doses of 0, 0.48/0.57, 6.5/7.7 and 160/190 mg/kg bw in males/females for chronic toxicity and carcinogenicity. Survival was decreased in all dose groups in males but was higher than 50 % in females. Terminal body weight was statistically significantly decreased in males at the highest dose level (14.4 %). An increase in the incidence of hepatocellular adenomas was observed in treated females at the mid dose outside the HCD. In the absence of dose-response, this neoplastic finding was not considered as treatment-related by the DS. An increase in thyroid follicular adenomas was observed at the highest dose level in both sexes. The incidence was above HCD in females only. With regards to non-neoplastic findings, an increase in follicular cysts with hyperplastic epithelium was noted at the top dose in females. This increase was also noted in males without a concomitant increase in thyroid adenomas. Therefore, for this lesion, the DS considered that it cannot be concluded that the findings in female rats are a treatment related effect". In conclusion, the DS proposed no classification.

Comments received during public consultation

No specific comments were received.

Assessment and comparison with the classification criteria

The table below presents a summary of non-neoplastic and neoplastic findings in the carcinogenicity study in rats based on the CLH report and JMPR, 2014.

***Table**: Summary of relevant lesions in the carcinogenicity study in rats (CLH dossier and JMPR, 2014)*

Tumour type	Tumour incidence (%) Dose (mg/kg bw)									
	M					F				
	0	0.48	6.5	160	Historical Control data: range (mean)	0	0.57	7.7	190	Historical control
Liver										
Hepatocellular adenomas	3.1	1.6	1.6	0	0-6.7 (2.3)	0	3.1	6.3	1.6	0-3.9 (1.0)
Thyroid										
Follicular cysts + hyperplastic epithelium	1.6	7.9	11	7.8	na	0	0	1.6	7.8	na
Squamous cysts	7.8	13	6.3	7.8	na	0	1.6	3.2	7.8	na
Follicular cell adenomas	0	1.6	4.7	1.6	0-11.5 (2.4)	0	1.6	1.6	6.3	0-3.9 (0.8)
Follicular cell adenocarcinomas	0	1.6	0	1.6	0-2	7.8	6.3	3.1	9.3	na
Total thyroid tumours	3.1	3.2	4.7	3.1	na	7.8	7.8	4.7	16	na
Uterus										
Uterine adenocarcinomas	--	--	--	--	--	0	7.8	1.6	4.7	0-5.8

na: information not available to RAC

No evidence of carcinogenicity was reported in the mice. In rats, thyroid follicular adenomas were outside historical control values in females at top dose (6.3 % *vs* 0 % in current controls and 0-3.9 % in historical control data). In males, the incidence of this finding was inside the historical control range. Adenocarcinomas were also increased outside the historical control data in females in all groups including controls, without dose-response (JMPR, 2014). Non-neoplastic findings in thyroid consisted in females of a dose-related increase in incidence and severity of follicular cysts with hyperplastic thyroid epithelium. RAC notes that in the RAR, this non neoplastic finding was considered potentially related to tyrosinaemia. With regard to general toxicity at top dose in females, no effects on survival were noted. Transient effects on mean body weight and decreased food utilisation were noted in females. Non neoplastic findings at top dose in females also included hepatocellular effects, thyroid effects, effects in sciatic nerves, kidney effects and ocular toxicity. Overall, RAC considers thyroid follicular potentially treatment-related effect as thyroid was a target organ in females. Nevertheless, considering the slight, non statistically significant increase in benign thyroid adenomas in one sex only and in a single species, in absence of evidence of mutagenicity, the concern is insufficient to classify mesotrione as carcinogenic.

Other neoplastic findings (hepatocellular adenomas and uterine adenocarcinomas) in females were not considered treatment-related as the findings were not dose-related and outside historical control values only in the low or mid dose groups.

Overall, RAC supports the DS's view that **classification for carcinogenicity is not warranted.**

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

Fertility

The DS based the evaluation of fertility and sexual function on a three-generation study in rats (Anonymous, 1977p, OECD TG 416, GLP-compliant), a single generation exacerbation study in rats (Anonymous, 1997q + 2000b, non-guideline, GLP-compliant), and a two-generation

reproductive toxicity study in mice (Anonymous, 1997r, OECD TG 416, GLP-compliant). The DS did not propose classification for fertility as no treatment-related effects were observed.

Developmental toxicity

The potential for mesotrione to induce effects on development was investigated in three developmental toxicity studies conducted according to OECD TG 414 and GLP-compliant in three species (rats, rabbits and mice). Additionally, one non guideline range-finding study in mice and one non guideline mechanistic developmental study in rabbits were available.

In the prenatal developmental toxicity studies in rats, mice and rabbits, reduced/delayed ossifications were observed. As the effects were not consistent and often without clear dose-response relationship, these findings were not considered of sufficient concern by the DS for classification. In the multigenerational and single generation studies in rats, an increase in total litter loss and pre-/ or post-natal death were observed. The single generation study aimed to evaluate the role of tyrosine on reduced litter size, pup survival and bilateral hydronephrosis. The DS concluded that reduced survival was correlated with the tyrosinaemia in dams resulting from dosing with mesotrione. Although the DS acknowledged that humans are expected to be less sensitive to the effects of mesotrione than rats, the DS proposed classification as Repr. 2; H361d for developmental toxicity as information is lacking in humans regarding the relative potency for reproductive toxicity.

Comments received during public consultation

Three MSCAs agreed to the proposal for classification as Repr. 2; H361d. This was based on total litter loss and pre/postnatal death observed in multigeneration studies and delayed ossification observed in developmental toxicity studies in the absence of clear maternal toxicity and given however unclear relevance to humans.

Industry disagreed with the classification proposal for developmental toxicity. Industry provided a position paper with the following main arguments for no classification:

- Developmental effects observed in multigenerational studies in rats were attributable to severe tyrosinaemia resulting in HPPD inhibition. This has been shown by data from the exacerbation one generation study in rats (Summarised in the Table below). Supportive evidence of dose-response for tyrosinaemia was derived from increased incidence and severity of ocular opacity (marker of tyrosinaemia) for increased plasma tyrosine concentration.

***Table**: Rat mechanistic (exacerbation) one generation study; litter parameters and plasma concentrations in the dam*

Group number	**Treatment (mesotrione ppm / concentration of tyrosine %)**						
	1	2	3	4	5	6	7
Effects	0 / 0	0 / 0.5	0 / 1.0	0 / 2.0	2 500 / 0	2 500 / 0.5	2 500 / 1.0
Total litter loss/total number of litters	0/19	1/19	2/14	1/17	0/18	4/17*	8/18**
% pups born live	97.8	96.2	96.3	92.5	93.3	92.2	86.2**
% pups live day 22	93.1	87.8	83.2	85.9	85.5	59.3**	31.6**
Plasma tyrosine day 3	182	200	209	293**	2051**	2644**	2012**
Plasma tyrosine day 51	109	124	146*	146*	2029**	2578**	2470**
Ocular opacity (dams)	0	0	0	0	12	19	19

* p<0.05, ** p< 0.01. Group 8 (mesotrione + 2 % tyrosine) : animals terminated on day 8-11 due to adverse clinical signs and bw decrease

- Similar effects on pup survival and pre-/post-natal death were observed in rat multigeneration studies conducted with other HPPD inhibitors (e.g. tembotrione) confirmed the association between tyrosinaemia and these effects.

- No effects on offspring survival and reproductive parameters were observed in the multigeneration study in mice which is considered the most relevant species for human health risk assessment of mesotrione as plasma tyrosine levels in humans would be equivalent to those in mice. Therefore, as no adverse effects on pups' survival were seen in mouse multigeneration study, no classification is warranted. Industry also noted that no effects were observed in mice, treated with topramezone (another HPPD inhibitor).

- Increased bilateral hydronephrosis in F1 and subsequent generations in rats was caused by tyrosinaemia and therefore not relevant to human. Similar kidney effects in F1 and subsequent generations were reported with different HPPD inhibitors (e.g. sulcotrione, isoxaflutole). Industry considers that, there may be a hereditary predisposition to the formation of hydronephrosis in neonatal/juvenile rats. Maternal dosing of mesotrione, with subsequent elevations of plasma/milk tyrosine concentrations, and the observation of an increased incidence/severity of hydronephrosis within offsprings, is consistent with this observation. They provided two hypotheses:
    a. increased amounts of proteins are excreted within the urine of neonates as a result of ineffective proximal tubular protein reabsorption;
    b. high levels of tyrosine in the urine of neonates could precipitate out of the urine causing a physical block in the urinary tract resulting in dilatation of the kidney pelvis.

Assessment and comparison with the classification criteria

Fertility

Effects in the multigenerational studies that could be possibly linked to fertility consisted of weight changes of the testis and epididymis in rats and mice and reduced successful mating in mice.

Effects on testis and in epididymis absolute weights were observed in both multigeneration studies in rats and mice. Effects were often not consistent between generations and without dose-response relationship. Moreover, as the effects were not correlated with histopathological findings or fertility effects, these changes are not considered of sufficient concern for classification. No effects were observed in the reproductive organs in other repeated-dose toxicity studies.

In the 2-generation study in mice, reduced successful mating was observed. Proportion of successful F0 mating was slightly lower at 350, 1 500 and 7 000 ppm; however values were similar to F1 controls. The proportion of successful F1 matings was slightly (but not statistically significantly) lower at 7 000 ppm only. In view of the absence of other findings on reproductive parameters in the generational studies in rats and mice, RAC supports the DS conclusion that **mesotrione does not need to be classified for effects on fertility and sexual development.**

Developmental toxicity

For the developmental endpoint, three developmental toxicity studies were available (one in rats, one in rabbits and one in mice), one range finding study in mice and one mechanistic developmental study in rabbits were available.

In rats (Anonymous, 1997s), dose-related decreases in body weight gain and food consumption were observed in dams at all doses tested (100, 300, 1 000 mg/kg). A decrease in skeletal ossification and an increase in pes and manus scores/litter was noted in all treated groups. A statistically significant decrease in mean foetal weight was also observed at 1 000 mg/kg (6 %).

In mice (Anonymous, 1999a), no maternal toxicity was noted up to the highest dose tested (600 mg/kg bw) in both the main and range-finding studies. In the range-finding study, delayed ossifications were reported at ≥ 300 mg/kg without a dose-response. In the main study, delayed ossifications were significantly increased at the top dose only without a clear dose-response. As mice developmental toxicity studies were not routinely performed in the laboratory, two controls were included in the main study. Some of the observed skeletal variations in the main study were above these two concurrent controls and the control of the range-finding study, suggesting a treatment-related effect. RAC also noted that reduced ossifications were also consistently observed in rat and rabbit studies.

In rabbits (Anonymous, 1997t), delayed ossifications were observed in all treated groups (0, 100, 250, 500 mg/kg). The incidence of extra normal length 13th ribs and 27 pre-sacral vertebrae were clearly in excess of the historical control range at ≥ 100 mg/kg. More importantly, slight increases in abortions were noted at 250 and 500 mg/kg (0 in control, 1 dam at 100 mg/kg and 2 dams at 250 and 500 mg/kg). Maternal toxicity consisted in a transient decrease in body weight at 500 mg/kg.

A non-guideline mechanistic study (Anonymous, 2000c) investigated the role of tyrosinaemia in developmental effects observed in the above rabbit study. Female rabbits were gavaged with mesotrione at 500 mg/kg on GD8-20. Animals were administered test material in conjunction with diet containing 0 or 1 % tyrosine. A further group was gavaged with water and administered 1% tyrosine in diet. Maternal toxicity was observed in the group treated with mesotrione and tyrosine (one dam killed, decreased weight loss). Plasma tyrosine level was significantly increased in all treated groups (mesotrione + tyrosine > mesotrione > tyrosine). Skeletal variants indicative of reduced/delayed ossification were increased in all treated groups. Severe blood vessels anomalies were noted in all treated groups (mesotrione + tyrosine > mesotrione > tyrosine). These results suggested that delayed ossification could be caused by tyrosinaemia. RAC notes that the effects were associated with plasma tyrosine concentration around 500 µM.

In the rat 3-generation study (Anonymous, 1997p), litter size was consistently reduced in all generations with the greatest effect in F3 animals. Pup survival was also significantly decreased at top dose in the three generations. Litter size was reduced at the top dose in all generations (see table below). No effects on pup body weights were noted.

***Table**: Litter size and pup survival in the developmental parameters in the rat 3-generation study (ref)*

Parameter	Generation	Dose Level (ppm)				
		0	2.5	10	100	2 500
Litter size (no. pups)	F1	11.7	12.4	10.9	10.3	9.2**
	F2	11.8	9.8	9.5	10.0	7.8**
	F3CT	10.6	10.8	8.4	8.5	5.5**
	F3R	11.7	10.5	9.6	9.2*	8.2*
Pup survival (%)	F1	92	90	85**	90	78**
	F2	84	89	80	76	48**
	F3CT	90	93	80	85	58*
	F3R	93	89	87	82	94

* significantly different to control ($p < 0.05$), ** ($p < 0.01$); CT: continuous treatment;
R: recovery
F3CT: animals continued with the same treatment
F3R: recovery animals

Other developmental effects in offspring consisted of eye lesions (corneal opacity) from 100 ppm in F1 males and F1 and F2 females and from 10 ppm in F2 males. Cataracts were also noted in

both sexes at the top dose only in F1 and F2. A dose-related increase in the incidence of bilateral hydronephrosis was seen at ≥ 100 ppm in all generations in both sexes and at all dose tested in F1 males (Table below). Severity of the effect was increased in F3 continuous treatment pups (F3CT). Recovery groups (F3R) showed evidence of reversibility of the observed effects. Hydronephrosis was associated with increases in kidney relative weights in F2 males and females at the top dose level.

Table: *% incidence of bilateral hydronephrosis in the rat 3-generation study (Anonymous, 1997p)*

		Dose Level of mesotrione (ppm) Incidence of bilateral hydronephrosis (%)									
		Males					Females				
	generation	0	2.5	10	100	2 500	0	2.5	10	100	2 500
Adults	F0	0	0	0	0	0	0	0	0	0	0
	F1	0	3.8	**38**	**52**	**80**	17	3.8	15	**58**	**50**
	F2 CT	0	0	17	**50**	**67**	0	0	0	17	8.3
	F2 R	7.7	14	7.7	36	36	0	7.1	0	21	**36**
Offspring	F1	5	7	15	**39**	**30**	4	2	17	**40**	**19**
	F2	11	11	21	**40**	**77**	11	14	14	**30**	**100**
	F3 CT	9	18	10	**58**	**63**	3	10	9	**44**	**90**
	F3 R	7	0	0	5	0	4	5	0	8	2

In bold: statistically significant; CT: continuous treatment; R: recovery.

In parental animals, no effects on mortality, body weight, body weight gain, and food efficiency were reported. Parental toxicity consisted of ocular toxicity in all parental generations from 100 ppm in F0 and 10 ppm in F1 (F2 were not examined histologically). Cataracts were also observed in F2 parental animals at the top dose. Evidence of recovery was observed for this effect. Effects in kidneys included increase in kidney relative weights from 10 ppm in males in all generations and at the top dose in females F0. Increase in bilateral hydronephrosis was observed at 10 ppm and above in F1 and F2 animals. Industry submitted a position paper during public consultation concluding that this effect was attributable to hypertyrosinaemia.

In the non-guideline single generation reproductive toxicity study in rats, one dose level (2 500 ppm) of mesotrione was tested with various concentrations of tyrosine (0, 0.5, 1 and 2 %). Group receiving mesotrione + tyrosine at 2 % was terminated at GD8-11 due to severe weight loss and clinical signs. Plasma tyrosine concentration was slightly increased in groups receiving tyrosine alone (0, 0.5, 1, 2 %) and markedly increased in groups receiving mesotrione + tyrosine. Ocular opacity was observed in all mesotrione treated groups. Pup survival was decreased and total litter loss was increased in all mesotrione + tyrosine groups. No effects on litter size were observed. These results suggest that the MoA for reduced pup survival is likely to be due to tyrosinaemia.

In the mice 2-generation study, pup body-weight was dose-dependently reduced in both sexes in both generations from day 8 throughout lactation (statistically significantly at all dose tested in F1A and at the highest doses of 1 500 ppm and 7 500 ppm in F2A). Developmental delay (preputial separation time) was observed in F1 and F2 (at mid and high dose in F1 and top dose in F2). Ocular opacity and cataracteous changes were observed in pups in both generations in all treated groups without clear dose-response but indicative of tyrosinaemia (ocular opacity). Plasma tyrosine levels were increased in both sexes in F1 parental and F2 pups at all doses tested. Tyrosinaemia was higher than 800 µM at 300 ppm and above. F2A pup survival was slightly but not statistically significantly reduced at the high dose tested (7 000 ppm). In parental animals, there were no effects on mortality. Decreased body weight was observed at high dose (7 000 ppm) in F0 females during gestation and in F0 and F1 females during lactation. This was

associated with reduced food consumption. No effect on body weight gain was reported in parental animals. Corneal opacity was increased in parental animals at the top dose only in F1. Cataracteous changes were observed at the top dose in both F1 and F0 animals.

Comparison with criteria

In prenatal developmental toxicity study, mesotrione consistently affects growth in rats, rabbits and mice. In rabbits and mice, growth effects occurred without maternal toxicity. Based on investigative studies in rabbits, the skeletal variations and severe blood vessel anomalies were likely to be associated with tyrosinaemia. Plasma tyrosine was around maximum 500 µM in this study.

In the multigeneration study in rats, decreased growth, prenatal survival and litter size was observed. Moreover, bilateral hydronephrosis was increased in pups and adults in F1 and subsequent generations. Decreased growth was observed in pups throughout lactation without effects on mortality, body weight gain or food efficiency in parental animals. Data available on tyrosineamia in pups and parental animals in the multigenerational studies showed no marked effects (rats, mice studies). Difference between NOAEL/LOAEL for developmental effects between rats and mice was lower than the difference in NOAEL/LOAEL observed in adult animals. This observation suggests that enzyme kinetics and susceptibility to HPPD inhibitor could differ in pups compare to adults. Indeed, according to EFSA conclusions (EFSA, 2016), offsprings NOAEL/LOAEL were set at 0.3/1 mg/kg for rats and 2/10 mg/kg in mice. In 90-day toxicity studies, the NOAEL/LOAEL of adult animals were 0.47/0.91 mg/kg in rats and 61.5/180 mg/kg in mice.

Overall, the growth and survival effects are not considered secondary to non-specific consequences of other toxic effects. The MoA (hypertyrosinaemia) proposed for foetal skeletal effects and postnatal survival has been shown to be relevant to human. Moreover, a direct effect of mesotrione cannot be excluded as some developmental effects were not investigated in exacerbation studies (e.g. kidney effects). As humans are less sensitive than rats, because of uncertainties on relative potency for developmental effects in human, RAC agrees with DS's proposal that **classification as Repr. 2; H361d is warranted.**

Effects on or via lactation

Although not discussed by the DS, the possibility that reduced pup survival (day 1-4) in the three-generation study in rats being a consequence of exposure via milk was raised by RAC. There is no data in the CLH dossier demonstrating a similar increase in plasma tyrosine level in dams and tyrosine level in milk. Moreover, as the effect occurred early in the post-natal period, RAC considered that the effects were likely the results of developmental toxicity i.e. pre-natal exposure to mesotrione. Thus, RAC considered no classification for adverse effects on or via lactation.

ENVIRONMENTAL HAZARD EVALUATION

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier Submitter's proposal

Mesotrione is a herbicide with an existing classification in Annex VI of the CLP Regulation as Aquatic Acute 1; H400 and Aquatic Chronic 1; H410. The DS proposed to confirm this classification and add M-factors for both categories. The proposal was based on the substance

being not rapidly degradable, not bioaccumulative and very toxic to aquatic organisms. The 7- and 14-day E_rC_{50} values for the macrophyte *Lemna gibba* were 0.028 and 0.0257 mg/L, respectively, which gave a short-term M-factor of 10 ($0.01 < LC_{50} \leq 0.1$ mg/L). The lowest chronic toxicity values are the 7-day NOE_rCs for frond number and dry weight of 0.002 mg/L and 14-day NOE_rC for dry weight of 0.002 mg/L. These NOECs are supported by the recalculated lowest 14-day dry weight E_rC_{10} value of 0.002 mg/L. The values are in the range $0.001 < NOEC \leq 0.01$ mg/L giving a long-term M-factor of 10 for a not rapidly degradable substance.

Degradation

There were two aqueous hydrolysis studies available showing that mesotrione is stable to hydrolysis. No major metabolites were detected. A GLP study performed according to the EPA Guideline (Pesticide Assessment Guidelines, Subdivision N, Series 161-1, 1989) was performed at pH 5, 7 and 9 at 25 °C. A non-GLP study according to OECD TG 111 at pH 4, 7 and 9 at 50 °C was performed additionally. A slight increase in degradation was observed at pH 4 and 9 against pH 7 after 5 days.

In an aqueous photolysis test performed according to the EPA guideline (Pesticide Assessment Guidelines, Subdivision N, Series 161-2, 1989) the half-life (DT_{50}) for mesotrione was from 81 to 88 days under summer conditions at circa 40 °N latitude. Using the conversion factor provided by the study author, the DT_{50} for mesotrione is from 89 to 97 days under summer conditions in Northern Europe. No major metabolites were found. In a study performed according to an OECD draft Guideline for Phototransformation of Chemicals in Water – Direct and Indirect Photolysis, a DT_{50} of 20 days in summer sunlight was estimated for latitudes 30, 40 and 50 °N. A large number of degradates were formed in low concentrations, the main products being MNBA and AMBA.

There were no ready biodegradability tests available for mesotrione.

In a simulation study on aerobic mineralisation in surface water (OECD TG 309, GLP), for the non-sterilized, viable test systems, the mean levels of parent compound decreased by between 80 and 84 % of applied radioactivity (AR) with resultant single first-order $DegT_{50}$[1] values ranging from 228 to 382 days. For the sterilised samples, mesotrione was found to be stable with 96 % remaining at 60 days after treatment (DAT). MNBA was the only metabolite found at concentrations ≥ 5 %, reaching a maximum level of 9.7 % at 60 DAT. AMBA and minor unknown metabolites were also detected. Ultimately, mesotrione was mineralised to carbon dioxide (< 1 % AR).

There were two aquatic water/sediment studies available for mesotrione. In the study performed according to EPA Guideline (Pesticide Assessment Guidelines, Subvision N, Series 162-4), total recovery from both systems was 88-109 % AR and more than 82 % of recovered mesotrione remained in the water phase at all sampling intervals. The maximum amount recovered from the sediment phase was 3.8 % AR. The only major metabolite was AMBA. In the other study performed according to the OECD TG 308 (GLP), the mean levels of parent compound decreased from 99 % AR at 0 DAT to < 1% AR at 102 DAT. AMBA was the major metabolite, reaching maximum levels of 16 % AR and 6 % AR in Calwich Abbey and Swiss Lake water, respectively. Metabolite SYN546974 was detected at up to 9 % AR at 29 DAT in the Swiss Lake water but remained at < 5 % AR in the Calwich Abbey water. A number of discrete metabolites, individually accounting for < 5 % in the total system, were also detected. Mean levels of parent compound in the sediment extracts increased to maximum values of 4 and 3 % AR at 1 DAT for the Calwich Abbey and Swiss Lake systems, respectively, before decreasing to 0 % AR at 4 DAT. AMBA was

[1] Degradation rate of the parent

the major metabolite. SYN546974 was detected at up to 6 % AR at 14 and 46 DAT in the Calwich Abbey sediment and up to 26 % AR at 102 DAT in the Swiss Lake sediment. All other metabolites accounted for < 5 % AR. Mineralisation (maximum 11 % AR) and degradation to bound residues (maximum 61 % AR) occurred under aerobic conditions. Mesotrione was not present in the sediment at significant levels.

In one additional study, a kinetic evaluation of the data from both water/sediment studies was done. The aerobic persistence endpoints were (as geomean values): DT_{50}[1] of 5.5 days (water) and 5.4 days (whole system); DT_{90} of 18.7 days (water) and 18.9 days (whole system).

The DS concluded that mesotrione was stable to hydrolysis and it did not readily biodegrade or ultimately degrade sufficiently rapidly to either CO_2 or to degradants which have been demonstrated not to fulfil the criteria for classification as hazardous in water/sediment systems. The substance was therefore considered 'not rapidly degradable' for hazard classification purposes.

Bioaccumulation

A number of log P_{OW} values were available for mesotrione. In unbuffered water, these ranged from 0.11 to 0.32 and all buffer values (pH 5-9) were negative values (≤ -1.0). No experimental bioconcentration data was available. The DS concluded that mesotrione was considered 'not bioaccumulative' for hazard classification purposes.

Aquatic toxicity

Table *Summary of relevant information on aquatic toxicity*

Test substance and guideline	Species, test conditions	Results
Fish		
Mesotrione Purity 95.1 % w/w EPA[1] ≈ OECD TG 203	*Oncorhynchus mykiss* Acute, static	96 hour LC_{50} > 120 mg/L[s] (nom) Nominal and mean measured concentrations: 0 and 120 mg a.s./L
Mesotrione Purity 95.1 % w/w EPA[1] ≈ OECD TG 203	*Lepomis macrochirus* Acute, static	96 hour LC_{50} > 120 mg/L[s] (nom) Nominal concentrations: 0 and 120 mg a.s./L; mean measured 108 % of the nominal
Mesotrione Purity 97.6 % w/w USEPA Guideline 72-4; GLP	*Pimephales promelas* Chronic, flow through	36 day NOEC: 12.5 mg/L (nom) physical symptoms* 36 day EC_{10}: ND 36 day EC_{20}: ND Nominal concentrations of 0, 12.5, 25, 50, 100 and 200 mg a.s./L; mean measured concentrations 88-98 % of the nominal.
Aquatic invertebrates		
Mesotrione Purity 96.8% w/w OECD TG 202 Part I	*Daphnia magna* Acute, static	48 hour EC_{50} > 622 mg/L (measured)** Nominal concentrations: 0, 130, 216, 360, 600 and 1 000 mg a.s./L; measured concentrations 104-107 % or the nominal

Mesotrione purity 96.8% w/w ASTM[2] ; OECD TG 202; GLP	*Daphnia magna* Chronic, semi-static	21 day NOEC 180 mg/L (measured) reproduction & length EC_{10}: ND EC_{20}: ND Nominal test concentrations 0, 100, 180, 320, 560 and 1 000 mg a.s./L; measured concentrations 37-100 % of the nominal
Algae		
Mesotrione Purity 95.1% w/w EPA[3] ≈ OECD TG 201	*Pseudokirchneriella subcapitata* Chronic, static	72 hour E_rC_{50}: 13 mg/L (nom) 72 hour $NOE_{r,b}C$: 0.75 mg/L (nom) 72 hour E_rC_{10}: 0.926 mg/L (nom) Nominal concentrations: 0, 0.38, 0.75, 1.5, 3.0, 6.0, 12, 24 and 49 mg a.s./L; measured concentrations within ± 20 % of the nominal
Mesotrione Purity 97.6% w/w EPA[3] ≈ OECD TG 201	*Navicula pelliculosa* Chronic, static	72 hour E_rC_{50}: 66 mg/L (nom) 72 hour $NOE_{r,b}C$: 48 mg/L (nom) 72 hour E_rC_{10}: 51.0 mg/L (nom) Nominal concentrations: 0, 0.75, 1.5, 3.0, 6.0, 12, 24, 48 and 96 mg a.s./L; measured concentrations within ± 20 % of the nominal
Aquatic macrophytes		
Mesotrione Purity 97.6% w/w EPA[3]	*Lemna gibba* 14 days, chronic, semi-static	E_rC_{50} (growth rate, frond no.): 0.0599 mg/L (nom) **E_rC_{50} (growth rate, dry weight): 0.0257 mg/L** (nom) NOEC (for frond no.): 0.008 mg/L (nom) NOEC (for dry weight): 0.002 mg/L (nom) E_rC_{10} (growth rate, frond no.): 0.0068 mg/L (nom) **E_rC_{10} (growth rate, dry weight): 0.002 mg/L** (nom) Nominal concentrations: 0, 0.50, 1.0, 2.0, 4.0, 8.0, 16, 32 and 64 µg a.s./L; measured concentrations within ± 20 % of the nominal
Mesotrione Purity 86.1 % w/w OECD TG 221 and US EPA/OPPTS 850.4400.	*Lemna gibba* 7 days, chronic, semi-static	**E_rC_{50} (growth rate, frond no.): 0.028 mg/L (nom)** **E_rC_{50} (growth rate, dry weight): 0.028 mg/L (nom)** **NOE_rC (growth rate, frond no.): 0.002 mg/L (nom)** **NOE_rC (growth rate, dry weight): 0.002 mg/L (nom)** Nominal concentrations: 0, 2.0, 4.0, 8.0, 16, 32 and 64 µg a.s./L; measured concentrations within ± 20 % of the nominal (one conc. at day 7: 122 %)

[1] EPA Pesticide Assessment Guideline Subdivision E, Section 72-1
[2] ASTM E1193-87 Standards Guide
[3] EPA Pesticide Assessment Guideline Subdivision J, Section 123-2
[s] single test concentration and a dilution control were used
[*] Physical symptoms were loss of balance, less activity, spinal deformity, skin lesions and internal bleeding
[**]All concentrations used in the test on *Daphnia magna*, apart from the lowest, were above the water solubility. For determination of EC_{50} see below

ND = Not Determined

Acute toxicity

There were two acute toxicity studies on fish available (similar to OECD TG 203). The tests followed the same guideline and were performed as limit tests. The fish species were rainbow trout (*Oncorhynchus mykiss*) and bluegill (*Lepomis macrochirus*). There were no mortalities or symptoms of toxicity at 120 mg/L in either of the tests. The nominal LC_{50} in both tests was > 120 mg/L.

In the one available *Daphnia* study (OECD TG 202), the test concentrations were 130, 216, 360, 600 and 1 000 mg/L. All concentrations used in the test, apart from the lowest, were above the water solubility. The measured concentrations were 104-107 % of the nominal concentrations. There was cloudiness due to precipitation at the highest concentration. The EC_{50} was stated to be 900 mg/L (95 % confidence interval 622-1 042 mg/L as mean measured), however, the DS considered it more appropriate to base the EC_{50} on the next lowest concentration were concentrations were still maintained. Mortality was 93 % at 1 000 mg/L and 0 % at 600 mg/L. The EC_{50} was > 622 mg/L based on mean measured concentrations.

There were two algae studies available. The test with *Pseudokirchneriella subcapitata* (formerly *Selenastrum capricornutum*) was performed according to EPA Pesticide Assessment Guideline Subvision J, Section 123-2. The nominal concentrations applied were 0.38, 0.75, 1.5, 3.0, 6.0, 12, 24 and 48 mg/L, and the measured concentrations were within ± 20 % of the nominal. The nominal 72-hour E_bC_{50} and E_rC_{50} values for mesotrione were 4.5 and 13 mg a.s./L, respectively. The same test guideline was used to study *Navicula pelliculosa*. The nominal concentrations were 0.75, 1.5, 3.0, 6.0, 12, 24, 48 and 96 mg/L, and the measured concentration were within ± 20 % of the nominal. The nominal 72-hour E_bC_{50} and E_rC_{50} values for mesotrione were 68 and 66 mg/L, respectively.

The effects of mesotrione on *Lemna gibba* were investigated according to EPA Pesticide Assessment Guideline Subdivision J, Section 123-2. The nominal concentrations employed were 0.50, 1.0, 2.0, 4.0, 8.0, 16, 32 and 64 µg/L. Actual concentrations were determined by chemical analysis on days 0, 5, 9 and 14. Although some individual samples at the lowest concentration were below the limit of detection, the mean measured concentrations ranged from 93-103 % of nominals. The *Lemna gibba* 14 days nominal EC_{50} values for frond growth (frond number) and dry weights for mesotrione were 0.022 and 0.0077 mg/L, respectively. Endpoints at 7 days were not determined, however, growth in controls appeared to continue satisfactorily up to 14 days. After a statistical reanalysis of the study, the 14-day nominal E_rC_{50} value for growth rate of 0.0257 mg a.s./L based on dry weight was seen as the most relevant recalculated endpoint for classification purposes. The applicant, however, suggested that the goodness of fit for the effects in this study was not satisfactory. The confidence limits were wide and effects did not follow a consistent concentration-dependant trend. A new study was performed.

The new study, following OECD TG 221 and US EPA/OPPTS 850.4400, was a 7-day semi-static growth inhibition test. The test solutions were renewed on days 3 and 5. The nominal concentrations employed were 2.0, 4.0, 8.0, 16, 32, and 64 µg/L. Actual concentrations of mesotrione were determined by chemical analysis on days 0 and 7. At the start of the test, the concentrations of test item were in the range 93-107 % of the nominal values and at the end of the test they were 87-122 %. The 7-day E_rC_{50}s (growth rate) for both frond number and dry weight were 0.028 mg/L based on nominal concentrations.

Chronic toxicity

There were chronic data available on fish, *Daphnia*, algae and *Lemna*.

In a 36 day fish early life stage flow-through test performed in accordance with USEPA Guideline 72-4 and GLP, fathead minnow (*Pimephales promelas*) hatchability, survival, growth and the appearance of physical symptoms in fish in early life stage were evaluated. The nominal concentrations were 12.5, 25, 50, 100 and 200 mg/L. Exposure levels were monitored for each test concentration and for the dilution water control on exposure days 0, 2, 3, 18, 24 and 31. Mean measured concentrations ranged from 88 to 98 % of the nominal values so results were based on nominal concentrations. The most sensitive indicator of toxicity was the appearance of physical symptoms. On the basis of symptoms seen at concentrations of 25 mg/L and higher, the NOEC for physical symptoms was set at 12.5 mg/L. No reliable EC_{10} or EC_{20} could be calculated from the measured parameters.

The chronic toxicity of mesotrione to *Daphnia magna* was assessed in a 21 day semi-static study performed according to ASTM E1193-87 and OECD TG 202. Effects on survival, reproduction and growth were evaluated. Test solutions were renewed three times a week. Concentrations of mesotrione were measured for each test solution and for the replaced one. Mean measured concentrations ranged from 97 to 100 % of nominal for test solutions at 100 and 180 mg/L. For test solutions at 320, 560 and 1 000 mg/L, concentrations measured at the beginning of the study were respectively 94, 60 and 37 % of nominal so results were expressed as measured concentrations; the LC_{50} was 230 mg/L. At concentrations up to 180 mg/L reproduction was not significantly reduced and four broods were completed, thus the NOECs for reproduction and growth (length) were set at 180 mg/L. No NOEC was proposed for dry weight, due to the variability in dry weight observed and to the overall reliability of this endpoint. Although several concentrations tested exceeded the water solubility and the pH was lower than recommended in the higher concentrations, the study was considered sufficiently reliable as effects and the NOEC occurred at concentrations where no problems with solubility and pH were noted. In the statistical reanalysis of the study (Liedtke, 2013a), the EC_{10} and EC_{20} could not be determined.

The two algae tests were described in relation to short-term toxicity. In the *Pseudokirchneriella subcapitata* study the 72-hour NOEC was 0.75 mg/L both for biomass and growth rate. The calculated 72-hour EC_{10} and EC_{20} values based on average specific growth rate were 0.926 and 1.66 mg/L respectively. The 72-hour NOEC in the *Navicula pelliculosa* study for both biomass and growth rate were 48 mg/L. In the statistical reanalysis the lowest calculated 72-hours EC_{10} and EC_{20} values were 51.0 and 53.2 mg/L, respectively.

The two *Lemna* studies were described in relation to short-term toxicity. The 14-day NOEC for frond growth was 0.008 mg/L and for dry weight 0.002 mg/L. The recalculated E_rC_{50} was 0.002 mg/L based on dry weight. As the applicant suggested that the goodness of fit for the effects in this study was not satisfactory a new 7-day *Lemna* study was submitted. The DS did not consider the 14-day study to be wholly unreliable, but thought that it was not of comparable quality to the other studies included. In the 7-day study, the NOEC for growth rate was determined to be 2.0 µg/L both for frond number and dry weight.

Comments received during public consultation

Three MSCAs supported the proposal. One MSCA suggested possible errors in toxicity values presented in the aquatic toxicity table. According to the DS, these may be typos or due to a different way of expressing the endpoints. However, they did not go back to the actual study reports to verify it since these values have no effect on the classification.

Assessment and comparison with the classification criteria

Mesotrione was stable to hydrolysis. The results of a simulation test and two water/sediment tests showed that mesotrione is not readily biodegrade and does not ultimately degrade

sufficiently rapidly to either CO_2 or to non-hazardous degradants in water/sediment systems. For classification purposes, mesotrione is considered not rapidly degradable.

Mesotrione has a low potential to bioaccumulate. There was no experimental BCF for fish available. The log P_{OW} values ranged from 0.11 to 0.32 in unbuffered water, and all buffer values were ≤ -1.0. These values are all below the CLP cut-off value of 4.

There were acute data available on fish, invertebrates, algae and *Lemna*. The lowest values were from two different *Lemna gibba* tests: 14-day E_rC_{50} (dry weight) of 0.0257 mg/L and a 7-day E_rC_{50} (dry weight, frond number) of 0.028 mg/L. RAC supports the use of the 7-day value as the basis for classification. The value of 0.028 mg/L fulfils the criteria for Aquatic Acute 1, i.e. < 1 mg/L. The value is in the range of $0.01 < L(E)C_{50} \leq 0.1$, thus giving an **M-factor of 10**.

There were chronic data on fish, invertebrates, algae and *Lemna*. The lowest values were from two different *Lemna gibba* tests: 14-day E_rC_{10} (dry weight) of 0.002 mg/L and a 7-day NOE_rC (dry weight, frond no.) of 0.002 mg/L. The value of 0.002 mg/L fulfils the criteria for Aquatic Chronic 1, i.e. ≤ 0.1 mg/L for a non-rapidly degradable substance. The value is in the range $0.001 < NOEC/EC_{10} \leq 0.01$, thus giving an **M-factor of 10**.

Overall, RAC agrees with the DS's proposal to confirm the current classification as **Aquatic Acute 1** and **Aquatic Chronic 1**, and to add an **M-factor of 10 for both acute and chronic classifications**.

Additional references

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ANNEXES:

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

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