Committee for Risk Assessment

RAC

Opinion

proposing harmonised classification and labelling
at EU level of

metribuzin (ISO); 4-amino-6-tert-butyl-3-methylthio-1,2,4-triazin-5(4*H*)-one; 4-amino-4,5-dihydro-6-(1,1-dimethylethyl)-3-methylthio-1,2,4-triazin-5-one

EC Number: 244-209-7
CAS Number: 21087-64-9

CLH-O-0000007008-77-01/F

Adopted

10 June 2021

P.O. Box 400, FI-00121 Helsinki, Finland | Tel. +358 9 686180 | Fax +358 9 68618210 | echa.europa.eu

10 June 2021

CLH-O-0000007008-77-01/F

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

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

Chemical name: **metribuzin (ISO); 4-amino-6-tert-butyl-3-methylthio-1,2,4-triazin-5(4H)-one**

EC Number: **244-209-7**

CAS Number: **21087-64-9**

The proposal was submitted by **Estonia** and received by RAC on **2 July 2020.**

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

PROCESS FOR ADOPTION OF THE OPINION

Estonia 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 **3 August 2020**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **2 October 2020**.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Michal Martínek**

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

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 **10 June 2021** by **consensus.**

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

	Index No	Chemical name	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATE	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	606-034-00-8	metribuzin (ISO); 4-amino-6-tert-butyl-3-methylthio-1,2,4-triazin-5(4H)-one	244-209-7	21087-64-9	Acute Tox. 4* Aquatic Acute 1 Aquatic Chronic 1	H302 H400 H410	GHS07 GHS09 Wng	H302 H410		M=10	
Dossier submitters proposal	606-034-00-8	metribuzin (ISO); 4-amino-6-tert-butyl-3-methylthio-1,2,4-triazin-5(4*H*)-one; 4-amino-4,5-dihydro-6-(1,1-dimethylethyl)-3-methylthio-1,2,4-triazin-5-one	244-209-7	21087-64-9	**Retain** Aquatic Acute 1 Aquatic Chronic 1 **Add** STOT RE 2 **Modify** Acute Tox. 4	**Retain** H302 H400 H410 **Add** H373 (blood, thyroid)	**Retain** GHS07 GHS09 Wng **Add** GHS08	**Retain** H302 H410 **Add** H373 (blood, thyroid)		**Retain** M=10 **Add** oral: ATE=322 mg/kg bw M=100	
RAC opinion	606-034-00-8	metribuzin (ISO); 4-amino-6-tert-butyl-3-methylthio-1,2,4-triazin-5(4*H*)-one; 4-amino-4,5-dihydro-6-(1,1-dimethylethyl)-3-methylthio-1,2,4-triazin-5-one	244-209-7	21087-64-9	**Retain** Aquatic Acute 1 Aquatic Chronic 1 **Add** STOT RE 2 **Modify** Acute Tox. 4	**Retain** H302 H400 H410 **Add** H373 (blood system)	**Retain** GHS07 GHS09 Wng **Add** GHS08	**Retain** H302 H410 **Add** H373 (blood system)		**Retain** M=10 **Add** oral: ATE=320 mg/kg bw M=10	
Resulting Annex VI entry if agreed by COM	606-034-00-8	metribuzin (ISO); 4-amino-6-tert-butyl-3-methylthio-1,2,4-triazin-5(4*H*)-one; 4-amino-4,5-dihydro-6-(1,1-dimethylethyl)-3-methylthio-1,2,4-triazin-5-one	244-209-7	21087-64-9	Acute Tox. 4 STOT RE 2 Aquatic Acute 1 Aquatic Chronic 1	H302 H373 (blood system) H400 H410	GHS07 GHS08 GHS09 Wng	H302 H373 (blood system) H410		oral: ATE=320 mg/kg bw M=10 M=10	

GROUNDS FOR ADOPTION OF THE OPINION

HUMAN HEALTH HAZARD EVALUATION

RAC evaluation of acute toxicity

Summary of the Dossier Submitter's proposal

Acute oral toxicity

The DS proposed Acute Tox. 4; H302, ATE= 322 mg/kg bw based on an acute oral toxicity study in rats.

Acute dermal toxicity

The DS proposed no classification based on LD_{50} values of >2000 mg/kg bw in rat acute dermal toxicity studies.

Acute inhalation toxicity

The DS proposed no classification based on an LC_{50} of >2.0 mg/l in a rat acute inhalation toxicity study.

Comments received during consultation

An MSCA and an industry commenter supported the DS's proposal.

Assessment and comparison with the classification criteria

Acute oral toxicity

Metribuzin currently has a minimum classification as Acute Tox. 4*; H302. An overview of the available studies is provided in the following table.

Species; year; reference number; purity	**Strain; vehicle**	**LD_{50} (mg/kg bw)**
Rat; 1998 M-018181 95.3%, 94.2%	Wistar Aqueous CMC and Tween 80	m 510 f 322
Rat; 1993 M-513530 97.8%	Wistar Peanut oil	m+f 2162
Rat; 1974 M-019421 92.0%	Sprague-Dawley Ethanol – propylene glycol (20:80)	m 1090 f 1206
Mouse; 1993 M-513531 97.8%	Swiss albino Peanut oil	m+f 1215
Guinea pig; 1974 M-019421 92.0%	Sprague-Dawley Ethanol – propylene glycol (20:80)	m 245 f 274

m = males, f = females

The lowest available LD_{50} is 245 mg/kg bw from a test in Guinea pigs (1974). Although this pre-guideline study appears reliable, it used a mixture of ethanol and propylene glycol as a vehicle. The current OECD TGs (420, 423, 425) specify that aqueous solutions, suspensions or emulsions are recommended wherever possible, followed in order of preference by a solution (or suspension, emulsion) in oil and then possible in other vehicles. In addition, rat is the preferred species for acute oral toxicity classification according to the CLP regulation.

Therefore, RAC agrees with the DS to give preference for a rat study using a water-based vehicle (1998; M-018181). Males and females showed a similar sensitivity in this study (male mortality 0-0-0-4-6, female mortality 0-0-0-6-6 out of 6 animals per sex and group at 0, 100, 200, 500 and 1000 mg/kg bw). The LD_{50} values were reported separately for each sex, 510 mg/kg bw for males and 322 mg/kg bw for females, a combined value is not available. Both values correspond to Category 4 (300 mg/kg bw < ATE ≤ 2000 mg/kg bw). The female LD_{50} from this study will be taken as the ATE after rounding to two significant figures in line with the standard RAC practice.

In conclusion, RAC agrees with the DS's proposal of **Acute Tox. 4; H302** with an **ATE** of **320 mg/kg bw** (rounded value) based on an acute toxicity study in rats.

Acute dermal toxicity

Three studies are available, all without mortalities or clinical signs of toxicity at doses of 2000 mg/kg bw or higher. Two of them (rat studies from 1998 and 1993) are standard studies conducted in line with OECD TG 402, the remaining one (a rat and rabbit study from 1972) is a pre-guideline study with some deviations and less detailed reporting.

As all available LD_{50} values are >2000 mg/kg bw, RAC agrees with the DS's proposal of **no classification**.

Acute inhalation toxicity

Three acute inhalation toxicity studies in rats are available, all without mortalities.

The only fully OECD TG-compliant study is the study from 2001 (M-136509). The exposure mode was nose-only, the test concentration was 2.0 mg/l, MMAD 3.7 µm, GSD 1.7 µm. 2 mg/l was the target concentration. The test substance group animals showed transient clinical signs of toxicity such as piloerection, bradypnea and laboured breathing. These clinical signs disappeared until day two post-exposure.

The study from 1986 (M-018207) with the highest attainable concentration of 0.65 mg/l had a slightly higher MMAD (5.1 µm) than specified by the OECD TGs (1 to 4 µm). Salivation during exposure was the only substance-related clinical sign.

The third study from 1993 (M-513533) with the highest attainable concentration of 0.53 mg/l did not specify the particle size. The exposure was to a liquid aerosol unlike the other two studies conducted with dust. The metribuzin-exposed animals showed nasal discharge and salivation shortly after exposure.

As no mortality was observed at 2 mg/l in a reliable rat acute inhalation toxicity study, RAC agrees with the DS's proposal of **no classification**.

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

Summary of the Dossier Submitter's proposal

The DS has not identified any specific, non-lethal target toxicity in the acute toxicity studies nor in an acute neurotoxicity study. Accordingly, they proposed no classification.

Comments received during consultation

An industry commenter supported the DS's proposal.

Assessment and comparison with the classification criteria

The acute toxicity studies did not report specific clinical signs of toxicity relevant for a STOT SE classification at non-lethal doses.

An acute oral neurotoxicity study (1999; M-009782) in F344 rats reported eye ptosis, decreased activity and other clinical signs of toxicity on the day of exposure mostly at the top dose of 100 mg/kg bw. Special investigations revealed increased incidence of gait incoordination, decreased reactivity, and reduced body temperature. There was no mortality in this study, a range-finding study reported deaths at 500 mg/kg bw.

Hypoactivity and ptosis were observed from 25 mg/kg bw/d and ataxia from 70 mg/kg bw/d after a single dose in a rat PNDT study (1986; M-108676). No mortality occurred at the top dose of 200 mg/kg bw/d.

Although ataxia (or gait incoordination) in the latter two studies could possibly indicate neurotoxicity, it may also represent a manifestation of general toxicity. RAC is of the view that the evidence is not sufficient to conclude that the effects represent neurotoxicity or other specific target organ toxicity. Consequently, RAC agrees with the DS's proposal of **no classification**.

RAC evaluation of skin corrosion/irritation

Summary of the Dossier Submitter's proposal

The DS proposed no classification based on negative dermal irritation tests in rabbits. Human patch tests were used as supporting information.

Comments received during consultation

An industry commenter supported the DS's proposal.

Assessment and comparison with the classification criteria

No irritation was observed in a rabbit study from 1993 (M-513568). Very slight erythema not meeting the classification criteria was reported in a rabbit study from 1998 (M-018182). Both studies were conducted under GLP and in line with OECD TG 404.

Two studies in human volunteers are also available (M-019714; M-019744), neither of them showing a skin-irritating potential. These human studies are described in detail in the section on skin sensitisation.

As no effects meeting the classification criteria were observed in the available studies, RAC agrees with the DS's proposal of **no classification**.

RAC evaluation of serious eye damage/irritation

Summary of the Dossier Submitter's proposal

The DS proposed no classification based on three rabbit studies showing mild eye irritation below the threshold for classification.

Comments received during consultation

An industry commenter supported the DS's proposal.

Assessment and comparison with the classification criteria

The *in vivo* studies from 2004 (M-538015) and 1998 (M-018184) reported conjunctival redness. The criterion for classification for conjunctival redness is a score of ≥ 2 in at least 2 of 3 tested animals. The maximum individual score (mean of 24, 48 and 72 h) was 1.7 and 0.7 in the 2004 and 1998 study respectively, the irritation resolved by day 4. Both studies were conducted in line with OECD TG 405 and under GLP.

The third *in vivo* study, from 1993 (M-513571), reported conjunctival redness and chemosis, the effect was reversible within 3 days. Individual animal scores are not provided in the study report but from the sums it is clear that the individual scores for redness and chemosis did not exceed 1.7 and 1 respectively, which is below the classification criteria (≥ 2 for both effects).

In conclusion, all available studies reported mild, reversible eye irritation not meeting the classification criteria. Therefore, RAC agrees with the DS's proposal of **no classification**.

RAC evaluation of respiratory sensitisation

Summary of the Dossier Submitter's proposal

In the absence of human or animal evidence of respiratory sensitisation, the DS proposed no classification for this hazard.

Comments received during consultation

An industry commenter supported the DS's proposal.

Assessment and comparison with the classification criteria

No specific data on respiratory sensitisation are available for metribuzin. The substance is not a strong skin sensitiser, so the likelihood of a respiratory sensitisation potential is relatively low. However, no specific test protocols exist to demonstrate the absence of this hazard. As there is no information meeting the CLP criteria for respiratory sensitisation, RAC agrees with the DS that **classification is not warranted**.

RAC evaluation of skin sensitisation

Summary of the Dossier Submitter's proposal

The DS proposed no classification based on three negative animal tests and on studies in humans.

Comments received during consultation

An industry commenter supported the DS's proposal.

Assessment and comparison with the classification criteria

Animal data

The available animal studies are summarised in the following table.

Study type; year; reference number	Result	Deviations from OECD TG 406 (1992)
GPMT; 2002 M-066574	Negative No reaction in the test substance group nor in the control group	No irritation on topical induction, SLS pretreatment not mentioned in the study report
Buehler test; 1994 M-513573	Negative	10 animals in the test group
Buehler test; 1989 M-018244	Negative	12 animals in the test group Positive control not mentioned in the study report

In the Guinea Pig Maximisation Test (2002), the test substance group consisted of 20 animals, and 10 animals were used as negative controls. The intradermal induction was performed with a 5% solution in polyethylene glycol 400, clear signs of irritation were observed at the injection site. The 50% suspension in PEG 400 used for topical induction and challenge was not irritating. No mention of SLS pre-treatment prior to topical induction can be found in the study report. No skin reactions were observed in the test substance group nor the control group upon challenge. Reliability was periodically checked with alpha hexyl cinnamic aldehyde, the latest check (5 months before the current study) showed a positive response in 100% of the animals.

In the Buehler test from 1994, the test substance (saturated solution) group and the negative control group consisted of 10 animals each. Finely ground metribuzin was made into a slurry with saline before application. No erythema or oedema was observed in the test substance group nor in the control group upon challenge. All 5 animals of a concurrent positive control group (dinitrochlorobenzene) did show a skin reaction.

The Buehler test from 1989 employed 12 test substance group animals and 12 negative control animals. The test substance was formulated with Cremophor EL in physiological saline solution (2%) as a 50% suspension. No skin reactions were observed in the test substance group nor the control group upon challenge. Positive control is not mentioned in the study report.

Human data

Two human patch tests are available, both from 1975. Study M-019744 reported skin reactions, mostly mild rash, in 9 field and production workers after exposure to metribuzin. To further investigate these reactions, the 9 subjects with previous skin problems were challenged with

metribuzin in petrolatum at concentrations of 1% and 5%. One person from this group showed a mild skin reaction (mild itching and macules without vesicles) at 5%. In addition, another group of 11 subjects with a possible previous contact with metribuzin but without dermal problems were challenged in the same way. One individual from this second group also showed a mild reaction at 5%.

Study M-019714 included a human repeated insult patch test. Metribuzin (unspecified concentration) was applied on dorsal skin of 7 volunteers for 6 hours peer application, 9 induction applications over 4 ½ weeks, challenge 10 days after the last induction. No skin reactions were observed in this study, but the number of volunteers was rather low and the form of application may not have been optimal (no mention of dilution or moistening the substance).

Conclusion

All three animal studies are negative. Each of them has a certain deficiency, but collectively they are considered to provide conclusive evidence of lack of a skin sensitisation potential in animals.

As to human data, there is a patch test reporting 2 positive individuals out of 20 tested. This finding raises some concern. On the other hand, the CLP regulation states that classification based on human data is warranted if there is evidence that the substance can lead to sensitisation by skin contact in a substantial number of persons, and that the positive human data should normally come from more than one dermatology clinic. Neither of these conditions is fulfilled here. In addition, the skin reactions were not severe, being limited to a mild rash.

In conclusion, there are negative animal studies and a patch test in humans reporting 2 cases of a mild dermal allergic reaction to metribuzin. As the available data are not considered to meet the CLP criteria for classification, RAC agrees with the DS's proposal of **no classification**.

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

Summary of the Dossier Submitter's proposal

The DS proposed classification with STOT RE 2; H373 (blood, thyroid) based on anaemia in dogs and thyroid effects in rats and rabbits. Although liver enzyme induction was acknowledged as a possible and rodent-specific mode of action (MoA) behind the thyroid effects, the DS emphasized that other MoAs, relevant for humans, could not be excluded.

Comments received during consultation

One MSCA supported the DS's proposal, whereas an industry commenter and an individual proposed no classification.

For the thyroid, industry submitted several recent mechanistic studies (see 'additional key elements') that in their view confirm uridine 5'-diphospho-glucuronosyltransferase (UGT) induction/inhibition as the sole MoA behind both T4 reductions and increases and its non-relevance for humans. In addition, they expressed doubts about severity of some of the thyroid findings used by the DS in support of classification, such as moderate T4 increases without thyroid weight changes or histopathological findings. Finally, they pointed out that metribuzin did not cause thyroid tumours in long-term rodent studies, which in their view indicates low potency. Industry provided a publication by Bomann *et al.* (2021), containing an analysis of the available information on the thyroid MoA of metribuzin.

Regarding haematotoxicity in the 90-day dog dietary study (2002), industry viewed the haematological effects in the two prematurely sacrificed animals as an isolated finding possibly related to infection or inflammation. The haematotoxicity in the 90-day dog capsule study (1998) was regarded as transient and not sufficiently adverse to warrant classification. The commenting individual considered the 2-year dog dietary study (1974) to be compromised by high mortality and pointed out that the surviving animals showed normal haematological parameters at termination.

In the response to the comments the DS maintained their original position.

Assessment and comparison with the classification criteria

Thyroid

The following table presents an overview of thyroid-related findings in the rat repeated-dose studies. More details on the individual studies including data tables can be found under 'additional key elements' (the 4-week UGT study) or 'supplemental information' (the rest of *in vivo* studies).

Thyroid-related findings in the rat studies			
Type of study; year; reference number	**Dose (mg/kg bw/d)**	**Thyroid-related findings**	**Liver, pituitary**
Oral gavage			
28-day; 1995 M-018443	150	↑ thyroid wt (f); colloidal vacuolation and altered colloidal staining; ↓ T4 (by ca. 60%), ↑ TSH (ca. 6-fold)	↑ liver wt (rel. by 10%/20% m/f)
	30	None	
	5	↑ T4 (m)	
Peripubertal assay; 2011 M-421281	120	↑ thyroid wt (m 1.5-fold); follicular cell hypertrophy (m); ↓ T4 (by ca. 70%/40% m/f), ↑ TSH (m ca. 3-fold)	↑ liver wt (rel. by ca. 20%)
	60	↓ T4 (m by ca. 40%), ↑ TSH (m ca. 2-fold)	↑ liver wt (rel. by ca. 10%)
PNDT; 1986 M-018676	200	↑ thyroid wt (1.6-fold); ↓ T4 (by 85%)	
	70	↓ T4 (by ca. 50%)	
Dietary			
4-week mechanistic; 2018 M-617607	87/102 m/f	↑ thyroid wt (ca. 1.5-fold); follicular cell hypertrophy; ↓ T4 (by ca. 50%), ↑ T3 (by ca. 30%), ↑ TSH (ca. 3-fold)	↑ liver wt (rel. by ca. 15%); hepatocellular hypertrophy; ↑ T4-glucuronidation (1.9/2.6-fold m/f), ↑ UGT1A5/6 activity (1.8/3.0-fold m/f)
	16/19 m/f	↑ T4 (by ca. 40%)	↑ liver wt (m rel. by ca. 10%); ↑ T4-

			glucuronidation (m 1.7-fold)
	2/3 m/f	↑ T4 (by ca. 60%)	No effect on UGT-related parameters in the liver
2-year; 1993 M-017948	42/54 m/f	↑ thyroid wt (ca. 1.3/1.1-fold m/f); follicular cell hyperplasia (m); T4 ↑ or unchanged	↑ liver wt (rel. by ca. 15%)
	14/18 m/f	↑ thyroid wt (ca. 1.2-fold m,f); ↑ T4 (by ca. 40%), ↓ T3 (by ca. 15%)	
	1.3/1.6 m/f	↑ T4 (by ca. 40%), ↓ T3 (by ca. 15%)	
9-week (males only); 1982 M-018468	65	Altered colloidal staining; ↑ T4	
	22	Altered colloidal staining; ↑ T4	
	7.5	Altered colloidal staining; ↑ T4; ↑ iodine uptake by the thyroid	
	2.4	Altered colloidal staining; ↑ T4; ↑ iodine uptake by the thyroid	
90-day; 1969 M-018365	28/22 m/f	↑ thyroid wt (ca. 1.4/1.7-fold m/f); follicular cell hypertrophy (m)	↑ liver wt (rel. by ca. 15%); pituitary hypertrophy (m)
Inhalation			
3-week; 1981 M-018391	720 mg/m^3	↑ thyroid wt; T4 not affected	↑ liver wt (rel. by ca. 10%)
	219 mg/m^3	↑ T4 (by ca. 20%)	
	93/90 mg/m^3	↑ T4 (by ca. 50%)	
	31 mg/m^3	↑ T4 (m by 50%)	

m = males, f = females

The rat studies show a relatively consistent pattern: a T4 increase from doses as low as 1-2 mg/kg bw/d, changing to a T4 reduction with a compensatory TSH and thyroid weight increase around 50 mg/kg bw/d. Both dose ranges are below the guidance values for a STOT RE classification. T3 alterations were also detected in both dose ranges, but the trends are not consistent across studies.

The Guidance on the application of the CLP criteria (CLP guidance; version 5.0) lists several examples of MoAs not relevant for humans. One of them is hypothyroidism secondary to hepatic UGT induction which has been postulated for the metribuzin-related decrease of T4 in the rat. As to other species, a 3-week dermal study in rabbits (1989; M-018488) reported increased T4 levels at 200 and 1000 mg/kg bw/d without an effect on thyroid weight or histopathology. No effect on the thyroid or thyroid hormones was found in the dog (e.g. 90-day dietary study, 2002, M-038758). It should be borne in mind that subtle changes in clinical chemistry values or organ weights may be difficult to detect in dog studies due to the low number of animals used (in this case 4/sex/group).

The 4-week mechanistic UGT study in rats (2018) has indeed shown increased UGT expression and T4-glucuronidation activity at a dose causing hypothyroidism (87/102 mg/kg bw/d m/f). It should be noted that also in one 90-day study in dogs (M-038758), UDP-GT was dose related increased in females, suggesting it is not rodent specific. A (weak) indication on thyroid activity in the second 90-day study in dogs was an increased thyroid weight in female dogs at 50/30 mg/kg (having no information whether thyroid hormones were estimated in the second study). In conclusion, uncertainty remains whether the UGT-related T4 increase is a rat-specific effect.

Other possible mechanisms have been investigated *in vitro*. Metribuzin did not inhibit rat microsomal thyroid peroxidase (study 2020; M-680749), recombinant rat and human deiodinases 1-3 (study 2019; M-664441), nor sodium-iodide symporter (Wang *et al.*, 2018). A brief description of these studies can be found under 'additional key elements'.

A caveat is that these *in vitro* assays do not reflect metabolic transformation. The information available in the draft Renewal Assessment Report (dRAR;2018) does not point to a specific metabolite being responsible for the antithyroid activity of metribuzin. Desaminodiketometribuzin (DADK-metribuzin), a representative of a quantitatively important group of metribuzin metabolites, showed a considerably weaker antithyroid potency than metribuzin in a 4-week rat study (1996; M-018441; for details see the dRAR). A minor metabolite with a demethylated thio-group (butylthione-metribuzin), whose structure shows some similarity to the potent anti-thyroid drug propylthiouracil, was found only in very small quantities (ADME study in rats, 1987; M-022071; see the dRAR).

Another uncertainty is that thyroid hormone levels can also be affected by other MoAs, for some of which standardized test methods may not exist (cf. Detailed review paper on thyroid hormone disruption assays, OECD series on testing and assessment no. 57, 2006).

UGT induction is likely to play a major role in the hypothyroidism observed at higher doses in the rat studies, but other MoA cannot be ruled out. MoA of the T4 increase at lower doses has not been elucidated. RAC does not find convincing evidence that the observed T4 increase at lower doses would be related to UGT inhibition (for details see 'additional key elements'). A possible involvement of a potential second MoA also at higher doses can therefore neither be excluded. In summary, the available data cannot exclude human relevance of the metribuzin-induced hypothyroidism.

Further, RAC was not able to draw a conclusion on whether the moderate T4 increase at lower doses in the rat studies should be considered adverse, and thus relevant for classification. While RAC discussed the issue that standard regulatory studies may not be able to detect adverse effects of abnormal high or low T4 hormone levels (e.g. on the brain development of offspring, cardiovascular dysfunction), industry's representative added to the discussion that no treatment-related (overt) damage to the heart has been seen in studies on several species. Although no classification is proposed, RAC emphasised the remaining concern with regard to the uncertainty on the relevance of the thyroid effects to humans.

RAC concluded that the thyroid-related findings in the rat and rabbit studies with metribuzin are not sufficient for a STOT RE classification. However, RAC agreed to highlight the concern about the unresolved MoA and toxicological significance of the T4 increases at low doses, as well as the possible contribution of this potential second MoA (besides the MoA via UGT induction) to the hypothyroidism at higher doses in the rat studies.

Haematotoxicity

Haematological effects warranting consideration for classification were observed only in dogs. No remarkable haematotoxicity was seen in rats. Only a mild effect on haematological parameters was seen in a chronic mouse study at a dose above the GV (2-year study 1981, M-018690).

90-day dietary study in dogs (2002; M-038758)

Beagle dogs (4/sex/group) were administered metribuzin at dietary concentrations of 0, 75, 300 and 1200 ppm (equivalent to ca. 0, 2, 8.5 and 28 mg/kg bw/d). Haematology and clinical chemistry determinations were conducted on study days 24, 63 and 87. Group-wise body weight gain was not significantly affected but 2 top dose animals (1 male and 1 female) started losing weight around day 49 and had to be sacrificed on day 59 and 58 respectively. Abnormal findings in these two animals are summarised in the table below (% or -fold changes are approximate and take into account both the pre-treatment individual value and the control mean; reticulocyte count and Heinz bodies not determined on day 58).

90-day study in dogs (2002; M-038758): findings in the two prematurely sacrificed animals (out of 8) at 1200 ppm		
Parameter	**Male BK3001** (sacrificed on day 59)	**Female BK3103** (sacrificed on day 58)
Body weight	Lack of bw gain From day 49 bw loss	Lack of bw gain From day 49 bw loss
Clinical signs	Day 56: thin, pale, yellow gingivae	Day 56: thin, pale, yellow gingivae Day 58: unthrifty, emaciated
Haematology	Day 24: normal Day 58: ↓ Hb (by 70%), ↓ erythrocyte count (by 70%), ↑ red cell distribution width (2-fold), anisocytosis (moderate), macrocytosis and hypochromasia (mild)	Day 24: normal Day 58: ↓ Hb (by 88%), ↓ erythrocyte count (by 89%), ↓ leukocyte count (by 35%), ↓ platelet count (by 96%), ↑ red cell distribution width (2-fold), anisocytosis (moderate), macrocytosis (minimal)
Clinical chemistry	Day 24: ↑ AST (5-fold), ↑ ALT (11-fold), ↑ GGT (9-fold), ↑ ALP (7-fold), ↑ bilirubin (13-fold), ↑ bile acids (10-fold) Day 58: ↑ ALT (4-fold), ↑ ALP (2-fold), ↑ bilirubin (7-fold)	Day 24: ↑ AST (2-fold), ↑ ALT (2-fold) Day 58: ↑ ALT (7-fold), ↑ bilirubin (3-fold)
Histopathology	Bone marrow: erythroid hyperplasia (moderate) Liver: extramedullary haematopoiesis (severe), inflammation chronic active (slight) Spleen: extramedullary haematopoiesis (moderate), granulocytic hyperplasia (marked) Small intestine: inflammation chronic (slight) Parathyroid: hyperplasia (moderate)	Bone marrow: hypoplasia (slight) Liver: microgranuloma (slight) Spleen: pigmentation (moderate) Kidney: inflammation (moderate), haemorrhage (slight) Heart: degeneration (slight), haemorrhage (slight) Thymus: involution (severe) Parathyroid: lymphocytic infiltrate (slight)

Another top dose male (BK3003) showed the following abnormalities:

- Haematology day 87: ↓ Hb (by 35%), ↓ erythrocyte count (by 35%), reticulocyte count normal, no Heinz bodies

- Clinical chemistry day 24: ↑ ALT (8-fold), ↑ GGT (3-fold), ↑ ALP (3-fold), ↑ bile acids (3-fold); days 63 and 87: normal
- Histopathology: liver – Kupffer cell aggregates with pigment

The rest of the top dose animals (2 males and 3 females) did not show any changes in haematology, clinical chemistry or histopathology.

The haematology parameters in the affected animals reflect erythrocyte destruction with some degree of regenerative response. The most severely affected animal (female BK3103) had also extremely low platelet count, reduced leukocyte count and bone marrow hypoplasia, which suggests an effect on bone marrow. It is interesting in this context that *in vivo* genotoxicity studies in mice reported effects on bone marrow (M-513623; M-513626).

All three animals affected by haematotoxicity showed increased ALT (an indicator of liver toxicity) while no such changes were observed in the rest of the group. The two sacrificed animals had inflammatory changes in the liver. Thus, there might be a link between hepatotoxicity and haematotoxicity. However, the available information is not sufficient to resolve whether haematotoxicity is secondary to hepatotoxicity.

<u>90-day oral (capsule) study in dogs (1998; M-513582)</u>

Beagle dogs (4/sex/group) were administered metribuzin in capsules at levels of 0, 5, 15 and 50 mg/kg bw/d. The top dose had to be reduced to 30 mg/kg bw/d from day 35 due to severe toxicity, particularly in males, who were losing weight, showed clinical signs of toxicity (weakness, dullness, blood tinged faeces), and one of them died (on 33). Body weights of top dose animals were by ca. 20% lower than controls at the end of the study in both sexes.

Haematology and clinical chemistry parameters were determined on days 45 and 90. The top dose males showed a reduction in erythrocyte count and haemoglobin by ca. 25% on day 45, no effect was apparent on day 90. Females were affected to a lesser extent than males, haemoglobin reduction at the top dose was ca. 15% and the effect persisted until the end of the study. In contrast to the 90-day dietary study (2002), some reduction in these parameters (Hb, erythrocyte count) was seen in all individual top dose animals. Clinical chemistry parameters were unaltered except a slight increase in bilirubin in both sexes on day 90. No treatment-related histopathological findings were identified. Haematology and clinical chemistry results are not available for the animal that died on day 33, and histopathological examination of this animal was not possible due to autolysis.

<u>2-year dietary study in dogs (1974; M-018381)</u>

Beagle dogs (4/sex/group) were administered metribuzin at dietary concentrations of 0, 25, 100 and 1500 ppm (equivalent to ca. 0, 0.8, 3.5 and 55 mg/kg bw/d). 3 animals at the top dose died with severe pneumonia, 3 other animals were sacrificed in a cachectic and very poor condition. Haematological findings in the individual animals are presented in the table below.

2-year study in dogs (1974; M-018381): haematological parameters in individual animals at 1500 ppm			
Animal no., sex	**Death**	**Haematology, 8 weeks**	**Haematology, last before death**
8183 m	Found dead, 16 weeks, pneumonia	↓ Hb by 55%, ↓ RBC by 65%, ↑ Ret 3-fold, ↑ sedimentation rate	See 8 weeks
8184 m	Scheduled sacrifice	↑ Ret 4-fold, ↑ sedimentation rate	24 months: ↑ Ret 2-fold, ↑ sedimentation rate

8188 m	Found dead, 7 weeks, pneumonia	–	–
8190 m	Sacrificed moribund, 15 months	↓ Hb by 75%[#], ↑ sedimentation rate	12 months: ↓ Hb by 65%, ↓ RBC by 87%, ↑ Ret 2-fold, ↑ sedimentation rate
8170 f	Found dead, 12 weeks, pneumonia	↓ Hb by 80%, ↓ RBC by 80%, ↑ Ret 5-fold, ↑ sedimentation rate	See 8 weeks
8171 f	Sacrificed moribund, 15 months	Ret ↑ 2-fold, ↑ sedimentation rate	12 months: normal, ↑ sedimentation rate
8176 f	Scheduled sacrifice	Ret ↑ 4-fold	24 months: Ret ↑ 4-fold
8191 f	Sacrificed moribund, 8 months	↓ Hb by 40%, ↓ RBC by 40%, ↑ Ret 15-fold	6 months: ↓ Hb by 40%, ↓ RBC by 40%, ↑ Ret 5-fold, ↑ sedimentation rate

m = male, f = female

Hb = haemoglobin, RBC = erythrocyte count, Ret = reticulocytes (absolute count)

[#] RBC reported to be normal, but this appears incorrect given the 4-fold haematocrit reduction

The pattern of haematological parameters is consistent with erythrocyte destruction and a regenerative response. There was a remarkable interindividual variability in susceptibility to the haematological effects.

Classification for haematotoxicity

Severe anaemia characterized by a reduction in haemoglobin and erythrocyte count by ca. 40-80% was observed in individual animals in the two dog dietary studies (one 2 year study where the effects were seen already at first measurement (8 weeks) and one 90 day study) at dose levels of 28 or 55 mg/kg bw/d, which is below the GV for Category 2 (100 mg/kg bw/d for a 90-day study). Most of the severely anaemic animals were sacrificed in moribund condition. This pattern of effects in principle meets the criteria for classification for haemolytic anaemia specified in the CLP guidance (3.9.2.5.2.), namely haemoglobin reduction by ≥ 20% and premature deaths in anaemic animals.

RAC acknowledges that the results of the 2-year study (1974) have to be interpreted with care due to the occurrence of pneumonia. On the other hand, haematotoxicity was also observed in the single anaemic survivor (BK3003) in the 90-day study (2002), whose clinical or pathology data do not indicate infection or inflammation.

Less severe haematotoxicity (haemoglobin reduction by ca. 25%/15% m/f) and a more uniform distribution of haematotoxicity across the individual animals was observed in the third dog study (1998), where the substance was administered via capsules, at 50 mg/kg bw/d. This dose level was not tolerated, leading to body weight loss and mortality, and probably affected the well-being of the animals also via other mechanisms besides haematotoxicity. Still, the results of this study indicate that the substance does affect blood.

Overall, RAC agrees to the DS's proposal of classification in Category 2, mainly based on severe haematotoxicity and mortality in a few dogs in the 90-day dietary study (2002), with haematotoxicity in the other two dog studies providing additional support.

In the present case effects related to haematotoxicity were observed in the spleen (haematopoiesis, pigmentation), bone marrow (hyperplasia, hypoplasia) and the liver (haematopoiesis, pigmentation). Therefore, the default designation "blood system" applies.

Conclusion

RAC agrees with the DS's proposal of a Category 2 classification with blood system as a target organ based on haematotoxicity in dog studies. As to the thyroid, RAC concluded not to specify thyroid as the second target organ, mainly due to doubts how to use changes in thyroid hormone levels for classification purposes, in addition to the unclarity of the MoA leading to these changes. Nevertheless, a concern remains because the MoA and toxicological significance of the effects on thyroid hormones in animal studies have not been fully elucidated.

In conclusion, RAC proposes to classify metribuzin with **STOT RE 2; H373 (blood system)**.

RAC evaluation of germ cell mutagenicity

Summary of the Dossier Submitter's proposal

The genotoxicity potential of metribuzin has been investigated in a range of *in vitro* and *in vivo* assays. All *in vivo* studies and the majority of *in vitro* studies were negative, and the substance does not show chemical structure activity relationship to known germ cell mutagens. Therefore, the DS proposed no classification.

Comments received during consultation

An industry commenter supported the DS's proposal.

Assessment and comparison with the classification criteria

In vitro assays

A battery of 3 recent, GLP- and OECD TG-compliant *in vitro* tests is available: an Ames test (2017; M-589742), a HPRT assay (2017; M-601206) and a micronucleus test (2017; M-604618). All three tests gave negative results and are considered acceptable. For details see the CLH report and the dRAR.

There are also a number of older *in vitro* studies. These were negative except two *in vitro* chromosome aberration assays (1989; M-018082 and 1990; M-018202), which were positive in the presence of metabolic activation and of some, but not excessive, cytotoxicity.

In vivo assays

The *in vivo* clastogenic potential of metribuzin in somatic cells was investigated in three oral bone marrow assays, summarized in the following table.

***In vivo* studies in somatic cells**			
Study type; year; reference number	**Method**	**Result**	**Remarks**
Micronucleus, mouse, bone marrow; 1982 M-018461	Strain: NMRI 2 oral (gavage) applications 24 h apart 200 or 400 mg/kg bw Vehicle aqueous Tylose (hydroxyethyl methyl cellulose)	Negative	No general toxicity No effect on PCE/NCE (polychromatic erythrocytes to normochromatic erythrocytes) ratio

	5/sex/group Sacrifice 6 h after the second application		OECD TG 474 (2016): sacrifice 18-24 h after the second application
Chromosomal aberration, mouse, bone marrow; 1994 M-513623	Strain: Swiss albino 2 oral (gavage) applications on two consecutive days 30, 100 or 300 mg/kg bw Vehicle peanut oil 5/sex/group Sacrifice 24 h after the second application	Negative	No general toxicity Mortality 1/10 at 400 mg/kg bw in a range-finding experiment Only the top dose evaluated A non-significant reduction in mitotic index in both sexes at the top dose
Micronucleus, mouse, bone marrow; 1997 M-513626	Strain: Swiss albino 2 oral (gavage) applications on two consecutive days 30, 100 or 300 mg/kg bw Vehicle peanut oil 5/sex/group Sacrifice 24 h after the second application	Negative	No general toxicity Mortality 1/10 at 400 mg/kg bw in a range-finding experiment Only the top dose evaluated Reduced PCE/NCE ratio in females

Bone marrow exposure in studies (1994; M-513623) and (1997; M-513626) is indicated by the effect on mitotic index and PCE/NCE ratio respectively. Bone marrow exposure under the conditions of the (1982; M-018461) study has been confirmed in a separate toxicokinetic study (2017; M-596009). One male NMRI mouse was administered radiolabelled metribuzin via gavage at a single dose of 400 mg/kg bw. The vehicle was 0.5% Tylose (hydroxyethyl methyl cellulose) in water containing 0.2% Tween 80 and 5% ethanol. Clinical signs of toxicity included semi-closed eyes, slow movements and ataxia. The animal was sacrificed 4 hours after administration. Mean concentrations in blood and bone marrow were 177 and 32 µg equivalents respectively.

Besides the bone marrow assays, several pre-guideline studies investigating genotoxic effects in germ cells are available. A spermatogonial chromosomal aberration test in Chinese hamsters (1974; M-018340) was negative. In addition, several dominant lethal assays are mentioned in the CLH report. These were negative as well but had significant methodological limitations.

Summary and conclusion

Reliable *in vitro* studies investigating the potential of the substance to induce point mutations were negative, whereas two older *in vitro* studies raised some concern about clastogenicity. However, the concern about this positive *in vitro* signal has been sufficiently addressed by other negative *in vitro* and *in vivo* studies investigating this endpoint (i.e. clastogenicity). Therefore, RAC agrees with the DS's proposal of **no classification** based on conclusive information.

RAC evaluation of carcinogenicity

Summary of the Dossier Submitter's proposal

The available animal studies did not show any evidence of neoplastic potential, and there was no convincing human evidence of metribuzin-related carcinogenicity. Therefore, the DS proposed no classification.

Comments received during consultation

An industry commenter supported the DS's proposal.

Assessment and comparison with the classification criteria

Animal data

One rat study and one mouse study are presented below. Three other studies are available (see the CLH report), all negative regarding carcinogenicity, but they used relatively low top doses, and therefore may not have fully explored the carcinogenic potential of the substance.

2-year dietary study in rats (1993; M-017948)

Fischer 344 rats (50/sex/group) were administered metribuzin at dietary levels of 0, 30, 300 or 900 ppm (top dose equivalent to 42/54 mg/kg bw/d m/f) for up to 2 years. An additional cohort of 10-20 animals per sex and group (20/sex/group at 0 and 900 ppm, 10/sex/group at 30 and 300 ppm) were sacrificed after 1 year of exposure. The top dose selection was based mainly on the body weight and thyroid effects at 1500 ppm in the 90-day study (1969; M-018365).

Terminal body weights at the top dose were reduced by 7%/13% (m/f), grand mean body weight gains were reduced by 14%/27% (m/f). There were no clinical signs of toxicity, survival was not affected. Absolute thyroid weights after 2 years were increased by 24%/14% (m/f), the majority of males (38 out of 50) showed follicular cell hyperplasia of minimal ('trace') severity. There were no other treatment-related histopathological findings. The study did not show evidence of a neoplastic potential.

2-year dietary study in mice (1981; M-018690)

CD1 mice (50/sex/group) were administered metribuzin at dietary levels of 0, 200, 800 or 3200 ppm (top dose equivalent to 438/567 mg/kg bw/d m/f). The rationale for dose selection is not provided in the study report.

Survival was not affected by treatment. No clinical signs of toxicity were observed and there was no significant effect on food consumption and body weight. Erythrocyte count and haemoglobin were slightly reduced at the top dose towards the end of the study. An increase in liver weight by ca. 50% was observed in top dose females (only a slight, non-significant increase was present in males), female kidney weight was increased by ca. 10%. No treatment-related neoplastic or non-neoplastic effects have been identified upon histopathological examination.

Human data

The DS summarized several epidemiology studies investigating associations between pesticide exposure and various types of cancer.

The case-control study by Hoar *et al.* (1986) reported a correlation between the use of triazines (including metribuzin) and the risk of non-Hodgkin's lymphoma based on 14 cases and 43

controls. However, when the use of phenoxyacetic acid herbicides was also controlled for, the risk was no longer significant.

The case-control study by Lee *et al.* (2005) found a positive association between the risk of glioma and exposure to several pesticides including metribuzin. However, the confidence in this finding is limited by small the small number of cases (9) and co-exposure to other pesticides.

No statistically significant associations between the use of metribuzin and cancer were observed in the rest of the studies presented in the CLH report, including a large prospective cohort study (DeLancey *et al.*, 2009).

In conclusion, the epidemiology studies do not provide convincing evidence of an association between metribuzin exposure and an increased risk of cancer.

Conclusion

No neoplastic findings were observed in the available rat and mouse carcinogenicity studies. The epidemiological studies do not provide convincing evidence of an association between metribuzin exposure and a risk of cancer. Therefore, RAC agrees with the DS's proposal of **no classification**.

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

Fertility

No effects related to sexual function and fertility were observed in the available studies (generational studies, studies on endocrine disruption, repeated dose studies). Accordingly, the DS proposed no classification for fertility.

Development

As the reliable studies showed no evidence of developmental toxicity besides foetal (or pup) weight reductions and delayed ossification at maternally toxic doses, the DS proposed no classification for development.

Lactation

In the absence of clear treatment-related pup toxicity in the available generational studies, the DS proposed no classification for effects on or via lactation.

Comments received during consultation

An industry commenter supported the DS's proposal.

Assessment and comparison with the classification criteria

Fertility

Two acceptable generational studies in rats are available, both conducted in line with the older version of the OECD TG 416 (1983). As certain endocrine-sensitive parameters were not investigated in these generational studies, a brief summary of data on the EAS modalities from the ED dataset is also included in this section.

2-generation dietary study in rats (1988; M-018517)

Crl:CD BR rats (30/sex/group) were administered metribuzin at dietary levels of 0, 30, 150 or 750 ppm (equivalent to ca. 0, 2, 11 or 52 mg/kg bw/d pre-mating), there was one mating per generation. Parental toxicity in top dose animals was manifest as reduced body weight (by about 9%/13% m/f), reduced food consumption and mild hepatocellular hypertrophy. There was no effect on reproductive performance. Histopathology of reproductive organs did not show any treatment-related effects.

3-generation drinking water study in rats (1998; M-493110)

The study was conducted in line with the OECD TG 416 (1983) modified to include a third generation. Wistar rats (30/sex/group) were exposed to metribuzin via drinking water at concentrations of 0, 30, 150 and 600 ppm (equivalent to ca. 0, 4.4, 19 and 67 mg/kg bw/d), there was one mating per generation. Top dose parental animals had lower body weights (males by ca. 10%, females by about 12%) and their food consumption was decreased compared to controls. Slight body weight reductions were also observed at the mid-dose.

There were no effects on fertility or sexual function. Post-implantation loss and pup mortality are discussed under developmental toxicity.

Studies on endocrine disruption

Only a brief summary of the results is provided here, the details can be found in the dRAR.

The following *in vitro* assays were negative: an estrogen receptor binding assay using rat uterine cytosol (2011; M-418673), an estrogen receptor transcriptional activation assay in human cell line HeLa-9903 (2011; M-416262), a human recombinant aromatase assay (2011; M-417742), and an androgen receptor binding assay using rat prostate cytosol (2011; M-418468). An H295R steroidogenesis assay (2011; M-419375) showed an increase in estradiol secretion.

An immature rat uterothrophic assay (2011; M-417703) and a Hershberger assay (2011; M-417700) were negative.

In a peripubertal study (2011; M-421281) following the US EPA guidelines OPPTS 890.1500 and 890.1450, juvenile Sprague-Dawley rats (15/sex/group) were administered metribuzin via gavage at levels of 0, 60 and 120 mg/kg bw/d by gavage. Males were exposed from PND 23 to 53, females from PND 22 to 42. The parameters investigated included preputial separation, vaginal opening, estrous cyclicity, serum testosterone (in males), and reproductive organ weights and histopathology. None of these parameters was significantly affected by treatment (details can be found in the dRAR).

Repeated dose studies

No adverse effects on reproductive organs were observed in the available repeated dose studies. Immature appearance of testes reported in the dog dietary studies (90-day study, decedents in the 2-year study) is a common finding in animals of young age.

Conclusion on fertility

No effects on fertility or sexual function were observed in the available generational studies, nor was there any indication of interference with the reproductive system in the *in vivo* studies on endocrine disruption or in the repeated dose studies. Therefore, RAC agrees with the DS's proposal of no classification for fertility.

Development

The acceptable prenatal developmental toxicity (PNDT) studies are summarized in the following table.

PNDT studies			
Study type; year; reference number	**Method**	**Maternal toxicity**	**Developmental toxicity**
Rat			
PNDT; 2015 M-530086	Strain: Wistar Oral gavage 0, 3, 15, 75 mg/kg bw/d Dosing GD 5-20 Vehicle: aqueous methylcellulose and Tween 80 24 females/group	75 mg/kg bw/d: transient clinical signs (hypoactivity, piloerection), ↓ body temperature, ↓ fc	75 mg/kg bw/d: slightly reduced foetal wt (by ca. 5%), retarded ossification
PNDT; 1986 M-018676	Strain: Crl:CD BR Oral gavage 0, 25, 70, 200 mg/kg bw/d Dosing GD 6-15 Vehicle: aqueous Emulphor 28 females/group	200 mg/kg bw/d: clinical signs (hypoactivity, ptosis, ataxia), ↓ fc (by 17% GD 15), ↓ corrected bw by 9%, ↓ T4 by 85%, ↑ thyroid wt 70 mg/kg bw/d: transient clinical signs (hypoactivity, ptosis, ataxia), ↓ fc (by 12% GD 15), ↓ corrected bw by 6%, ↓ T4 by 52% 25 mg/kg bw/d: transient clinical signs (hypoactivity, ptosis), ↓ fc (by 10% GD 15)	200 mg/kg bw/d: ↓ foetal wt by 18%, wavy ribs, delayed ossification 70 mg/kg bw/d: ↓ foetal wt by 5% 25 mg/kg bw/d: ↓ foetal wt by 5%
PNDT; 1997 M-493058	Strain: Wistar Oral gavage 0, 10, 40, 150 mg/kg bw/d Dosing GD 6-15 Vehicle: peanut oil At least 20 pregnant females/group	150 mg/kg bw/d: ↓ fc by 33% (GD 6-15)	150 mg/kg bw/d: hydronephrosis (developmental retardation, 4 foetuses from a single dam), small fetuses (4%), retarded ossification
Rabbit			
PNDT; 2015 M-537608	Strain: NZW Oral gavage	100 mg/kg bw/d: ↓ defecation, ↓ fc by 34% (GD 7-29), ↓ bwg (by	100 mg/kg bw/d: ↓ foetal wt by 11%

	0, 10, 30, 100 mg/kg bw/d Dosing GD 7-28 Vehicle: aqueous methylcellulose and Tween 80 25 females/group	42% GD 7-29), 1 dam sacrificed *in extremis*	
PNDT; 1989 M-018201	Strain: American Dutch Oral gavage 0, 10, 30, 85 mg/kg bw/d Dosing GD 6-18 Vehicle: aqueous carboxymethyl cellulose and Tween 80 17 females/group	85 mg/kg bw/d: ↓ fc by ca. 35% (dosing period)	85 mg/kg bw/d: none
PNDT; 1995 M-493061	Strain: NZW Oral gavage 0, 10, 30, 100 mg/kg bw/d Dosing GD 6-18 Vehicle: carboxymethyl cellulose 15 females/group	100 mg/kg bw/d: none	100 mg/kg bw/d: no clear treatment-related effect (several anomalies without a clear dose-response relationship and mostly within the HCD range)

The PNDT studies showed no evidence of a teratogenic potential. The rabbit study (1995) and the rat study (1997), coming from the same laboratory, showed deficiencies in reporting and some of the findings (anomalies) are therefore difficult to interpret. They are given less weight in the assessment than the new rat and rabbit studies from 2015.

The main effects consistently observed in several studies were foetal weight reduction and delayed ossification in the presence of maternal toxicity (clinical signs, reduced food consumption). These mild, reversible effects that are likely to be secondary to maternal toxicity do not warrant classification.

<u>2-generation dietary study in rats (1988; M-018517)</u>

The DS discussed an apparent slight increase in stillborn + dead pups at the top dose (750 ppm) in F0. However, this was largely due to a single dam with all pups stillborn after a very prolonged delivery. Further, mean litter size and mean number of implantation sites seemed to be slightly reduced compared to control at the mid- and top dose in F1 without a clear dose-response relationship. Comparison with F0 data show that this apparent reduction can be explained by high control values in F1 (see Table 33 and Table 34 in the CLH report).

<u>3-generation drinking water study in rats (1998; M-493110)</u>

A statistically significant increase in post-implantation loss was observed in the P generation. However, no significant increase in post-implantation loss was present in F1 or F2. In addition,

the increased numbers at the top dose in P generation were around or below the F1 and F2 control values, and thus well within background variability (see Table 42 in the CLH report).

Another finding was an apparent increase in pup mortality. Litter-based incidence of pup mortality calculated from the individual data are provided in the table below. Despite the high variability and relatively high background incidence, a treatment-related effect cannot be excluded at the top dose in the P generation. On the other hand, no increase in pup mortality was observed in the F2 generation.

Pup mortality in the 3-generation study (1998; M-493110)				
Dose (ppm)	**0**	**30**	**150**	**600**
Dose (mg/kg bw/d)	0	4.4	19	67
P/F1				
Total no. of pups born	319	352	379	333
Stillborn pups (%/litter)	2.0	1.1	6.8	5.8
Pup mortality PND 1-4 (%/litter)	0.6	4.3	2.9	5.0
Pup mortality PND 5-21 (%/litter)	0.5	2.2	3.8	8.5
F1/F2				
Total no. of pups born	337	380	365	328
Stillborn pups (%/litter)	3.5	5.2	0.5	8.5
Pup mortality PND 1-4 (%/litter)	2.6	5.4	1.5	1.6
Pup mortality PND 5-21 (%/litter)	1.4	0.8	0	4.9
F2/F3				
Total no. of pups born	320	325	336	305
Stillborn pups (%/litter)	5.8	3.1	1.1	4.0
Pup mortality PND 1-4 (%/litter)	0.8	4.2	3.8	2.5
Pup mortality PND 5-21 (%/litter)	5.4	0.9	4.9	0.9

Conclusion on development

The PNDT studies have not shown any evidence of a teratogenic potential. The only finding potentially relevant for classification is increased pup mortality in one of the cohorts of the 3-generation study (1998). However, as the effect was not consistently observed across generations, and no increase in pup mortality was observed in the reliable 2-generation study (1988), RAC agrees with the DS's proposal of no classification for developmental toxicity.

Lactation

The only finding potentially relevant for classification is increased pup mortality in the 3-generation study (1998; M-493110). As explained in the section on developmental toxicity, this effect was not consistent across generations and between studies, and therefore is not sufficient for classification. RAC agrees with the DS's proposal of no classification for effects on or via lactation.

Overall conclusion on reproductive toxicity

RAC agrees with the DS's proposal of **no classification** for reproductive toxicity.

ENVIRONMENTAL HAZARD EVALUATION

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier Submitter's proposal

Metribuzin is an active substance with herbicidal activity for weed control. It is a selective triazinone herbicide acting as an inhibitor of photosynthesis. Metribuzin has an existing harmonised classification in Annex VI of the CLP Regulation as Aquatic Acute 1 and Aquatic Chronic 1 with an acute M-factor of 10. The Dossier Submitter proposed to maintain the classification as Aquatic Acute 1, M=10 and add an M-factor of 100 to the Aquatic Chronic 1 classification. The proposal for the acute classification was based on an acute E_rC_{50} value of 0.0265 mg/L for *Pseudokirchneriella subcapitata* ($0.01 < E_rC_{50} \leq 0.1$). The chronic classification proposal was based on the substance being not rapidly degradable and on the NOE_rC of 0.000205 mg/L for *Lemna gibba* (0.0001 mg/l < NOEC ≤ 0.001 mg/l).

Degradation

The environmental phototransformation half-life of metribuzin in water was estimated by simulation models and studied in sterile water. Model calculations resulted in environmental direct photolysis half-lives shorter than one day. In natural waters, metribuzin degraded rapidly with an experimental half-life of 0.63 hours corresponding to a calculated experimental half-life of approximately 0.15 days under intensive solar conditions. The major degradation product observed was DA-metribuzin. It was concluded that solar radiation significantly contributed to the degradation of metribuzin in aquatic environmental systems.

There were two studies on hydrolysis available. In a non-guideline, non-GLP study from 1986 metribuzin was stable at pH values 5, 6, 7 and 9 at 25 °C over 34 days. In a GLP study following OECD TG 111, metribuzin was stable at pH 4 and 7 at 50 °C. At pH 9, hydrolysis amounted to ca. 50%. Metribuzin was found to be stable at neutral and acidic pH at room temperature and was slowly degraded (calculated DT_{50}: 1317 days at 20°C) under basic conditions.

There was no ready biodegradability test available for metribuzin.

The route and rate of degradation of [5-^{14}C] metribuzin in surface water was investigated under defined laboratory conditions according to OECD TG 309 (GLP). For this purpose, the radiolabelled test item was applied to 500 mL of natural pond water at concentrations of 0.1 and 0.01 mg/L. The study period was of 56 days; sampling intervals were 0, 7, 14, 21, 28, 45 and 56 days. The mean recoveries of both test concentrations were within the range of 99.4 % to 107.4 % of the applied radioactivity (AR). Only amounts < 5 % AR were detected as evolved $^{14}CO_2$ and the amounts of organic volatiles detected were only traces (< 1 % AR) throughout the study. No degradation of test item could be observed throughout the study, and the mineralisation rate was negligible under tested conditions. The DT_{50} was > 10000 days for both concentrations of metribuzin tested and metribuzin is regarded as stable in natural surface water under the prevailing study conditions in the dark at 20 °C.

The optimised degradation parameters of metribuzin in total water and sediment systems, in the water phase, and in the sediment phase under laboratory conditions at 20 °C in the dark were investigated by two studies in 4 test systems (EPA Pesticide Assessment Guidelines, Subdivision N: § 161-4, GLP; Council Directive 91/414/EEC 1995, GLP). The geometric mean SFO-DT_{50} value of metribuzin in the total system was 42.2 days, in the water phase 31.2 days and in the sediment phase 55.2 days, indicating slow primary degradation. Mineralisation to carbon dioxide was observed at a rather low level, only. As a substantial result of the transformation processes, the stable major metabolite DA-metribuzin was formed.

The Dossier Submitter also shortly presented results from four aerobic and two anaerobic studies in soil. In addition, a photo-transformation study in soil was described.

Based on the results of degradation simulation studies in surface water and in water-sediment system, the Dossier Submitter considered metribuzin to be not rapidly degradable as the substance was not demonstrated to be ultimately degraded in either surface water or in aquatic sediment with a half-life of < 16 days.

Bioaccumulation

There were no estimated or experimental data on bioconcentration available. Log Pow of 1.8 was reported at all three pH values at 25°C in an OECD TG 117 test. The Dossier submitter concluded that metribuzin has a low potential for bioaccumulation.

Aquatic toxicity

Acute aquatic toxicity

***Table**: Summary of reliable acute aquatic studies*

Method	Species	Test material	Exposure	Results(* mg a.s./L	Reference(**
Fish					
OECD TG 203 GLP	*Oncorhynchus mykiss*	Metribuzin (94.3%)	96 hours, static	LC_{50} = 74.6 (mm)	M-046241-01-1
OECD TG 203 GLP	*Oncorhynchus mykiss*	Metribuzin (95.3%)	96 hours, static	LC_{50} = 80.3 (nom)	M-513878-01-1
OECD TG 203 GLP	*Leuciscus idus melanotus*	Metribuzin (93.5%)	96 hours, static	LC_{50} = 141.6 (nom)	M-013913-01-2
OECD TG 203 GLP	*Leuciscus idus melanotus*	Metribuzin (95.3%)	96 hours, static	LC_{50} = 169.4 (nom)	M-513882-01-1
FIFRA/ASTM GLP	*Cyprinodon variegatus*	Metribuzin (92.6%)	96 hours, static	LC_{50} = 85 (mm)	M-013993-01-1
OECD TG 203 GLP	*Cyprinus carpio*	Metribuzin (93.7%)	96 hours, static	LC_{50} > 100 (nom)	M-104023-01-1
Aquatic invertebrates					
OECD TG 202 GLP	*Daphnia magna*	Metribuzin (94.3%)	48 hours, static	EC_{50} = 49.6 (nom)	M-021792-04-1
OECD TG 202 GLP	*Daphnia magna*	Metribuzin (95.3%)	48 hours, static	EC_{50} = 49.0 (nom)	M-513889-01-1
Algae					
OECD TG 201 GLP	*Pseudokirchneriella subcapitata*	Metribuzin (99.0%)	72 hours, static	E_rC_{50} (1 = 0.0265 (nom)	M-042548-01-1 (recalculation of M-013856-01-1)
OECD TG 201 GLP	*Desmodesmus subspicatus*	Metribuzin (95.3%)	72 hours, static	E_rC_{50} (2 = 0.02657 (nom)	M-468327-01-1 (recalculation of M-493049-01-1)
OECD TG 201 GLP	*Desmodesmus subspicatus*	Metribuzin (93.9%)	72 hours, static	E_rC_{50} = 0.0664 (mi)	M-456768-01-1
Aquatic plants					
OECD TG 221 GLP	*Lemna gibba G3*	Metribuzin (94.4%)	7-days, semi-static	E_rC_{50} frond no.: 0.0385 frond area: (** 0.029 dry weight 0.0161 (3 (nom)	M-455636-01-1

OECD TG 239 GLP Water-Sediment test	*Myriophyllum spicatum*	Metribuzin tech. (91.9%)	14-days, semi-static	E_rC_{50} total shoot length: 0.154 fresh weight: 0.0457 dry weight: 0.0313 (nom) [4]	M-663178-01-1

[* mean measured concentrations were ~ 80 – 120 % of nominal in all tests

[** Reference in the Renewal Assessment Report under Regulation (EC) 1107/2009. Volume 3 – B.8 and Volume 3 B.9 (AS). September 2019.

mm=mean measured concentrations, mi= measured initial concentrations

[1 5-day test, recalculated for growth rate and 72-hour endpoint

[2 recalculated using state of the art statistics (most recent OECD TG 201)

[3 endpoint given by industry in the consultation

[4 mm 88.7 – 123% of nominal in overlying water; mm 22.7 -47.5% of nominal in pore water; mm > LOQ except in the lowest conc. of 1.03 µg/L in sediment

The Dossier Submitter also presented supplementary studies on three algae tests, on a *Lemna minor test* and on a *Xenopus laevis* test. Details on these studies can be found in the CLH Report.

Algae and aquatic plants were most sensitive in acute aquatic studies. The lowest acute toxicity value for algae was an E_rC_{50} of 0.0265 mg/L for *Pseudokirchneriella subcapitata* in a 120-hour static test conducted according to OECD TG 201. The green algae were exposed to a dilution water control, a solvent control and to nominal concentrations of 0.0025, 0.005, 0.01, 0.02 and 0.04 mg a.s./L, which corresponded to mean measured concentrations of 0.00233, 0.00469, 0.00943, 0.0182 and 0.0365 mg a.s./L. The treatment groups and the controls consisted of 3 replicates each containing 3000 cells/mL at test initiation. Assessment of growth was conducted daily. To fulfil the reporting requirements of OECD TG 201, additional calculations for growth rate based on cell density between 0 and 72 h were performed. The recalculated endpoints based on nominal concentrations after 72 hours were: E_rC_{50} 0.0265 mg a.s./L, LOErC 0.005 mg a.s./L and NOE_rC 0.0025 mg a.s./L. The Dossier Submitter used the E_rC_{50} value for proposing Aquatic Acute 1; H400, M=10.

Chronic aquatic toxicity

Table: *Summary of reliable chronic aquatic toxicity studies*

Method	Species	Test material	Exposure	Results(* mg a.s./L	Reference(**
Fish					
OECD TG 210 US EPA OCSPP 850.1400 Non-GLP	*Oncorhynchus mykiss*	Metribuzin (94%)	95 days, flow-through	EC_{10} = 4.432 (mm, body length)	M-042516-01-1
OECD TG 210 GLP	*Pimephales promelas*	Metribuzin (93.9%)	36 days, flow-through	NOEC = 13.1 (mm, survival, growth)	M-073884-01-1
Aquatic invertebrates					
OECD TG 211 GLP	*Daphnia magna*	Metribuzin (93.0%)	21 days, flow-through	NOEC = 1.29 EC_{10} = 1.44 (mm, offspring per adult per repro day)	M-654189-01 (recalculation of M-013774-01-1)

OECD TG 211 GLP	*Daphnia magna*	Metribuzin (95.3%)	21 days semi-static	NOEC=0.32 EC_{10} =0.29 (nom, weight)	M-604351-01-1 (recalculation of M-513894-01-1)
Algae					
OECD TG 201 GLP	*Pseudokirchneriella subcapitata*	Metribuzin (99.0%)	72 hours, static	NOE_rC (1 = 0.0025 (nom)	M-042548-01-1 (recalculation of M-013856-01-1)
OECD TG 201 GLP	*Desmodesmus subspicatus*	Metribuzin (95.3%)	72 hours, static	E_rC_{10}(2 = 0.00516 NOE_rC = 0.001 (nom)	M-468327-01-1 (recalculation of M-493049-01-1)
OECD TG 201 GLP	*Desmodesmus subspicatus*	Metribuzin (93.9%)	72 hours, static	E_rC_{10} = 0.0147 NOE_rC = 0.0116 (mi)	M-456768-01-1
Aquatic plants					
OECD TG 221 GLP	*Lemna gibba G3*	Metribuzin (94.4.0%)	7-days, semi-static	E_rC_{10}: frond no: 0.0059 frond area: 0.00678 growth rate of biomass: 0.00506 NOE_rC = 0.000205 (nom)	M-455636-01-1
OECD TG 239 GLP Water-Sediment test	*Myriophyllum spicatum*	Metribuzin tech. (91.9%)	14-days, semi-static	E_rC_{10}: total shoot length: 0.00764 fresh weight: 0.00713 dry weight: 0.00507 NOE_rC: 0.00295 (nom)(3	M-663178-01-1

(* The mean measured concentrations were ~ 80 – 120 % of nominal in all tests

(** Reference in the Renewal Assessment Report under Regulation (EC) 1107/2009. Volume 3 – B.8 and Volume 3 B.9 (AS). September 2019.

mm=mean measured concentrations, mi= measured initial concentrations

(1 5-day test, recalculated for growth rate and 72-hour endpoint

(2 recalculated using state of the art statistics (most recent OECD TG 201)

(3 mm 88.7 – 123% of nominal in overlying water; mm 22.7 -47.5% of nominal in pore water; mm > LOQ except in the lowest conc. of 1.03 µg/L in sediment

The Dossier Submitter also presented 3 algae tests and a *Lemna minor* test as supplementary studies. Details of these studies are available in the CLH Report.

Algae and aquatic plants were most sensitive also in chronic toxicity tests. The lowest 72-hr E_rC_{10} values were 0.00516 mg /L for *Desmodesmus subspicatus* and 0.0059 mg/L for *Lemna gibba*. The E_rC_{10} values for *Myriophyllum spicatus* were of same magnitude from the water/sediment test. The lowest NOE_rC value was 0.000205 mg/L for *Lemna gibba*.

A 72-hour static test on the green alga *Desmodesmus subspicatus* was conducted according to OECD TG 201. The green algae were exposed to a dilution water control and to nominal concentrations of 0.00032, 0.001, 0.0032, 0.01, 0.032, 0.1 and 0.32 mg a.s./L. The measured concentrations of the active substance at the beginning of the test were in the range of 76 -126 % of nominal and at the end of the test they were in a range of 87 - 98 % of nominal. The results were derived from recalculations of the same data by using state of the art statistics. The recalculated results relevant for classification were, based on nominal concentrations, E_rC_{10} 0.00516 mg a.s./L and NOE_rC 0.001 mg a.s./L for growth rate.

A 7-day semi-static toxicity laboratory test on *Lemna gibba* was conducted according to OECD TG 221. The plants were exposed to a dilution water control and to nominal concentrations of 0.000205, 0.000512, 0.00128, 0.0032, 0.008, 0.02 and 0.05 mg a.s./L. There were no visual effects observed in any of the test concentrations. Chemical analysis of metribuzin was performed for all freshly prepared test levels on day 0, 2, and 4 ranging between 100 and 118% of nominal concentrations and additionally, for all aged test levels on day 2, 4, and 7 of the exposure period ranging between 98 and 114% of nominal. The E_rC_{10} and NOE_rC on mean growth rate of frond number were 0.0059 mg/L and 0.000205 mg/L, respectively. The Dossier Submitter used the NOE_rC value as the basis for proposing Aquatic Chronic 1; H410, M=100.

Comments received during consultation

Two Member States supported the classification Aquatic Acute 1, M=10 and Aquatic Chronic 1, M=100. One MS supported the classification and requested additional information on the key study for chronic classification on *Lemna*. At the request of the MS, the Dossier Submitter gave more details on the test.

Industry provided test reports on several environmental studies. The aquatic toxicity studies provided, in addition to the ones presented by the Dossier Submitter, were metabolite and formulation studies presenting results way above the limits for classification with the exception of a new test[1] on *Navicula pelliculosa* giving a 72h- E_rC_{50} of 0.034 mg a.s./L and a 72h- NOE_rC of 0.001 mg a.s./L. The test report also gave a 72-hr E_rC_{10} value of 0.00655 mg a.s./L. These results are in line with the other study results presented in the CLH Report.

Industry also pointed out that the overall lowest E_rC_{50} in the acute dataset was not obtained in the *Pseudokirchneriella subcapitata* test but was obtained for *Lemna gibba* with another endpoint that was mentioned in Table 63 of the CLH Report, namely E_rC_{50} (dry weight) of 0.0161 mg/L. The Dossier Submitter refers to the ECHA Guidance Document on CLP and on Information Requirements and Chemical Safety Assessment concerning their choice to use frond number as the basis for classification.

Industry also questioned the use of the *Myriophyllum* test for classification considering that the test is performed according to the OECD TG 239 which is a water-sediment test. They would prefer to use *Lemna* species to present macrophytes in order to ensure a harmonised approach. The DS explained that the test was included in the CLH Report because it was a well performed test conducted according to the guideline with no deviations and could be considered as supplementary information.

A national authority supported the M-factor of 10 for chronic classification based on the ErC_{10} values in the range from 0.001-0.01 mg/L from studies on three different species.

[1] GLP, OECD TG 201, EPA Guideline 712-C-006: OCSPP 850.4500

Assessment and comparison with the classification criteria

Degradation

RAC agrees with the DS to consider metribuzin as not rapidly degradable, as:

- there was no ready biodegradability test available
- no degradation of metribuzin was observed in a surface water simulation study, mineralisation rate was negligible
- metribuzin was stable to hydrolysis at neutral and acidic pH values and slowly degraded (DT50 1317 days) under basic conditions
- metribuzin showed slow primary degradation (42.2 days in total system) in 2 water/sediment studies, mineralisation at low level, stable metabolite DA-metribuzin was formed

Bioaccumulation

RAC agrees with the DS's conclusion to consider metribuzin having a low potential for bioaccumulation based on the log Pow of 1.8 in the absence of experimental data on bioconcentration.

Acute aquatic toxicity

There were reliable acute toxicity data available on three trophic levels showing that algae and macrophytes were the most sensitive in acute tests. All relevant tests gave E_rC_{50} values in range of $0.01 < E_rC_{50} \leq 0.1$ mg/L.

The *Lemna gibba* study (M-455636-01-1) gave results for frond number (E_rC_{50} 0.0385 mg/L), frond area (E_rC_{50} = 0.029 mg/L) and dry weight (E_rC_{50} 0.016 mg/L). The Dossier Submitter was of the opinion that according to the CLP Guidance frond number should be used for classification. In RAC's opinion the CLP Guidance states the primary endpoint but according to the OECD TG 221 at least one other measurement variable including total frond area, dry weight or fresh weight should also be measured because some substances may affect other measurement variables more than the frond number. RAC is of the opinion that the lowest 7-day E_rC_{50} value to be used for classification was 0.016 mg/L based on the dry weight in the OECD TG 221 *Lemna gibba* test.

RAC considers the water-sediment *Myriophyllum* study as supportive evidence in this case. Although the concentrations of metribuzin in overlaying water in aged media ranged between 88.7 and 110%, the pore water concentrations ranged between 22.7 and 47.5%. A sediment-rooted aquatic macrophyte as *Myriophyllum* takes up contaminants from pore water directly through the roots and it is not possible to conclude that the effect is solely based on metribuzin in overlaying water.

Chronic aquatic toxicity

There were reliable chronic data available on three trophic levels showing that algae and macrophytes were the most sensitive also in chronic tests. Altogether there were four tests on algae. In three of those tests, and in the *Lemna* test, both NOE_rC and E_rC_{10} values were available. In the *Pseudokirchneriella* test, only a NOE_rC of 0.0025 mg/L was available. The lowest NOE_rC and E_rC_{10} values were in range of $0.001 < E_rC50 \leq 0.01$ mg/L except for two NOE_rC values for *Lemna gibba* (0.000205 mg/L) and for *Navicula pelliculosa*[1] (0.001 mg/L). RAC considers, however, that the E_rC_{10} values of 0.00506 mg/L (*Lemna gibba*) and 0.00655 mg/L (*Navicula pelliculosa*) from those same tests take precedence. According to the CLP Guidance, EC_{10} values

[1] New data received in the consultation

are preferred as these are statistically derived from the entire dataset, and less dependent on test design considerations than NOEC values.

RAC's opinion on the *Myriophyllum* test can be found under Acute toxicity chapter above.

Comparison with the criteria

Aquatic Acute

RAC agrees with the Dossier Submitter to classify metribuzin as **Aquatic Acute 1, H400 (M=10)** based on lowest acute E_rC_{50} value of 0.016 mg/L for *Lemna gibba* which is below the cut-off value of 1 mg/L. M-factor of 10 should be assigned because the E_rC_{50} is in range 0.01 mg/L < $L(E)C_{50}$ ≤ 0.1 mg/L.

Aquatic Chronic

RAC disagrees with the Dossier Submitter proposal and agrees to classify metribuzin as **Aquatic Chronic 1, H410 (M=10).** Metribuzin is not rapidly degradable and the lowest chronic toxicity values are below the cut-off ≤ 0.1 mg/L. The lowest E_rC_{10} value is 0.00506 mg/L for *Lemna gibba*. An M-factor of 10 should be assigned because the E_rC_{10} is in range 0.001 mg/L < EC_{10} ≤ 0.01 mg/L, rather than an M-factor of 100 as proposed by the DS.

Additional references

Anonymous (1996) DADK (desaminodiketometribuzin) – Study for subacute oral toxicity in rats. Bayer, Gewrmany. Report no. 24800. M-018441

Anonymous (2018) 4-week toxicity study by the oral route (dietary admixture) in rats. Citoxlab, France. Study no. 452222 TSR. M-617607

Bomann *et al.* (2021) Metribuzin-induced non-adverse liver changes result in rodent-specific non-adverse thyroid effects via uridine 5'-diphospho-glucuronosyltransferase (UDPGT, UGT) modulation. Regulatory Toxicology and Pharmacology 122:104884

Estonia (RMS) and Germany (Co-RMS) (2018) Metribuzin, Renewal Assessment Report under Regulation (EC) 1107/2009. 1st draft, April 2018

Higgins (2019) Evaluate the potential for metribuzin to inhibit recombinant rat and human deiodinases. Concept Life Sciences, Scotland. Study no. CLS4_0010_0002. M-664441

Richert and Parmentier (2020) Inhibition of rat microsomal thyroid peroxidase by metribuzin. KaLy-Cell, France. Study no. KLC-BA20-01. M-680749

Richert and Routhier (2020) In vitro CYP and UGT induction in human and Wistar rat hepatocytes by metribuzin. KaLy-Cell, France. KLC-BA20-04. M-753595

Wang *et al.* (2018) High-throughput screening and quantitative chemical ranking for sodium-iodide symporter inhibitors in ToxCast Phase I chemical library. Environmental Science and Technology 52:5417-5426

Renewal Assessment Report under Regulation (EC) 1107/2009. Volume 3 – B.8 (AS). September 2019

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).

Annex 3 Comments received on the ad hoc consultation, response to comments provided by RAC