CLH report

Proposal for Harmonised Classification and Labelling

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

International Chemical Identification:

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

Index Number: 606-034-00-8

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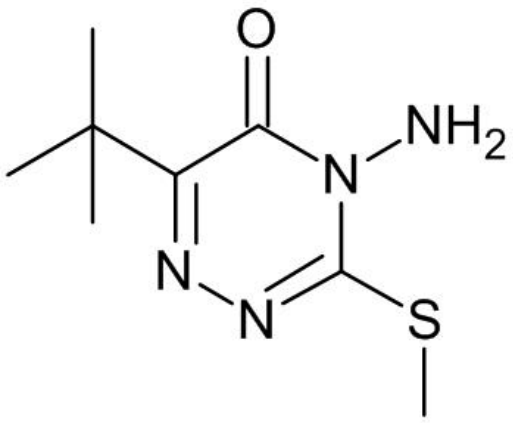
Version number: 3 **Date: July 2020**

CONTENTS

1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

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

Name(s) in the IUPAC nomenclature or other international chemical name(s)	Metribuzin (ISO); 4-amino-6-tert-butyl-3-methylthio-1,2,4-triazin-5(4H)-one; 4-amino-4,5-dihydro-6-(1,1-dimethylethyl)-3-methylthio-1,2,4-triazin-5-one
Other names (usual name, trade name, abbreviation)	Metribuzin
ISO common name (if available and appropriate)	Metribuzin (ISO)
EC number (if available and appropriate)	244-209-7
EC name (if available and appropriate)	N/A
CAS number (if available)	21087-64-9
Other identity code (if available)	CIPAC N° 283 CLP Annex VI Index N° 606-034-00-8
Molecular formula	$C_8H_{14}N_4OS$
Structural formula	
SMILES notation (if available)	Canonical SMILES CC(C)(C)C1=NN=C(N(C1=O)N)SC Isomeric SMILES No data
Molecular weight or molecular weight range	214.3 g/mol
Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	The active substance as defined in the ISO common name and as reflected in the CA name has no isomers.
Description of the manufacturing process and identity of the source (for UVCB substances only)	N/A
Degree of purity (%) (if relevant for the entry in Annex VI)	min. 93 %

1.2 Composition of the substance

Table 2: Constituents (non-confidential information)

Constituent (Name and numerical identifier)	Concentration range (% w/w minimum and maximum in multi-constituent substances)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)
Metribuzin (ISO) (EC no 244-209-7)	min. 93 %	Acute Tox. 4* H302 Aquatic Acute 1 H400 Aquatic Chronic 1 H410	Acute Tox. 4* H302 Aquatic Acute 1 H400 Aquatic Chronic 1 H410 STOT SE 3 H336 Acute Tox. 3 H331

2 PROPOSED HARMONISED CLASSIFICATION AND LABELLING

2.1 Proposed harmonised classification and labelling according to the CLP criteria

Table 3

	Index No	Chemical name	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATEs	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(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* 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	
Resulting entry in Annex VI if adopted by RAC and agreed by Commission	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, thyroid) H400 H410	GHS07 GHS08 GHS09 Wng	H302 H373 (blood, thyroid) H410		oral; ATE=322 mg/kg bw M=10 M=100	

Table 4: Reason for not proposing harmonised classification and status under public consultation

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

Hazard class	Reason for no classification	Within the scope of public consultation
Aspiration hazard	data lacking	No
Hazardous to the aquatic environment	harmonised classification proposed	Yes
Hazardous to the ozone layer	hazard class not assessed in this dossier	No

3 HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING

Metribuzin is a an approved substance under Commission Regulation (EC) No. 1107/2009. Currently the renewal of assessment is in progress under EFSA`s procedure. The EFSA Scientific Report (2006) 88, 1-74, "Conclusion regarding the peer review of the pesticide risk assessment of the active substance metribuzin" available at https://www.efsa.europa.eu/en/efsajournal/pub/rn-88 (referred as EFSA, 2010). The EFSA 2006 assessment concluded that metribuzin has a moderate acute oral toxicity and low dermal and inhalation toxicity. The classification as R22 (Harmful if swallowed) has been proposed. It was considered that metribuzin potentially reached the requirement for classification as [R22/48] since in a number of studies, serious adverse effects were noted at doses of >50 mg/kg bw/day. The issue was then flagged to ISPRA for consideration. Metribuzin did not exhibit a genotoxic and carcinogenic potential. A possible classification as R62/63 was considered and forwarded to ISPRA based on delays in skeletal ossification, considered to be related to decreased bodyweight, rather than developmental toxicity, and alterations in kidneys and ureter, together with the increased pup mortality in the multigeneration study.

According to the classification and labelling legislation Dangerous Substances Directive (67/548/EEC) the substance had classification: Xn; R22 ("Harmful if swallowed.") and N; R50-53 ("Very toxic to aquatic organisms and May cause long-term adverse effects in the aquatic environment").

The substance is listed in Annex VI to CLP with harmonised classification for Acute Tox. 4*, Aquatic Acute 1 and Aquatic Chronic 1, M factor = 10. According to ECHA`s Summary of Classification and Labelling metribuzin, in addition to the harmonised classification, has been self-classified also as STOT SE 3 H336 by 13 notifiers and as Acute Tox. 3 H331 by 128 notifiers[1].

4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

Metribuzin is an active substance in the meaning of Regulation EC 1107/2009 and there is no requirement for justification that action is needed at Community level (Article 36 CLP Regulation).

5 IDENTIFIED USES

Metribuzin is an active substance with herbicidal activity used for weed control in different agricultural crops, such as potatoes, soybean etc. Metribuzin is a selective triazinone herbicide acting as an inhibitor of photosynthesis, specifically the inhibition of the photosynthetic electron transfer in the stage of the second light reaction.

[1] https://echa.europa.eu/et/information-on-chemicals/cl-inventory-database/-/discli/details/68849 (on 13.12.2019)

6 DATA SOURCES

The data presented in this dossier has been submitted by the applicant as part of the renewal process. Some of the data was submitted and evaluated during the first approval while other data was submitted for the first time for the purpose of renewal of approval. All data is presented in the Renewal Assessment Report (RAR) publicly available at https://www.efsa.europa.eu/en/consultations/call/public-consultation-active-substance-metribuzin-1 (30.09.2019) prepared by Rapporteur Member State (RMS) Estonia which is being submitted to EFSA.

Where necessary, the confidential original study reports relevant for classification and labelling of metribuzin provided to the eCA by the owner of the data were reviewed.

7 PHYSICOCHEMICAL PROPERTIES

Table 5: Summary of physicochemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
Physical state at 20 °C and 101,3 kPa	Physical state: Fine needles Colour: White Odour: Weak, not characteristic	M-464873-01-1	Observed (OPPTS 830.6302, 830.6303, OPPTS 830.6304) Purity 99.3 %
	Physical state: Powder Colour: Off white Odour: cabbage-like	M-467560-01-1	Observed (OPPTS 830.6302, 830.6303, OPPTS 830.6304) Purity 94.4 %
Melting/freezing point	The test item had a melting point of 125.9 °C.	M-412991-01-1	Measured (EC A.1, OECD 102) Purity 99.3 %
Boiling point	The test item had no boiling point; the test item decomposed fist starting at a temperature of 230 °C.	M-412991-01-1	Measured (EC A.2, OECD 103) Purity 99.3 %
Relative density	D_4^{20} = 1.28	M-469763-01-1	Measured (OECD 109, EC A.3, OPPTS 830.7300) Purity 94.4 %
Vapour pressure	1.7 x 10^{-4} Pa for 20 °C, 3.4 x 10^{-4} Pa for 25 °C, 7.0 x 10^{-3} Pa for 50 °C.	M-462079-01-1	Vapour pressure values extrapolated (vapour pressure balance method) (EC A.4, OECD 104) Purity 99.3 %
Surface tension	σ = 63.6 mN/m at 20 °C	M-464893-01-1	Measured (EC A.5, OECD 115) Purity 99.3 %
Water solubility	Water solubility = 1.07 g/L (in distilled water at 20 °C, final pH = 6.3). The partition coefficient of Metribuzin pure a.s. was found to be pH independent in the pH range of pH 4 – pH 9 (M-464868-01-1). Consequently the water solubility is also pH independent in that pH range.	M-464871-01-1	Measured (EC A.6, OECD 105, OPPTS 830.7840) Purity 99.3 %

Property	Value	Reference	Comment (e.g. measured or estimated)
	Therefore the water solubility was determined in distilled water only.		
Partition coefficient n-octanol/water	At 25 °C Pow log Pow pH 4 63 1.8 pH 7 63 1.8 pH 9 63 1.8	M-464868-01-1	Measured (EC A.8, OECD 117, (HPLC method)) Purity 99.3 %
Flash point	-	-	Not applicable. The active substance is a solid; its melting point is > 40 °C.
Flammability	Metribuzin is not highly flammable in the sense of EC guideline A.10.	M-008050-01-1	Measured (EC A.10) Purity 94.3 %
	Metribuzin is not highly flammable in the sense of EC guideline A.10.	M-513259-01-1	Measured (EC A.10) Purity 93 %
Explosive properties	Metribuzin is not explosive in the sense of EC guideline A.14.	M-008050-01-1	Measured (EC A.5, OECD 115) Purity 99.3 %
	Not explosive in the sense of EC Guideline A.14.	M-513262-01-1	Calculation
Self-ignition temperature	No self-ignition temperature was observed until the maximum temperature of 410 °C. The test item melted at about 103 °C.	M-008050-01-1	Measured (EC A.16) Purity 94.3 %
	No self-ignition temperature was observed until the maximum temperature of 400 °C. The test item just melted.	M-513261-01-1	Measured (EC A.16) Purity 93 %
Oxidising properties	Metribuzin has no oxidizing properties in the sense of EC guideline A.17.	M-462082-01-1	Measured (EC A.17) Purity 94.4 %
Granulometry	No data	-	Not applicable according to EC 283/2013
Stability in organic solvents and identity of relevant degradation products	Solubility at 20 °C in n-Heptane 0.84 g/L Xylene 60 g/L Dichloromethane > 250 g/L 2-Propanol > 250 g/L 1-Octanol 54 g/L Polyethyleneglycol > 250 g/L Acetone > 250 g/L Ethylacetate > 250 g/L Acetonitrile > 250 g/L Dimethylsulfoxide > 250 g/L	M-021343-01-1	Measured (OECD 105) Purity 94.8 %
	Solubility at 22 °C in Toluene 117.3 g/L Methanol 259.9 g/L Acetone 449.4 g/L Ethylacetate 336.0 g/L 1,2-Dichloroethane 426.9 g/L n-Heptane 0.8 g/L	M-513255-01-1	Measured Purity: 98.3 %
Dissociation constant	The dissociation constant pKa of Metribuzin purified a.s. in water: $pKa_1 = 1.3$	M-469947-01-1	Measured (OECD 112, OPPTS 830.7370) Purity 99.3 %

Property	Value	Reference	Comment (e.g. measured or estimated)
	pKa_2 = 12.8		
Viscosity	Not applicable	-	The substance is a solid.

8 EVALUATION OF PHYSICAL HAZARDS

Not addressed in this dossier.

9 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

Table 6: Summary table of toxicokinetic studies

Method	Results	Remarks	Reference
The metabolism and excretion of Metribuzin in rats: Radiolabelled Metribuzin (5-^{14}C and S-methyl-^{3}H) in 50 % ethanol (20 mg/kg bw) or 0.5 % aqueous gum tragacanth (50/100 mg/kg bw) was administered by a single oral gavage dose to groups of male and female rats as follows: Group 1: 20 mg/kg bw to 1 male and 1 female (1st excretion monitoring / low dose), Group 2: 100 mg/kg bw to 2 males (2nd excretion monitoring / high dose), Group 3: 50 mg/kg bw to 8 males and 8 females (tissue residue and metabolism monitoring).	Excretion was essentially quantitative in urine and faeces. Tritium activity was found to be eliminated in increasing amounts in air suggesting S-demethylation and oxidation of the methyl group. Male rats had a more rapid clearance of the radioactivity. Approximately 35 % of residues in urine were identified as Metribuzin and its three metabolites with an additional 32 % being made organosoluble by hydrolysis treatments. A metabolic pattern in tissues from organosoluble to water-soluble to insoluble residues was observed which was secondary to rapid elimination of total residues. Treatment of these residues by enzyme and chemical hydrolysis confirmed the metabolic pathway to be: Metribuzin → DA-metribuzin[2] → DADK-metribuzin[3] → conjugation.	No guideline available at that time, non-GLP, Acceptable	M-020240-02-1
The excretion and metabolism of Metribuzin by rats: Radio-labelled Metribuzin was dissolved in ethanol/propylene glycol and administered by oral gavage at different dose levels to groups of 2, 5 or 6 male and female rats as shown below:	In all groups dosed a total of 27.3 to 43.4 % was excreted via the urine and 55.8 to 71.5 % was excreted via the faeces and less than 1 % remained in the tissues. There were no significant differences in the	EPA Pesticide Assessment Guidelines, Subdivision F, § 85-1, General Metabolism, Rat (November 1982),	M-022071-03-1

[2] DA-metribuzin - Desaminometribuzin

[3] DADK-metribuzin - Desaminodiketometribuzin

Method	Results	Remarks	Reference

Test group	Dose	Sex / No. of animals	Type of study
Prelim.	5 mg/kg bw	2 females	to detect the presence of expired radioactivity
B	5 mg/kg bw	5 males, 5 females	single low dose
C	5 mg/kg bw/day for 14 days with unlabeled Metribuzin, followed by a single application of 5 mg/kg bw radioactive compound	6 males, 6 females	multiple low dosing
D	500 mg/kg bw	6 males, 6 females	single high dose

rates and routes of radiocarbon elimination between the male and female rats in the groups B and D, however in the group B the total excreted radioactivity reached a plateau faster (48 h) compared to the group D (72 h). In the group C a difference in the rate of elimination between the sexes was observed in the faeces, the male rats' elimination of radiocarbon reached a plateau by 48 hours, whereas the female rats' faeces elimination of radioactivity did not reach a plateau until 72 hours post-administration of ^{14}C-Metribuzin.
There does not appear to be an accumulation of radiolabeled material in the various tissues due to a repeated dosing regime in comparing groups B and C.
Metabolism of Metribuzin appeared to involve deamination, dethioalkylation, hydroxylation of the t-butyl sidechain and conjugation. The predominant metabolite in the excreta of all dose groups was cystein-DA-metribuzin (12.9 to 23.7 % of the administered radioactivity) followed by DADK-metribuzin (4.8 to 10.3 %), DA-metribuzin (4.1 to 9.2 %), DK[4]-metribuzin (3.1 to 6.3 %), t-BuOH-DADK-metribuzin (3.0 to 7.0 %), 3-amino-DA-metribuzin (2.8 to 5.3 %) and t-BuOH-DA-metribuzin (0.8 to 2.5 %). None of the remaining four identified compounds exceeded 1.5 % of the administered radioactivity. In total, 52.3 to 61.6 % of the administered radioactivity was identified. At least 6 unidentified compounds were detected in the urine and faeces, none of which individually exceeded 8.6 % of the administered radioactivity. Although significant amounts of radioactivity remained unidentified, it should be noted that these amounts are the sum

GLP, Acceptable

[4] DK-metribuzin - Diketometribuzin

Method	Results	Remarks	Reference
	of several smaller fractions from different extractions and therefore consist of several smaller degradation products. The evidence indicates that glucuronidation and sulfation do not play a major role in metabolism or excretion. In contrast, conjugation with glutathione (GSH) followed by conversion to mercapturic acid derivatives appears to play a major role in detoxification and excretion.		
a) Metabolism study of ^{14}C-labelled metribuzin after single oral and intravenous (i.v.) administration to Sprague-Dawley rats; b) Metribuzin - ADME-study in rats. In a metabolism study, the metabolism profile of ^{14}C-labelled Metribuzin after single intravenous injection and single oral administration in rats was determined. Technical metribuzin, spiked with radiolabelled metribuzin, was administered once to four groups of Sprague-Dawley rats as follows: Group I (A&E): 4/sex, 0.57 mg/kg bw by i.v. injection (vehicle water), Group II (A&E): 4/sex, 0.57 mg/kg bw/day by oral gavage (vehicle 0.5 % aqueous tylose), Group III (A, M&E): 4/sex, 0.57 mg/kg bw/day plus 200 mg/kg bw/day (non-labelled) by oral gavage (vehicle 0.5 % aqueous tylose), and Group IV (D): 8 females, 0.57 mg/kg bw/day by i.v. injection (vehicle water).	For both oral and i.v. administrations the excretion of radioactivity at low dose groups I and II was rapid, the majority being excreted in urine and faeces within 24 h. For group III most of the excretion occurs within 48 h. Since more than half of the administered dose was present in faeces, excretion with bile can be considered to be a major route of elimination. Additionally, the concentrations of radioactivity in blood were found to be almost always higher than the ones in other tissues at all time-intervals after i.v. administration, especially significant at later sampling points (24 h and 7 d); which indicates the absence of accumulation. The metabolite pattern of the urine samples of i.v.- and oral-low dose groups I and II were different from samples of the oral-high dose group III: based on the total excretion of radioactivity the main metabolites were determined to be DA-metribuzin and 6-*tert*-butyl-4,5-dihydro-l,2,4-triazin-5-one-3-mercapturic acid (69.0 and 44.6 %, respectively for low and high dose) and t-BuOH-DA-metribuzin (15.4 to 12.2 %, respectively).	The in life phase and sample collection were reported in M-493116-01-1 and the ADME method and results were reported in M-493124-01-1, Purity of unlabelled Metribuzin: 99 %; ^{14}C-labelled Metribuzin: > 98 %, GLP, Acceptable	a) M-493116-01-1, b) M-493124-01-1
Comparative *in vitro* metabolism of [^{14}C]-metribuzin using rat and human liver microsomes: The *in vitro* metabolism of [^{14}C]-metribuzin was investigated in pooled rat liver microsomes (males and females), obtained from Wistar rats, and	After incubation in liver microsomes the majority of the radiolabelled active substance was recovered as unchanged metribuzin. However, comparing both incubation	Commission Regulation (EU) No 283/2013, 5.1.1, Purity ^{14}C-labelled Metribuzin: 98.7 %,	M-466743-01-1

Method	Results	Remarks	Reference
pooled human liver microsomes (males and females). [^{14}C]-metribuzin was incubated for 2 h at a concentration 10 µM with rat and human liver microsomes in a NADPH regeneration system with a microsomal protein concentration of 1.0 mg/mL. Activity of microsomes was confirmed by parallel incubation with a standard substrate ([^{14}C] testosterone). At the end of each incubation the metabolic reaction was stopped by precipitation of the protein, the supernatant isolated by centrifugation and metabolite pattern investigated by HPLC and on-line radioactivity detection.	systems, [^{14}C]-metribuzin was faster and more extensively metabolised in rat than in human liver microsomes.	GLP, Acceptable	
In vitro metabolism of [^{14}C]-Metribuzin by Beagle dog liver microsomes: Metabolic activity of the microsomes towards a standard substrate (testosterone) was determined and found appropriate. Conditions were developed for which the microsomal metabolism of [^{14}C]-metribuzin was linear with protein concentration. The final incubations with dog liver microsomes were conducted for 120 minutes at a [^{14}C]-metribuzin concentration of 10 µM and a microsomal protein concentration of 1.0 mg/mL.	[^{14}C]-metribuzin accounted for the majority of radioactivity in the sample. Two metabolites were formed and detected by HPLC with on-line radioactivity detection. These metabolites could be assigned to DK-metribuzin and DA-metribuzin after spiking of dog microsomal samples with reference compounds.	Commission Regulation (EU) No 283/2013, 5.1.1, Purity ^{14}C-labelled Metribuzin: 98.7 %, GLP, Acceptable	M-466751-01-1

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

Studies on absorption, distribution, metabolism and excretion were conducted with ^{14}C-labelled metribuzin at 5-position and 6-position using different doses. The biokinetics and metabolism of metribuzin following oral or intravenous administration in rats was investigated in four studies (M-020240-02-1, M-022071-03-1, M-493116-01-1, M-493124-01-1). Additionally, two *in vitro* metabolism studies were performed with [^{14}C]-metribuzin. One is in rat and human liver microsomes (M-466743-01-1) and a second in dog liver microsomes (M-466751-01-1).

Absorption:

The absorption of metribuzin from the gastrointestinal tract (GIT) was rapid and nearly complete (EFSA, 2010). The systemic bioavailability can be considered as 100 % by comparing low dose oral and i.v. administrations in rats. (M-493116-01-1, M-493124-01-1)

The highest concentration of radioactivity was detected in almost all tissues and plasma sampled four hours after oral administration. For both oral and i.v. administrations the excretion of radioactivity was rapid, the majority being excreted in urine and faeces within 24 h. The amount of radioactivity present in urine and faeces was similar irrespective of administration routes. Although there was a tendency towards higher amounts excreted in faeces in the higher oral dose group, essentially the same metabolic profile was found in urine and faeces. (M-493116-01-1, M-493124-01-1) This is an indication that the metabolites detected in the faeces must have been excreted via the bile into the GIT. It can be therefore concluded that the absorption of metribuzin from GIT was rapid and complete.

Distribution:

The radioactivity administered with metribuzin is widely distributed in the body but with the highest concentrations in liver and kidney (EFSA, 2010). The elimination half-life in plasma was 19.1 – 27.2

hours and in other tissues the elimination half-lives ranged between 18.4 to 33.6 hours (M-020240-02-1).
After the comparison of the single- and repeat-dose groups, changes in toxicokinetics (TK) due to enzymatic induction/inhibition after repeated exposure to unlabeled metribuzin can be ruled out. At 96 h after sacrifice the residue levels found in blood were significantly higher than the ones in other tissues irrespective of dose levels; which indicates that accumulation of radioactivity in the tissues is unlikely. Additionally, the concentrations of radioactivity in blood were found to be almost always higher than the ones in other tissues at all time-intervals after i.v. administration, especially significant at later sampling points (24 h and 7 d); which further indicates the absence of accumulation. (M-022071-03-1, M-493116-01-1, M-493124-01-1)
Metabolism:
Metabolism of metribuzin is extensive (EFSA, 2010). The metabolic profiles in urine and faeces were essentially the same. Based on the isolation and characterisation of the metabolites in faeces and urine the sequence of metribuzin biotransformation proceeded via the following steps (M-022071-03-1):

- deamination,
- dethioalkylation,
- hydroxylation of the t-butyl sidechain,
- conjugation.

In study M-022071-03-1, the predominant metabolite in the excreta of all dose groups was cystein-DA-metribuzin (12.9 to 23.7 % of the administered radioactivity) followed by DADK-metribuzin (4.8 to 10.3 %), DA-metribuzin (4.1 to 9.2 %), DK-metribuzin (3.1 to 6.3 %), t-BuOH-DADK-metribuzin (3.0 to 7.0 %), 3-amino-DA-metribuzin (2.8 to 5.3 %) and t-BuOH-DA-metribuzin (0.8 to 2.5 %). None of the remaining four identified compounds exceeded 1.5 % of the administered radioactivity. In total, 52.3 to 61.6 % of the administered radioactivity was identified. Although significant amounts of radioactivity remained unidentified, it should be noted that these amounts are the sum of several smaller fractions from different extractions and therefore consist of several smaller degradation products. DK- and DA-metribuzin were also detected as metabolites of metribuzin in *in vitro* study with [^{14}C]-Metribuzin in Beagle dog liver microsomes (M-466751-01-1).
The evidence indicates that glucuronidation and sulfation do not play a major role in metabolism or excretion. In contrast, conjugation with glutathione (GSH) followed by conversion to mercapturic acid derivatives appears to play a major role in detoxification and excretion.
Elimination:
For both oral and i.v. administrations the excretion of radioactivity was rapid, the majority being excreted in urine and faeces within 24 h (M-493124-01-1). In general, more than 95 % of the administrated radioactivity was excreted via urine and faeces within 72 hours after dosing (EFSA, 2010). Less than 0.1 % of the orally administered radioactivity was excreted in expired air (M-022071-03-1). Data also indicate the absence of enterohepatic circulation.
Since more than half of the i.v. administrated dose was present in faeces, excretion with bile can be considered to be a major route of elimination (M-493124-01-1).

10 EVALUATION OF HEALTH HAZARDS

Acute toxicity

10.1 Acute toxicity - oral route

Table 7: Summary table of animal studies on acute oral toxicity

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance (purity)	Dose levels, duration of exposure	Value LD_{50}	Reference
Mouse acute	Swiss albino mice,	Metribuzin	Single oral gavage at	1215 mg/kg bw (95 %	M-

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance (purity)	Dose levels, duration of exposure	Value LD_{50}	Reference
oral toxicity OECD 401 GLP Acceptable	5/sex/dose level	technical (97.8 %)	doses of 400, 600, 900 and 1200 mg/kg bw, in vehicle (peanut oil) at a dose volume of 10 mL/kg bw.	confidence limits 729 and 2025 mg/kg bw).	513531-01-1
Acute oral toxicity study in Wistar rats OECD 401 GLP Acceptable	Wistar rats, 5/sex/dose level	Metribuzin technical (97.8 %)	Single oral gavage of 1000, 1600, 2500 mg/kg bw, in peanut oil	2162 mg/kg bw (1332 – 3508 mg/kg bw 95 % confidence limit)	M-513530-01-1
Acute oral toxicity study in rats OECD 401 GLP Acceptable	Wistar rats (Crl:WI(Glx/BRL/Han)GSBR), 6/sex/dose level	Metribuzin / Metribuzin technical (95.3 % (September 1997); 94.2 % (June 1998)	Single oral gavage nominal doses of 0, 100, 200, 500 (measured dose 530 for males/520 for females), 1000 (measured dose 980 for males/990 for females) mg/kg bw in vehicle (0.5 % (w/v) methylcellulose and 0.4 % (w/v) Tween 80 in deionised water (10 mL/kg dose volume).	510 mg/kg for males and 322 mg/kg for females. The no observed effect level (NOEL) is less than 100 mg/kg for both sexes.	M-018181-01-1
The acute oral toxicity to rats and guinea pigs OECD 401 Deviations regarding numerous reporting deficienciesNo information on GLP. Supplementary	Sprague-Dawley rats and albino guinea pigs, 4/sex/dose level	Metribuzin Technical (92.0 %), metabolites DA-metribuzin, DK-metribuzin and DADK-metribuzin and impurities	Single or multiple gavage administrations depending of the volume for a single adminis-tration, dissolved in ethanol-propylene glycol (20:80). Dose levels: Metribuzin: Rat: 593, 889, 1333, 2000 mg/kg; Guinea pig: 196, 246, 307, 384 mg/kg; DA-metribuzin: Rat: Males: 800, 1000, 1250, 1531, Females: 366, 467, 607, 800 mg/kg; DK-metribuzin: Rat: Males: 266, 400, 600, 900; Females: 177, 266, 400, 600 mg/kg; Guinea pig: 158, 198, 250 and 315 mg/kg;	**Rat:** Metribuzin: Males: 1090 mg/kg; Females: 1206 mg/kg; DA-metribuzin: Males: 1118 mg/kg; Females: 468 mg/kg; DK-metribuzin: Males: 541 mg/kg; Females: 266 mg/kg DADK-metribuzin: Males: 701 mg/kg; Females: 822 mg/kg; N-methyl impurity: Males: 1230 mg/kg; Females: 1586 mg/kg; desmethyl impurity: Males: 1074 mg/kg; Females: 1012 mg/kg; **Guinea pig:** Metribuzin: Males: 245 mg/kg; Females:274 mg/kg;	M-019421-01-1

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance (purity)	Dose levels, duration of exposure	Value LD_{50}	Reference
			DADK-metribuzin: Rat: Males: 539, 701, 912, 1185; Females: 592, 770, 1000, 1300 mg/kg; N-Methyl Impurity: Rat: Males: 667, 1000, 1500, 2250; Females: 1200, 1500, 1875, 2343 mg/kg; Desmethyl Impurity: Rat: Males: 359, 621, 1074, 1858; Females: 746, 895, 1074, 1289 mg/kg;	DK-metribuzin: Males: 217 mg/kg; Females: 251 mg/kg	

10.1.1 Short summary and overall relevance of the provided information on acute oral toxicity

Acute toxicity studies with single oral administration were conducted in rats, mice and guinea pigs. Clinical signs were seen in mice from 900 mg/kg bw and in rats already from the lowest doses tested of 100 mg/kg bw.

In the oral acute toxicity study in mice (M-513531-01-1), no toxicity symptoms were observed at doses 400, 600 and 900 mg/kg bw, except for lethargy in one mouse at 900 mg/kg bw on day 2; in high dose (1200 mg/kg bw) lethargy was common while some mice had excessive salivation on day 1 of treatment. Surviving mice have gained body weight. Dead mice either maintained or lost body weight marginally. Except for lung petechiae in a mouse at 900 mg/kg bw dose no other abnormalities were evident.

In the acute oral toxicity study in rats (M-513530-01-1), all rats were lethargic on days 1 and 2 of administration; some had nasal discharge; few had lacrimation. One each in mid (1600 mg/kg bw) and high (2500 mg/kg bw) dose groups had tremors and/or ataxia. Two in high dose (2500 mg/kg/bw) group were weak. No toxicity symptoms were seen beyond 5th day of administration.

All surviving rats had gained body weight. Only one dead rat, gained body weight, while all other dead rats had lost body weight. In the low dose group (1000 mg/kg bw) one rat and in the mid dose group (1600 mg/kg bw) three rats and in high dose (2500 mg/kg bw) group two rats exhibited lung petechiae/congestion. Liver congestion was seen in one of two rats of high dose (2500 mg/kg bw) group.

In the second acute oral toxicity study in rats (M-018181-01-1), compound-related clinical signs were evident in males and females at all dose levels. Occurrence of signs generally increased with dose in both sexes and consisted of the following: decreased activity, fecal staining, labored breathing, red lacrimal staining (males only), red nasal staining, red oral staining, red perigenital staining (males only), salivation, tremors and urine staining (males only). Compound-related signs were first apparent on the day of treatment and generally resolved in surviving animals by day 4. Body weight gain was reduced in surviving males and females at all dose levels. No compound-related gross lesions were observed at necropsy for animals that were sacrificed at term. In the animals that died prior to terminal sacrifice, the following findings were ascribed to treatment to the test substance: evidence of salivation, lacrimation, nasal discharge, ventrum wet/stained, discolored lungs, discolored zones in the stomach, prolapsed penis, and red urine in the bladder.

In the supplementary acute oral toxicity study in rats and guinea pigs (M-019421-01-1), the animals exhibited symptoms of profound non-arousable sedation and a coarse coat, although the incidence was not specified.
The LD50 values in the different acceptable studies and species ranged from the lowest LD50 of 322 mg/kg bw up to 2162 mg/kg bw.

10.1.2 Comparison with the CLP criteria

Classification criteria in CLP Regulation define substances with oral LD50 over 300 and up to and including 2 000 mg/kg bw as Acute Toxicity Category 4 (H302, harmful if swallowed). The studies provided herein for metribuzin confirm the lowest LD50 value is 322 mg/kg bw after oral administration to rats based on mortality. According to the lowest LD50 value in the most sensitive species tested, an ATE of 322 mg/kg bw is warranted.

10.1.3 Conclusion on classification and labelling for acute oral toxicity

The data on the acute toxicity potential of metribuzin are conclusive. Based on the lowest oral LD50 of 322 mg/kg bw after acute oral administration to rats, an acute toxicity classification is warranted according to CLP Regulation, as Acute Toxicity Category 4 (H302, harmful if swallowed). Based on table 3.1.1 of the CLP regulation, an ATE of 322 mg/kg bw is warranted. The minimum classification (Acute Tox. 4*; H302) can therefore be confirmed and the * removed.

10.2 Acute toxicity - dermal route

Table 8: Summary table of animal studies on acute dermal toxicity

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance (purity)	Dose levels, duration of exposure	Value LD_{50}	Reference
Acute dermal toxicity study in rats OECD 402 GLP Acceptable	Wistar rats, 5/sex/dose level	Metribuzin technical (97.8 %)	Single limit dose of 2000 mg/kg bw, animals were exposed for 24 h (slurry with distilled water on a 6 x 10 cm aluminium foil).	> 2000 mg/kg bw (limit test, no mortalities).	M-513532-01-1
Acute dermal toxicity study in rats OECD 402 GLP Acceptable	Wistar rats (Crl:WI(Glx/BRL/Han)GSBR), 6/sex/dose level	Metribuzin technical (95.3 % (September 1997); 94.2 % (June 1998))	Single dermal application of 0 (deionised water) or 5000 mg/kg bw, animals were exposed for 24 h (pads of 36, 40 or 44 cm2 were used depending on the total body surface area).	> 5000 mg/kg bw for males and females (limit dose, no mortalities). The no observed effect level (NOEL) is less than 5000 mg/kg bw for males and 5000 mg/kg bw for females.	M-018180-01-1
Acute dermal toxicity study in rats and rabbits. Non-guideline study.	Sprague-Dawley rats and New Zealand White rabbits, 4/sex/group	METRIBUZIN Technical and METRIBUZIN 50 % Wettable Powder	20000 mg/kg bw METRIBUZIN Technical or 20000 mg formulation/kg	> 20000 mg/kg bw for males and females to both rats and rabbits (limit	M-019688-01-1

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance (purity)	Dose levels, duration of exposure	Value LD_{50}	Reference
No information on GLP Not acceptable		Purity: not reported	bw METRIBUZIN 50 % Wettable Powder, animals were exposed for 24 h.	dose, no mortalities). The no observed effect level (NOEL) is greater than 20000 mg/kg bw for both sexes.	

10.2.1 Short summary and overall relevance of the provided information on acute dermal toxicity

Two acceptable acute dermal toxicity studies with rats were reported (M-513532-01-1 and M-018180-01-1). No mortalities, clinical signs or specific organ damages were observed at the tested doses, only slight effects on the body weight. The lowest resulting dermal LD50 value was > 2000 mg/kg bw.

10.2.2 Comparison with the CLP criteria

According to classification criteria in CLP Regulation, substances with LD50 > 2000 mg/kg bw after dermal exposure do not warrant classification into acute dermal toxicity hazard class.

10.2.3 Conclusion on classification and labelling for acute dermal toxicity

The data on the acute dermal toxicity potential of metribuzin are conclusive. Based on the LD50 values of > 2000 mg/kg bw after acute dermal administration to rats, according to the CLP criteria acute dermal toxicity classification is not warranted.

10.3 Acute toxicity - inhalation route

Table 9: Summary table of animal studies on acute inhalation toxicity

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance (purity), form and particle size (MMAD)	Dose levels, duration of exposure	Value LC_{50}	Reference
Acute inhalation toxicity study in rats OECD 403 GLP Acceptable	Wistar rats, strain Hsd Cpb:WU (SPF), 5/sex/group	DIC 1468 (93.9 %), micronized metribuzin dust, MMAD 3.65 µm (GSD 1.68)	0 and 2.045 mg/L air (nominal concentration 2 mg/L), head-only for 4 hours.	> 2.045 mg/L air in male and female rats (limit test, no mortalities). NO(A)EC < 2.045 mg/L.	M-136509-01-1
Acute inhalation toxicity study in rats In main accordance to OECD 403 Deviations regarding the	Sprague-Dawley rats, 10/sex/group	Metribuzin / Metribuzin (92.6 %), dust, MMAD 5.1 µm (GSD 2.1)	0 and 0.648 mg/L (20.5 mg/L nominal) (highest attainable concentration), single 4-hour head-only exposure.	> 0.648 mg/L for male and female rats (limit test, no mortalities). The no-observable-effect concentration (NOEC) in the	M-018207-02-1

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance (purity), form and particle size (MMAD)	Dose levels, duration of exposure	Value LC_{50}	Reference
particle size and exposure chamber temperature, reporting deficiencies. Non-GLP Supplementary				current study was less than 0.648 mg/L.	
Acute inhalation toxicity study in rats OECD 403 Deviations regarding age of rats, acclimatisation to test apparatus, particle sizing was not performed, reporting of justification for whole-body exposure and other major reporting deficiencies. GLP Not acceptable	Wistar rats, 5/sex/group	Metribuzin technical (97.8 %), aerosol in cyclohexanone (30 % w/v)	0 and 0.532 mg/L (maximum achievable concentration), 4-hour whole-body exposure.	> 0.532 mg/L air (maximum attainable concentration, no mortalities).	M-513533-01-1

10.3.1 Short summary and overall relevance of the provided information on acute inhalation toxicity

One acceptable (M-136509-01-1) and one supplementary (M-018207-02-1) acute inhalation toxicity study in rats are available, from which the LC50 values resulted > 0.648 mg/L and > 2.045 mg/L, respectively, since there were no mortalities observed at the tested doses. Compound-related signs were limited to mild and transient respiratory tract irritation and transient salivation in males and females. There was no effect on body weights. There were no macroscopic abnormalities at examination post mortem.

10.3.2 Comparison with the CLP criteria

The CLP Regulation, classifies substances with LC50 over 1.0 and up to and including 5.0 mg /L after acute inhalational exposure to dusts and mists as acute inhalation toxicity Category 4 (harmful if inhaled). In the provided studies no mortalities were observed at any of the tested doses (up to the highest tested dose of 2.045 mg/L).

10.3.3 Conclusion on classification and labelling for acute inhalation toxicity

The data on the acute inhalation toxicity potential of metribuzin suggest that based on the LD50 values of > 2.045 mg/L after acute inhalative administration to rats, with no mortalities and no severe clinical signs, according to the CLP Regulation, acute inhalation toxicity classification is not warranted.

10.4 Skin corrosion/irritation

Table 10: Summary table of animal studies on skin corrosion/irritation

<table>
<tr><th>Method, guideline, deviations if any</th><th>Species, strain, sex, no/group</th><th>Test substance (purity)</th><th>Dose levels, duration of exposure</th><th>Results
-Observations and time point of onset
-Mean scores/animal
-Reversibility</th><th>Reference</th></tr>
<tr><td>Rabbit skin irritation study OECD 404. Deviations from the guideline regarding minor noncompliance of the reporting and test conditions (temperature range, applications of test substance etc.)
GLP
Acceptable</td><td>New Zealand white rabbits, 2 males and 1 female</td><td>Metribuzin technical (97.8 %)</td><td>500 mg metribuzin, moistened with 0.5 ml distilled water, for 4 h to a dorsolateral thoracic area, on a 4 x 4 cm aluminium foil patch. The skin reaction was assessed at 1, 24, 48 and 72 hours after the removal of the test patch.</td><td>No deaths, clinical signs, differences in bodyweight gain, gross pathological changes or local skin reactions were observed at any time following treatment.
Metribuzin technical is not irritating to rabbit skin.</td><td>M-513568-01-1</td></tr>
<tr><td>Rabbit skin irritation study OECD 404. Deviations from the guideline regarding minor deficiencies of reporting and conduct (age of animals, initial reading at 30 minutes etc)
GLP
Acceptable</td><td>Albino Hra:(NZW)SPF rabbits, 3 males and 3 females</td><td>Metribuzin technical (95.3 %)</td><td>500 mg metribuzin, moistened with 0.5 ml distilled water, to an area of clipped skin on the back, approximately 6.25 cm^2, under a semi-occlusive dressing, approx. 4-h exposure period. Scores were taken at 30 min, 24, 48 and 72 hours after the removal of the test patch.</td><td>Two females were noted with very slight erythema at the 48-h observation interval, no signs of skin irritation by 72-h observation point.
<table>
<tr><td></td><td></td><td colspan="2">Mean score of 24, 48 and 72 h</td></tr>
<tr><td></td><td>Animal number</td><td>Erythema</td><td>Oedema</td></tr>
<tr><td rowspan="3">Males</td><td>1</td><td>0</td><td>0</td></tr>
<tr><td>2</td><td>0</td><td>0</td></tr>
<tr><td>3</td><td>0</td><td>0</td></tr>
<tr><td rowspan="3">Females</td><td>4</td><td>0.3</td><td>0</td></tr>
<tr><td>5</td><td>0</td><td>0</td></tr>
<tr><td>6</td><td>0.3</td><td>0</td></tr>
</table>
Metribuzin was considered only slightly irritating.</td><td>M-018182-01-1</td></tr>
</table>

Table 11: Summary table of human data on skin corrosion/irritation

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

Type of data/report	Test substance (purity)	Relevant information about the study (as applicable)	Observations	Reference
Study on skin reactions after metribuzin exposure in male and female volunteers. Supplementary	Metribuzin samples (not reported)	1) Single application of 500 mg/person (on 2.5 x 2.5 cm of the dorsal skin) for 6 hours; 3 males. Observation period 7 days, 2) Repeated applications of 500 mg/person (on 2.5 x 2.5 cm of the left-hand side of dorsal skin), 6 hours/day, 2 days/week, 4 1/2 weeks (total of 9 exposures) + 10th treatment for 6 hours 10 days after the 9th treatment; 5 males and 2 females.	No skin reactions after 1) and 2).	M-019714-01-2
Patch test in two groups of volunteers Supplementary	Metribuzin (92.1 %) or Analytical Standard (99.6 %)	Patch test in a group of 9 volunteers which worked with metribuzin and had showed skin reactions following the exposure to metribuzin and in another group of 11 volunteers which also worked with metribuzin, but had not shown any skin reaction. Both groups were treated on the forearm with 1 % formulations of metribuzin technical or analytically pure substance with petrolatum (two patches) over 48 hours and observed after that on day 4th and 7th. A second treatment was performed after 14 days with a 5 % formulation of both test substances on those individuals who had negative reaction to the first treatment.	Positive reactions were observed in one person of each group after first application as well as after second application. Differences between both test substances were not found.	M-019744-01-1

10.4.1 Short summary and overall relevance of the provided information on skin corrosion/irritation

Two skin corrosion/irritation studies in rabbits were conducted. One study (M-513568-01-1) did not show signs of skin irritation, whereas in the second rabbit study (M-018182-01-1) two females were noted with very slight erythema at the 48-hour observation interval, which was reversible by the 72-h observation point. Patch tests in human volunteers also did not show skin irritation potential of metribuzin in humans.

10.4.2 Comparison with the CLP criteria

According to CLP Regulation, the criteria for the skin irritation Category 2 classification are: mean score of ≥ 2.3 - ≤ 4.0 for erythema/eschar or for oedema in at least 2 of 3 tested animals from gradings at 24, 48 and 72 hours after patch removal. The Guidance on the Application of the CLP Criteria states, that in the case of 6 rabbits the following applies: classification as skin irritant – Category 2 if at least 4 out of 6 rabbits show a mean score per animal of ≥ 2.3 - ≤ 4.0 for erythema/eschar or for oedema. Two skin corrosion/irritation studies in rabbits were conducted with metribuzin. One study did not show signs of skin irritation, whereas in the second rabbit study 2 female rabbits showed a mean score of 0.3 for erythema. This degree of skin irritation does not warrant classification. This is supported by patch tests in human volunteers which did not show skin irritation potential of metribuzin in humans.

10.4.3 Conclusion on classification and labelling for skin corrosion/irritation

The study results on metribuzin are conclusive and do not warrant classification as skin irritant according to the CLP Regulation. Two skin corrosion/irritation studies in rabbits did not show signs of skin irritation to a degree that would require classification as skin irritant. This is supported by studies in human volunteers which also did not indicate skin-irritating potential in humans and give supplementary information that no classification is warranted.

10.5 Serious eye damage/eye irritation

Table 12: Summary table of animal studies on serious eye damage/eye irritation

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance (purity)	Dose levels, duration of exposure	Results - Observations and time point of onset - Mean scores/animal - Reversibility	Reference
Rabbit eye irritation study, OECD 405. Deviations regarding deficiencies of reporting and conducting, no pretreatment of animals with analgesic. GLP Acceptable	New Zealand White rabbits, 2 males and 1 female	Metribuzin technical (97.8 %)	100 mg of metribuzin technical was placed into the conjunctival sac of the left eye of each rabbit. After 24 h contact period the treated and the control eyes were irrigated with distilled water to remove the residual test compound in the treated eye. Approximately 1, 24, 48, and 72 hours after instillation of the test material, the eyes were observed for ocular irritation.	All animals showed redness and chemosis of the conjunctivae 1 and 24 hours after treatment and this persisted until 48 hours in 2 of the rabbits. Corneal opacity was observed in 1 animal after 24 hours. The eyes of all animals appeared normal within 72 hours. Thus, metribuzin technical is considered to be moderately irritating to the rabbit eye. No individual mean scores presented.	M-513571-01-1
Rabbit eye irritation study,	Albino Hra:(NZW)SPF rabbits, 3	Metribuzin Technica	45.9 mg of Metribuzin technical was	There was no obvious indication of pain and no cornea findings in any animal during the course of the study. The iris of 3 animals showed	M-018184-01-1

<table>
<tr>
<td>OECD 405. Deviations regarding deficiencies in conducting, no pretreatment of animals with analgesic. GLP Acceptable</td>
<td>males and 3 females</td>
<td>l (95.3 %)</td>
<td>placed into the everted lower right eyelid of each rabbit.Approximately 1, 24, 48, and 72 hours after instillation of the test material, the eyes were observed for ocular irritation. Corneal injury was assessed using sodium fluorescein dye on all animals at the 24-hour observation interval and on any animal with a positive result, until resolution.</td>
<td>irritation (score = 1) at the 1 hour reading. Of these animals, all 3 were noted with circumcorneal injection and 1 was noted with congestion. These findings had resolved by the 24 hour reading. At the 1 hour reading, conjunctival redness (score = 1) was noted in all animals. These animals also exhibited chemosis (score = 1 or 2). Discharge (score = 1) was noted in 2 animals. Conjunctival redness (score 1) persisted in all the animals at the 24 hour reading and in 1 animal at the 48 hour reading. There were no additional ocular findings noted for the remainder of the study.
Metribuzin Technical is considered to be mildly irritating to the rabbit eye.
<table>
<tr><td></td><td colspan="4">Mean score of 24, 48 and 72 h</td></tr>
<tr><td>Animal number</td><td>Corneal opacity</td><td>Iritis</td><td>Conjunctival redness</td><td>Conjunctival oedema</td></tr>
<tr><td>1</td><td>0</td><td>0</td><td>0.67</td><td>0</td></tr>
<tr><td>2</td><td>0</td><td>0</td><td>0.33</td><td>0</td></tr>
<tr><td>3</td><td>0</td><td>0</td><td>0.33</td><td>0</td></tr>
<tr><td>4</td><td>0</td><td>0</td><td>0.33</td><td>0</td></tr>
<tr><td>5</td><td>0</td><td>0</td><td>0.33</td><td>0</td></tr>
<tr><td>6</td><td>0</td><td>0</td><td>0.33</td><td>0</td></tr>
</table>
</td>
<td></td>
</tr>
<tr>
<td>Rabbit eye irritation study, OECD 405. GLP Acceptable</td>
<td>New Zealand albino rabbits, 3 females</td>
<td>Metribuzin Technical (97.3 %)</td>
<td>0.1 mL/animal (0.04 g) instillation into the conjunctival sac of the right eye, no rinsing. Ocular irritation was evaluated at 1, 24, 48 and 72 hours and at 4 days post-instillation.</td>
<td>Regarding eye effects there was no corneal opacity or iritis observed during this study. One hour after test substance instillation, all three treated eyes exhibited conjunctivitis. The overall incidence and severity of irritation decreased gradually with time. All animals were free of ocular irritation by Day 4 (study termination). Metribuzin Technical is considered to be mildly irritating to the eye.
<table>
<tr><td></td><td colspan="4">Mean score of 24, 48 and 72 h</td></tr>
<tr><td>Animal number</td><td>Corneal opacity</td><td>Iritis</td><td>Conjunctival redness</td><td>Conjunctival oedema</td></tr>
<tr><td>1</td><td>0</td><td>0</td><td>1.0</td><td>0</td></tr>
<tr><td>2</td><td>0</td><td>0</td><td>1.7</td><td>0</td></tr>
<tr><td>3</td><td>0</td><td>0</td><td>1.0</td><td>0</td></tr>
</table>
</td>
<td>M-538015-01-1</td>
</tr>
</table>

10.5.1 Short summary and overall relevance of the provided information on serious eye damage/eye irritation

Three valid eye irritation studies (M-513571-01-1, M-018184-01-1 and M-538015-01-1) in rabbits are available, where metribuzin was considered to be mildly to moderately irritating to the rabbit eye. In all the studies in all rabbits conjunctival redness was observed at 1 h and 24 h after treatment. By 72 h or 4 days after treatment all rabbit eyes appeared normal in all studies. In all studies chemosis was observed 1 h after treatment, which resolved by 24-72 h. Corneal opacity was observed in one study (M-513571-01-1) 24 h after treatment and in one study (M-018184-01-1) irritation of the iris was observed 1 h after treatment.

10.5.2 Comparison with the CLP criteria

According to the grading criteria as described in CLP Regulation, substances that produce in at least 2 of 3 tested animals, a positive response of: (a) corneal opacity ≥ 1 and/or (b) iritis ≥ 1, and/or (c) conjunctival redness ≥ 2 and/or (d) conjunctival oedema (chemosis) ≥ 2 calculated as the mean scores following grading at 24, 48 and 72 hours after installation of the test material, and which fully reverses within an observation period of 21 days shall be classified as Category 2 eye irritant.
In all the studies the mean scores of 24, 48 and 72 h in all animals were < 1 for corneal opacity and iritis and ≤ 1 for conjunctival oedema, the mean scores for conjunctival redness were < 2 in all animals. Based on the available data the mean eye irritation scores for metribuzin do not fulfil the criteria given in the CLP.

10.5.3 Conclusion on classification and labelling for serious eye damage/eye irritation

The results of the three valid eye irritation studies in rabbits are conclusive. Metribuzin technical was characterized as mildly to moderately irritating to the rabbit eye but does not fulfil the aforementioned criteria and does not warrant an eye irritation classification according to the CLP Regulation.

10.6 Respiratory sensitisation

Table 13: Summary table of animal studies on respiratory sensitisation

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels, duration of exposure	Results	Reference
Acute oral, dermal, inhalative toxicity, eye and dermal irritation studies.				No evidence of a respiratory sensitisation potential.	See Sections 10.1-10.5

10.6.1 Short summary and overall relevance of the provided information on respiratory sensitisation

No relevant human data are available. No formally recognised and validated animal or *in vitro* tests currently exist for respiratory sensitisation.
In the acute oral, dermal, inhalative toxicity, eye and dermal irritation studies in animals conducted with metribuzin (see Sections 10.1-10.5), no evidence of respiratory tract irritation (local cytotoxic effects) or of functional impairment of the respiratory system was observed. In the provided skin sensitisation studies (2 Buehler assays and 1 guinea pig maximisation test, see Section 10.7) metribuzin showed no skin sensitising potential. It is stated in the Guidance on the Application of the CLP Criteria that where there is convincing negative data in the LLNA on a substance, the lack of potential for respiratory allergy is also most probable. No evidence of a respiratory sensitisation potential of metribuzin exists. Also human studies in volunteers did not indicate an irritating potential on skin and mucous membranes, a respiratory irritation potential of metribuzin is therefore not considered likely.

10.6.2 Comparison with the CLP criteria

According to the classification criteria in CLP Regulation for respiratory sensitisation, effects seen in either humans or animals will normally justify classification in a weight of evidence approach for respiratory. No formally recognised and validated animal or *in vitro* tests currently exist for

respiratory sensitisation. The Guidance on the Application of the CLP Criteria states that data from some animal studies may be indicative of the potential of a substance to cause respiratory sensitisation in humans.
In the available animal studies with metribuzin, no evidence of respiratory sensitisation was observed. Furthermore, human volunteer studies with single and repeated dermal application did not indicate a severe irritating or corrosive potential on skin and mucous membranes, so that a respiratory irritation potential of metribuzin is not considered likely.

10.6.3 Conclusion on classification and labelling for respiratory sensitisation

Since there is no relevant human data available and in the animal studies with metribuzin no evidence of respiratory tract irritation was obvious there are no grounds for a respiratory sensitisation classification of metribuzin according to the CLP Regulation.

10.7 Skin sensitisation

Table 14: Summary table of animal studies on skin sensitisation

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance (purity)	Dose levels, duration of exposure	Results	Reference
Guinea pig skin sensitisation (Buehler test) OECD 406. Major deviations: Animal number and dose level selection not according to the guideline, strain of guinea pigs not reported, application of test substance on aluminum foil not cotton pad, reporting deficiencies. GLP Acceptable	Guinea pigs: Albino, test group: 3 males and 7 females; vehicle control group: 4 males and 6 females; positive control (dinitrochlorobenzene) group: 2 males and 3 females	Metribuzin technical (97.8 %)	500 mg of the test compound slurry with normal saline on a 4 cm x 4 cm aluminium foil to prepared area (2 x 2 cm). After 3 inductions with 7-day intervals the animals were challenged, 14 days after the last induction. Contact period for induction and challenge: 6 hours. Skin reaction was evaluated 1 and 24 h after induction and 24, 48 and 72 h after challenge.	No skin sensitisation, no dermal reactions in any test or control animal observed.	M-513573-01-1
Guinea pig skin sensitisation (Maximisation Test according to Magnusson and Kligman) OECD 406 GLP Acceptable	Guinea pigs (Hsd Poc:DH), test group: 20 females; control group: 10 females; dose range finding group: 2 females for challenge and 5 females for induction; positive control: alpha-Hexylzirntaldehyd	DIC 1468 technical (94.3 %)	5 % solution (20 mg/animal) in polyethylene glycol 400 (PEG 400) for the induction via 3 intradermal injections and 50 % solution (250 mg/animal) in PEG 400 for	There were no signs of skin sensitisation following challenge with DIC 1468.	M-066574-01-1

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance (purity)	Dose levels, duration of exposure	Results	Reference
			the topical induction and challenge. Injection sites were visually assessed 2 and 7 days after injection. Topical induction was performed 1 week after the intradermal induction and challenge was 3 weeks after the intradermal induction. Contact period for induction and challenge applications were 48 and 24 hours, respectively. After removal of the patches, the challenge sites were evaluated after 48 and 72 hours.		
Guinea pig skin sensitisation study (Buehler test) OECD 406 Major deviations: there are no results included for a positive control substance/sensitivity and reliability test, marginal deviations of temperature range, only 12 guinea pigs were used, reporting deficiencies. GLP Supplementary	Guinea pigs (DHPW), test group: 12 males; control group: 12 males; dose range finding group: 5 females	DIC 1468 technical (93.5 %)	Induction topical: 0.5 mL of 50 % (w/v) suspensions with Cremophor EL in physiological saline solution (2 % v/v) 3x in 7-day intervals. Challenge topical: 0.5 mL of 50 % (w/v) suspensions with Cremophor EL in physiological saline solution (2 % v/v) 2 weeks after last induction	No skin sensitisation, no dermal reactions in any test or control animal.	M-018244-01-1

Table 15: Summary table of human data on skin sensitisation

Type of data/report	Test substance (purity)	Relevant information about the study (as applicable)	Observations	Reference
Study on skin reactions after metribuzin exposure in male and female volunteers Supplementary	See Table 11	See Table 11	No skin reactions after 1) and 2) and thus no signs of skin sensitisation.	M-019714-01-2
Patch test in two groups of volunteers Supplementary	See Table 11	See Table 11	Positive reactions were observed in one person of each group. The test substance is not a strong skin sensitiser.	M-019744-01-1

10.7.1 Short summary and overall relevance of the provided information on skin sensitisation

Three studies on skin sensitisation in guinea pigs are available: two skin sensitisation studies using the Buehler Test (M-513573-01-1, M-018244-01-1) and one Guinea Pig Maximisation test according to Magnusson and Kligman (M-066574-01-1).

In the Guinea Pig Maximisation test (M-066574-01-1), 5 % metribuzin in polyethylene glycol 400 (PEG 400) was injected intradermally and after one week, 50 % metribuzin in PEG 400 was applied topically for 48 h, and after two weeks, a challenge dose of 50 % metribuzin was applied topically for 24 h. No signs or effects on body weight were observed. There were no signs of skin sensitisation. Thus, under the conditions of the Guinea Pig Maximisation test, metribuzin did not show any sensitising potential in guinea pigs.

In one Buehler Test (M-513573-01-1), 500 mg of metribuzin slurry in normal saline was topically applied for 6 h once a week for three weeks and two weeks after the last induction dose, the challenge dose was applied. There were no treatment-related effects as a result of the induction or the challenge in the vehicle control or metribuzin treated animals. No mortality, toxicological symptoms or abnormal body weight gains were observed. Challenge of the positive control group with 0.4 mL of 0.15 % DNCB produced clear evidence of skin sensitisation.

In the second, supplementary Buehler Test (M-018244-01-1) with metribuzin, a 50 % formulation of the test substance in Cremophor EL in physiological saline solution (2 % v/v) was applied topically for 6 h once a week for three weeks and after a two-week rest period a challenge dose at the highest non irritating concentration (HNIC, 50 %) was applied. There were no dermal reactions as a result of the challenge exposure in the control or test animals.

Therefore, under the conditions of the provided tests metribuzin did not show sensitising potential in guinea pigs.

10.7.2 Comparison with the CLP criteria

According to The Guidance on the Application of the CLP Criteria, effects seen either in animals or in humans will normally justify classification in a weight of evidence approach for skin sensitisers. According to CLP there are three standard animal test methods used to evaluate skin sensitisation for substances: the mouse local lymph node assay (LLNA), the guinea pig maximisation test (GPMT) and the Buehler assay.

For metribuzin three animal studies did not indicate a skin sensitising potential, which is further supported by no evidence of a sensitising potential in humans.

10.7.3 Conclusion on classification and labelling for skin sensitisation

No evidence for metribuzin of a sensitising potential in humans and also no evidence from animal studies exists, the data are conclusive and do not warrant a skin sensitisation classification of metribuzin according to the CLP Regulation.

10.8 Germ cell mutagenicity

Table 16: Summary table of mutagenicity/genotoxicity tests *in vitro* in microbial cells

Method, guideline, deviations if any	Test substance (purity)	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
Salmonella typhimurium reverse mutation assay (Ames Test) OECD 471 GLP Acceptable	Metribuzin technical (91.9 % w/w) dissolved in DMSO	*Salmonella typhimurium* strains TA1535, TA1537, TA98, TA100, and TA102. Pre-experiment/Experiment I (incorporation test): 3; 10; 33; 100; 333; 1000; 2500; and 5000 µg/plate. Experiment II (pre-incubation test): 10; 33; 100; 333; 1000; 2500; and 5000 µg/plate. Both experiments were performed with and without liver microsomal activation (S9 mix).	Negative No substantial increase in revertant colony numbers of any of the five tested strains at any dose level, neither in the presence nor absence of metabolic activation (S9 mix). In experiment I, an increase in revertant colony numbers was observed in strain TA1535 with S9 mix. Since the threshold of thrice the number of the corresponding solvent control was not reached and the effect was not reproduced in experiment II, it can be stated that these increases are based on biological fluctuations and do not indicate a true mutagenic potential.	M-589742-01-1
Mutagenicity test on bacterial systems: (1) Rec-assay: *Bacillus subtilis* (NIG17 and NIG45 strains) (2) Reversion assay: with metabolic activation using four *Salmonella typhimurium* strains (TA1535, TA1537, TA98 and TA100) Pre-OECD 471 Deviations regarding strains used, insufficient concentrations of test material used, no information on no of replicates, other major deficiencies in	Technical grade BAY 94337 (Metribuzin) (93.7 %) dissolved in DMSO	(1) Rec assay: 3, 30, 300 µg/disc. (2) Reversion assay: 0; 2; 20; 500 µg/plate + S9 of liver homogenate from rats or mice were added.	Negative BAY 94337 showed no mutagenic action to the two strains of *Bacillus subtilis* in the rec-assay (1) and to the four strains of *Salmonella typhimurium* in the reversion assay (2) with the S-9 fraction of rat or mouse liver homogenate.	M-021844-01-1

Method, guideline, deviations if any	Test substance (purity)	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
conducting and reporting. Non-GLP Supplementary				
Mutagenicity test on bacterial systems: (1) Rec-assay using *Bacillus subtilis* strains H17 and M45; (2) Reversion assay with metabolic activation using *Escherichia coli* strain WP2 hcr and *Salmonella typhimurium* strains (TA98, TA100, TA1535, TA1537 and TA1538) Pre- OECD 471. Deviations regarding insufficient information on test item, only duplicates tested, other deficiencies in reporting. Non-GLP Supplementary	Metribuzin (93.3 %) dissolved in DMSO	(1) Rec-assay: 0, 20, 100, 200, 500, 1000 and 2000 µg/disc. (2) Reversion assay: 0, 10, 50, 100, 500, 1000 and 5000 µg/ plate with and without activation (S9 mix).	Negative (1) Rec-assay: With metribuzin, the growth of H 17 and M 45 strains of *B. subtilis* was not inhibited. (2) Reversion assay: With metribuzin, no increase of reversed colonies was identified. It was concluded that under the experimental conditions metribuzin gave negative results in rec-assay and reversion assay.	M-021853-01-1
S. cerevisiae D7 test for determination of point mutations. *Saccharomyces cerevisiae* D7 OECD 481 GLP Supplementary	DIC 1468 (94.7 % w/w); dissolved in DMSO.	Test substance was used in concentrations of 625, 1250, 2500, 5000 and 10000 µg/mL both with and without metabolic activation.	Negative Concentrations up to 10000 µg/mL did not produce an increase in the mutant frequency. Evidence for induction of mitotic recombination by metribuzin was not found.	M-017982-01-1

Table 17: Summary table of mutagenicity/genotoxicity tests *in vitro* in mammalian cells

Method, guideline, deviations if any	Test substance (purity)	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
In vitro Gene Mutation Assay in Chinese Hamster V79 Cells (V79/HPRT) OECD 476 GLP Acceptable	Metribuzin, (91.9 % w/w) dissolved in DMSO	Experiment I: 37.5, 75.0, 150.0, 300.0, 600.0, 1000.0 µg/ mL. Experiment II: 25.0, 50.0, 100.0, 200.0, 400.0, 600.0, 800.0, 1000.0 µg/mL.	Negative No substantial and reproducible dose dependent increase of the mutation frequency was observed in the main experiment with and without metabolic activation.	M-601206-01-1

Method, guideline, deviations if any	Test substance (purity)	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
			Metribuzin did not induce gene mutations at the HPRT locus in V79 cells and thus is considered to be non-mutagenic in this HPRT assay.	
In vitro Micronucleus Test in Human Lymphocytes OECD 487 GLP Acceptable	Metribuzin technical (91.9 % w/w) dissolved in DMSO	The assay was conducted at dose levels of 14.1; 24.7; 43.3; 75.8; 133; 232; 406; 711; 1243; 2176 µg/mL.	Negative In the absence of S9 mix, the highest evaluated concentration (1243 µg/mL) showed an increase in the number of micronucleated cells above the historical control data range. The value is considered not statistically significant and there is no dose-dependency, the finding is regarded as biologically irrelevant. In Experiment IB in the presence of S9 mix, all evaluated concentrations were slightly above the historical control data range, however, most of the values were not statistically significantly increased and no dose-dependency was visible. One isolated statistically significant increase in the number of micronucleated cells (1.53 %) was observed after treatment with 406 µg/mL of the test item, however, without any dose-dependency. In Experiment II in the absence and presence of S9 mix, no relevant increases in micronucleated cells were observed. Therefore, Metribuzin technical is considered to be non-mutagenic in this *in vitro* micronucleus test, when tested up to precipitating concentrations.	M-604618-01-1
CHO/HGPRT mutation assay in the presence and absence of exogenous	Metribuzin, (92.6 %) dissolved in acetone	The assay was conducted at dose levels of 1000, 900, 800, 700 and 600 µg/mL in the non-activated study and	Negative	M-018190-01-1

Method, guideline, deviations if any	Test substance (purity)	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
metabolic activation OECD 476 Deviations regarding insufficient information on cell line, inappropriate concentrations of test substance and number of cells, reporting deficiencies. GLP Supplementary		at 200, 175, 150, 100 and 50 µg/mL in the presence of Aroclor-induced rat liver S-9 activation system.		
Unscheduled DNA synthesis in rat primary hepatocytes OECD 482 (Guideline deleted 2014) GLP Supplementary	METRIBUZIN technical (92.6 %) in ethanol	At seven concentrations of 0, 0.07, 0.7, 6.7, 20, 100 and 200 µg/mL	Negative None of the test compound doses caused a significant increase in the mean net nuclear counts.	M-018186-01-1
Chromosome aberrations in Chinese hamster ovary (CHO) cells OECD 473 Deviations regarding insufficient information on cell line and no historical control data, inadequate number of cells tested, aneuploidy not addressed. GLP Supplementary	Metribuzin technical (93 %) dissolved in DMSO	Test substance concentrations used: **Preliminary cytotoxicity study**: 9 concentrations: 0.5, 1.5, 5, 15, 50, 150, 500, 1510 and 5000 µg/mL; **In absence of S9 mix:** **Initial experiment:** 100, 200, 400, and 800 µg/mL; **In presence of S9 mix:** **Initial experiment:** 200, 400, 800 and 1600 µg/mL; **In presence of S9 mix:** **Confirmatory experiment:** 1024, 1280, 1600 and 2000 µg/mL;	Negative in the non-activated system (-S9). Positive in the S-9 activated system (+S9), only at dose levels toxic to the cells. No increase in chromosome aberrations was observed in any test compound-treated group for the non-activated test system. In the S-9 activated studies, statistically significant increases were observed only at dose levels toxic to the cells as demonstrated by cell cycle delay in the range finding study and altered morphology of the cell monolayer at the time of harvest in the definitive studies.	M-018082-01-1
In vitro cytogenetic assay measuring chromosomal aberration frequencies in Chinese hamster ovary (CHO) cells OECD 473 Deviations regarding deficient measurement	Metribuzin technical (93.0 %) dissolved in ethanol	Concentrations of 0.584 µg/mL to 1750 µg/mL in a half-log series was tested in the range finding assays with and without metabolic activation. Cell cycle delays or marked toxicity was observed in the nonactivation assay at doses	Negative (-S9) Positive (+S9, only at a dose level toxic to the cells) No significant increase in chromosomally aberrant cells was observed without S9 mix. An increase in chromosomally aberrant	M-018202-01-1

Method, guideline, deviations if any	Test substance (purity)	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
of cytotoxicity, inadequate number of cells scored, aneuploidy not addressed, no historical control data and other deficiencies in conducting and reporting. GLP Supplementary		from 175 µg/mL. In the assay with activation, cell cycle delay or marked toxicity was observed at doses from 58.4 µg/mL. Hence, in the main study, replicate cultures of CHO cells were incubated with 99.7 to 598 µg/ml for 20 hours without metabolic activation, and with 50 to 200 µg/mL with metabolic activation. Concentrations of 25 to 50 µg/mL were tested with a 10 hour exposure with metabolic activation.	cells was observed at the highest dose with analysable cells with S9 mix, severe toxicity was exhibited at this concentration with unhealthy cell monolayer, floating dead cells, severe reduction in visible mitotic cells and reduction of -70 % in the cell monolayer observed at the time of harvest and the presence of dead cells and sparse metaphases on the prepared slides. The test article, METRIBUZIN Technical, was considered negative for inducing chromosomal aberrations in CHO cells under the nonactivation conditions of this assay. In the metabolic activation portion of this assay, a significant increase in chromosomally aberrant cells was observed at the highest dose with analyzable cells.	

Table 18: Summary table of mutagenicity/genotoxicity tests in mammalian somatic or germ cells *in vivo*

Method, guideline, deviations if any	Test substance (purity)	Relevant information about the study (as applicable)	Observations	Reference
Micronucleus test on the Mouse (5 male and 5 female Bor: NMRI (SPF Han)/group) Pre-OECD 474 Deviations regarding insufficient number of cells tested and sampling time of bone marrow, no	DIC 1468 (93.3 %) in 0.5 % aqueous Tylose solution (hydroxyethyl methyl cellulose)	Metribuzin was evaluated for possible mutagenic effect on the chromosome in bone marrow polychromatic erythrocytes of NMRI mice orally at doses of 2 x 200 mg/kg bw or 2 x 400 mg/kg bw.	Negative. The results for DIC 1468 showed that no indications of mutagenic effects were found in doses up to 2 x 400 mg/kg bw. The treated mice showed no signs of damage and all survived. There were no compound-induced mortalities and no indications of mutagenic effects. Erythrocyte formation, measured by the ratio of polychromatic to normochromatic erythrocytes, was also not adversely affected.	M-018461-01-1

Method, guideline, deviations if any	Test substance (purity)	Relevant information about the study (as applicable)	Observations	Reference
historical control data, reporting deficiencies. Non-GLP Supplementary				
Micronucleus test in Swiss albino mice (5 male and 5 female young Swiss albino mice/dose) OECD 474 Deviations regarding insufficient number of cells scored, only high dose evaluated, no historical control data. GLP Supplementary	Metribuzin technical (97.8 %) in peanut oil	The test substance was administered by gavage at 0, 30, 100 and 300 mg/ kg bw for two consecutive days. The occurrence of micronuclei was studied in the erythrocytes sampled from the bone marrow.	Negative The number and percentage of micronucleated PCEs, NCEs and RBCs at 300 mg/kg bw were comparable to their respective control values. The PCE:NCE ratio was reduced at 300 mg/kg bw (statistically significant only in females), which is indicative of the cytotoxicity of the test compound and indicates that the test article had systemically reached the bone marrow cells.	M-513626-01-1
In vivo mammalian bone marrow cytogenetic test – Chromosomal analysis in Swiss albino mice (5 male and 5 female young Swiss albino mice/dose) OECD 475 Deviations regarding inadequate dosing and sampling times, insufficient number of cells analysed, only high dose evaluated, no historical control data. GLP Supplementary	Metribuzin technical (97.8 %) in refined groundnut oil	The test substance was administered by oral gavage at 0, 30, 100 and 300 mg/kg bw once a day for 2 consecutive days.	Negative No increase in the incidence of individual aberrations or total number of metaphases with aberrations in either males or females at the highest dose tested, slight (not statistically significant) reduction in mitotic index at the high dose in both sexes and for the sexes combined. Metribuzin technical is not mutagenic up to 300 mg/kg bw in the *in vivo* chromosomal aberration study in mice. NOAEL (structural changes in chromosomes) > 300 mg/kg bw	M-513623-01-1

Method, guideline, deviations if any	Test substance (purity)	Relevant information about the study (as applicable)	Observations	Reference
In vivo cyto-genetic test on spermatogonia of Chinese hamster (8 males/dose) Pre-OECD 483 Deviations regarding inadequate number of metaphases examined, only one treatment group, only one sampling time, no information on test material stability. Non-GLP Supplementary	Metribuzin (99.5 %) in 0.5 % aqueous Tylose suspension	The doses were 2 x 100 mg/kg bw at an interval of 24 hours, orally.	Negative Aberrations occurred in the untreated negative control group (1.85 % incl. gaps, 0.62 % less gaps), no translocations in the control group. In the test substance group, the frequencies of the aberrant metaphases practically corresponded to the control results (1.85 % incl. gaps, 0.86 % less gaps). Thus, the results did not differ significantly from the control data. Under these test conditions, Metribuzin had no mutagenic effects on Chinese hamster spermatogonia.	M-018340-01-1
Dominant Lethal test in female mice NMRI mice (38/dose, 37/control) Pre- OECD 478 Deviations regarding testing in females instead of males. Non-GLP Supplementary	Metribuzin (99.5 %) in 0.5 % Cremophor emulsion	The test substance was administered at 300 mg/kg bw orally. Immediately after administration, the females were mated with untreated males.	Negative No indication of a mutagenic effect at the test concentration and no effects on ovulation, fertilization, cleavage and early blasto-cysts. Mild drowsiness in the females on the day of application was seen which did not have any adverse effects on the fertilisation quota, number of corpora lutea per female, implantation quota, or on the number of living and dead embryos per female. The statistical analysis of the pre- and post-implantation losses revealed that the treated group had significantly smaller losses than the control group, which was considered to be fortuitous. Thus, no indication of the test substance having a mutagenic effect at the dose level of 300 mg/kg bw.	M-018335-01-1
Dominant lethal study on male mice to test for mutagenic effects NMRI mice (22/dose, 20/control)	Metribuzin (99.5 %) at 1.5 % in a 2.5 % Cremophor emulsion.	Single dose of 300 mg/kg bw administered by oral gavage to male mice. After dosing, each male was mated with 3 untreated females. To examine the successive germ cell stages of the males, at the end of each week the females were	Negative No significant differences were seen between the Metribuzin-treated group and the control group with respect to the fertilization quota (% fertlized females of total number on females), post-implantation loss, pre-implantation loss, total implantations. Thus, no indication of a mutagenic effect of Metribuzin at	M-018338-01-1

Method, guideline, deviations if any	Test substance (purity)	Relevant information about the study (as applicable)	Observations	Reference
Pre-OECD 478 Deviations regarding mating of animals, only one treatment group, no information on test material stability or on positive control. Non-GLP Supplementary		removed from the cage and replaced by another group of 3 untreated females for insemination by the respective male which continued for 8 consecutive weeks. The examination of the uteri of the females (480 per test group) was performed about Gestation Day 14.	the acute oral dose of 300 mg/kg bw was observed. 300 mg/kg bw was considered a level of slight toxicity to the animals (light, brief somnolence after administration of Metribuzin).	
Additional Dominant lethal study on male mice to test for mutagenic effects by an improved method (50/group) Pre-OECD 478 Deviations regarding inadequate number and duration of mating periods, only one treatment group, no information on test material stability or on positive control. Non-GLP Supplementary	Metribuzin (99.5 %) at 3.0 % in a 1.0 % aqueous Cremophor emulsion	Single oral dose of 300 mg/kg bw was administered. Test with a new methodology for dominant lethal assays: 4-day mating schedule and a 1:1 mode of mating, which is an improvement over the previous procedure (8-day mating schedule, 1:3 mating mode) and increases the sensitivity of the test. This study comprised only 5 mating periods (20 days) after test compound administration since this is the most sensitive phase in the dominant lethal test on male mice.	Negative This additional dominant lethal test on the male mouse by a more sensitive method provided no indication of a mutagenic effect at the acute oral Metribuzin dose of 300 mg/kg bw.	M-018344-01-1

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

The mutagenicity and genotoxicity of metribuzin has been investigated in a range of unpublished tests that included *in vitro* tests in microbial cells, *in vitro* tests in mammalian cells as well as *in vivo* tests in mammals.

4 *in vitro* tests, in microbial systems were provided, including an Ames test (M-589742-01-1) from 2017 with *Salmonella typhimurium* strains TA1535, TA1537, TA98, TA100, and TA102. The other *in vitro* studies were older and considered as supplementary studies, including two pre-OECD 471

guideline bacterial reverse mutations assays (M-021844-01-1, M-021853-01-1), and a mitotic gene conversion assay in *S. cerevisiae* (D7 strain) (M-017982-01-1). In these studies, liver S9 mix from rats or mice was used for metabolic activation. The results of the Ames tests and *S. cerevisiae* point mutagenic test were negative. The studies are compiled in Table 16.

6 *in vitro* mammalian cells tests (compiled in Table 17) were provided, including a Gene Mutation Assay in Chinese Hamster V79 Cells (V79/HPRT) according to OECD TG 476 from 2017 (M-601206-01-1), which was negative, and also a Micronucleus Test in Human Lymphocytes according to OECD TG 487 (M-604618-01-1), which was also negative. In addition, an older *in vitro* Gene Mutation Assay in Chinese Hamster V79 Cells (M-018190-01-1) and an Unscheduled DNA synthesis assay in rat primary hepatocytes (M-018186-01-1) were provided, which gave negative results but were considered supplementary because of deviations from current guidelines. In two *in vitro* Chinese hamster ovary cell assays (M-018082-01-1; M-018202-01-1), chromosomal clastogenic effects were observed at cytotoxic doses in the presence of S9 activation, however these results were not confirmed in *in vivo* tests.

Genotoxicity of metribusin was assessed in 3 *in vivo* rodent studies in somatic cells, and 4 *in vivo* rodent studies in germ cells (compiled in Table 18). All of the *in vivo* studies were considered supplementary because of deviations. The chromosome aberration test in mouse bone marrow cells (M-018461-01-1) and the two micronucleus tests in erythrocytes from mouse bone marrow (M-513626-01-1, M-513623-01-1) were negative. Also, the cytogenetic test on spermatogonial cells from Chinese hamster (M-018340-01-1), and the three dominant lethal tests in mice (M-018335-01-1, M-018338-01-1, M-018344-01-1) were negative.

In addition, the literature review revealed three publications comprising non-GLP, non-guideline *in vitro* genotoxicity tests. Two of them investigated genotoxicity of metribuzin without or with metabolic activation by *Vicia faba* roots / root extracts (S10-fraction) in human lymphocytes via sister chromatid exchange (SCE) or Comet assay, respectively. The publication on SCE´s was reliable with restrictions, the one on the Comet assays was not reliable. In both cases, genotoxic effects were only observed after incubation of lymphocytes with metribuzin with metabolic activation by *Vicia faba* roots / root extracts (S10-fraction), and this test system is non-relevant for the evaluation of mammalian genotoxicity and for the pre-emergence or pre-sowing application of metribuzin. The third publication, a micronucleus test *in vitro*, was also not reliable, and, as such, not relevant (M-520018-01-1, M-520024-01-1, M-520026-01-1).

10.8.2 Comparison with the CLP criteria

According to CLP Regulation, the classification in germ cell mutagens Category 1A is based on positive evidence from human epidemiological studies. The classification in Category 1B is based on positive results from *in vivo* heritable germ cell mutagenicity tests in mammals; or *in vivo* somatic cell mutagenicity tests in mammals; or from tests showing mutagenic effects in the germ cells of humans, without demonstration of transmission to progeny. The classification in Category 2 is based on positive evidence obtained from somatic cell mutagenicity tests *in vivo*, in mammals; or other *in vivo* somatic cell genotoxicity tests which are supported by positive results from *in vitro* mutagenicity assays.

There is no positive evidence for mutagenicity/genotoxicity of metribuzin coming from epidemiological studies; or positive results from *in vivo* heritable germ cell mutagenicity tests in mammals or *in vivo* somatic cell mutagenicity tests in mammals. Also, the majority of the *in vitro* genotoxicity tests were negative. The negative results from available guideline-compliant *in vitro* assays were supported by negative results from supplementary *in vivo* somatic and germ cells mutagenicity assays. Classification criterion for substances which show chemical structure activity relationship to known germ cell mutagens is also not met since metribuzin does not show a chemical structure activity relationship to known germ cell mutagens.

Therefore, based on the available evidence, metribuzin does not meet the criteria for germ cell mutagenicity classification.

10.8.3 Conclusion on classification and labelling for germ cell mutagenicity

The study results on metribuzin are conclusive and do not warrant classification for germ cell mutagenicity according to the CLP Regulation. Results of the conducted genotoxicity studies with metribuzin did not reveal a genotoxic potential.

10.9 Carcinogenicity

Table 19: Summary table of animal studies on carcinogenicity

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance (purity), dose levels, duration of exposure	Results (Effects statistically significantly different unless stated otherwise)	Reference
A Combined Chronic Toxicity/ Oncogenicity Feeding Toxicity Study in the Rat Fischer 344 rat (50/dose/sex, interim sacrifice: 10 or 20/dose/sex for low-/mid-dose and high-dose, respectively) OECD 453 Deviations regarding epididymides and uterus not weighed and prothrombin and activated partial thromboplastin times not investigated. GLP Acceptable	Technical grade metribuzin (92.1-93.0 %) dissolved in ethanol, dietary concentrations of 0, 30, 300 and 900 ppm to groups of 50 animals/dose/sex (equivalent to 0, 1.3, 13.8 and 42.2 mg/kg bw/d for males and 0, 1.6, 17.7 and 53.6 mg/kg bw/d for females). Interim sacrifice groups of 20/sex (control and 900 ppm) or 10/sex (30 and 300 ppm) were treated up to two years.	**30 ppm (males 1.3 and females 1.6 mg/kg bw/day):** Clinical chemistry: ↓ AlP (males -11% at day 364); ↓ ALT (males -11% at day 91 and at day 364); ↑ cholesterol (males +9% at day 182); ↓ T3 (males -17% at day 91; -19% at day 182; -15% at day 364; females -16% at day 91; -23% at day 182; -26% at day 546); ↑ T4 (males +57% at day 91; +48% at day 182; +41% at day 364; +21% at day 546; females +28% at day 91; +27% at day 182; +93% at day 364; +22% at day 546; +42% at day 728); **300 ppm (males 13.8 and females 17.7 mg/kg bw/day):** ↓ bodyweight (females -4% at day 182, 6% at days 364, 546 and 728); ↓ bw gain (males 4%, females 12 %); Clinical chemistry: ↓ AlP (males -7% at day 182; -13% at day 364; -26% at day 546; females -15% at day 91; -23% at day 546; -15% at day 728); ↓ ALT (males -15% at days 91, 182 and 364; females -25% at day 91; -24% at day 546); ↓ AST (males -10% at day 91, -13% at day 182; -13% at day 364; females -15% at day 91; -21% at day 546); ↑ cholesterol (males +18% at day 91; +19% at day 182; females +17% at day 91; +19% at day 182; +13% at day 364; +14% at day 546); ↓ T3 (males -37% at day 182; -24% at day 364; -16% at day 546; -22% at day 728; females -16% at day 182; -13% at day 546; -16% at day 728); ↑ T4 (males +25% at day 91; +41% at day 182; +43% at day 364; females +30% at day 91; +31% at day 182; +84% at day 364; +32% at day 546; +57% at day 728); Pathology: Enlarged thyroid (males 7/50, females 1/50 animals); Enlarged adrenals (males 3/50; females 1/50 animals); ↑ absolute thyroid wt. (females +19%); Histopathology: thyroid follicular cell hyperplasia (males 4/10 animals, 1-yr); **900 ppm (males 42.2 and females 53.6 mg/kg bw/day):** ↓ bodyweight (males -3% at day 364, -6% at day 546, -7% at day 728 and females -10% at days 182 and 364, -12% at day 546, -13% at day 728); ↓ bw gain (males -14% and females -27%); Hematology: ↑ platelet counts (males +6% at day 182; females +8% at day 182, +5% at day 546); Clinical chemistry:	M-017948-02-1

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance (purity), dose levels, duration of exposure	Results (Effects statistically significantly different unless stated otherwise)	Reference
		↓ AlP (males -12% at day 91; -14% at day 182; -12% at day 364; -29% at day 546; females -18% at day 91; -17% at day 182; -35% at day 546; -29% at day 728); ↓ ALT (males -16% at days 91and 182; -23% at day 364; -16% at day 546; females -20% at day 91;); ↓ AST (males -10% at day 91; -25% at day 182; -12% at day 364; females -16% at day 91; -20% at day 182); ↑ cholesterol (males +31% at day 91; +44% at day 182; +19% at day 364; females +27% at day 91; +19% at day 182; +28% at day 364; +44% at day 546); ↓ T3 (males -17% at day 182; -27% at day 728; females -11% at day 182); ↑ T4 (males +7% at day 182, +27% at day 364; +29% at day 546; females +47% at day 364; +36% at day 728); Pathology: Enlarged thyroid (males 8/50, females 3/50 animals); Enlarged adrenals (males 5/50 animals); ↑ absolute thyroid wt. (males +24% and females +14%); Histopathology: thyroid follicular cell hyperplasia (males 11/20 animals, 1-yr; 38/50 animals, 2-yr). **Neoplastic findings:** No evidence of metribuzin-induced neoplasia was found in this study. For the entire study, a minimal, diffuse follicular hyperplasia of the thyroid gland was the single lesion observed microscopically that was attributed to exposure to the test substance.	
Combined chronic toxicity and carcinogenicity study in Wistar rats (50/dose/sex, interim sacrifice: 20/10 (control)/ dose/sex) OECD 453 Deviations regarding deficiencies in recording of food and water consumption, insufficient clinical, tissue and organ weight analysis. GLP Acceptable	Technical grade metribuzin (91.1 %), In drinking water at concentrations of 0, 20, 100 and 500 ppm (equivalent to 0/0, 1.6/ 2.3, 7.4/ 9.4 and 32.3/ 38.4 mg/kg bw/day for males and females, respectively) for 24 months.	One female of the high dose interim sacrifice group died pre-terminally. Treatment did not affect mortality and survival rates of the animals in the main study groups compared to the control group **20 ppm (males 1.6 and females 2.3 mg/kg bw/day):** No toxicologically significant treatment-related effects **100 ppm (males 7.4 and females 9.4 mg/kg bw/day):** lower bodyweight (males -6% at 2 weeks, -10% at 23 months; females -5% at 2 weeks, -9% at 18 months); ↓ bw gain (males -12% at month 24; females -6% at month 24), ↓ food consumption (males -6% on day 5, -5% on day 10, -11% on day 65; females -7% on day 6, -7% on day 26, -11% on day 65); Clinical chemistry: ↑ AST (males +26% at 12 months); ↑ total bilirubin (males +41% at 6 months, +30% at 24 months; females +43% at 6 months, +22% at 12 months); ↓ glucose conc. (males -22% at 6 months, -13% at 12 months, -17% at 18 months); ↑ albumin (females +10% at 18 months); **500 ppm (males 32.2 and females 38.4 mg/kg bw/day):** lower bodyweight (males from week 1 to 23 months -5...-16%; females from week 1 to 23 months -9...-19%); ↓ bw gain (males -	M-513629-01-1

<table>
<tr><th>Method, guideline, deviations if any, species, strain, sex, no/group</th><th>Test substance (purity), dose levels, duration of exposure</th><th>Results (Effects statistically significantly different unless stated otherwise)</th><th>Reference</th></tr>
<tr><td></td><td></td><td>8% at month 24; females -15% at month 24), ↓ food consumption (males on weeks 1, 2, 5, 6, 13, 39, 78, from -4 to -20%; females on weeks 1, 2, 3, 5, 6, 9, 10, 12, 26, 39, 52, 65, from -6 to -28%); ↓ water consumption (males -20%, females -28%),
Clinical chemistry:
↑ BUN (males +25% at 6 months, +38% at 18 months; females +28% at 6 months, +49% at 18 months); ↑ total protein (males +11% at 18 months); ↑ AST (males +37% at 12 months); ↑ total bilirubin (males +38% at 6 months, +55% at 24 months; females +68% at 6 months); ↓ glucose conc. (males -19% at 12 months); ↑ albumin (females +9% at 18 months); ↑ GGT (females ~2-fold at 18 months);

Relative organ weights: interim sacrifice groups at 12 months: ↑ kidneys (males +15%; females +13%); ↑ liver (males +16%; females +12%); ↑ ovaries (+29%);

Neoplastic findings:
No treatment-related changes in neoplastic findings at any dose level.

Incidence of tumours and tumour types for all fates and combined sex:
<table>
<tr><td>Incidence</td><td>Control</td><td>20</td><td>100</td><td>500</td></tr>
<tr><td>No. of rats examined</td><td>100</td><td>93</td><td>93</td><td>100</td></tr>
<tr><td>No. of rats with tumours</td><td>54</td><td>46</td><td>49</td><td>50</td></tr>
<tr><td>No. of tumours per tumour bearing rat</td><td>1.54</td><td>1.20</td><td>1.27</td><td>1.48</td></tr>
<tr><td>No. of rats with benign tumours</td><td>51</td><td>34</td><td>42</td><td>45</td></tr>
<tr><td>No. of benign tumours per tumour bearing rat</td><td>1.35</td><td>1.21</td><td>1.23</td><td>1.42</td></tr>
<tr><td>No. of rats with malignant tumours</td><td>12</td><td>13</td><td>9</td><td>9</td></tr>
<tr><td>No. of malignant tumours per tumour bearing rat</td><td>1.17</td><td>1.08</td><td>1.00</td><td>1.11</td></tr>
<tr><td>No. of rats with metastatic tumours</td><td>4</td><td>5</td><td>2</td><td>1</td></tr>
<tr><td>No. of metastatic tumours per tumour bearing rat</td><td>1.00</td><td>1.00</td><td>1.00</td><td>1.00</td></tr>
</table></td><td></td></tr>
<tr><td>Carcinogenicity study in Swiss albino mice (HsdOla: MF1 mice) (50/dose/sex) OECD 451. Deviations regarding partly insufficient</td><td>Metribuzin (91.1 %), Via drinking water at concentrations of 0, 20, 100 and 500 ppm (equivalent to 0, 3.4, 17.1 and 76.3 mg/kg bw/d in males and to 0,</td><td>20 ppm (males 3.4 and females 3.0 mg/kg bw/day):
lower bodyweight (males from -6% to -7% months 11-13; females -4% weeks 11-12); lower bodyweight gain (males -14% after 12 months);

100 ppm (males 17.1 and females 15.1 mg/kg bw/day):
lower bodyweight (males from -5% to -7% months 11-14 and 17; females from -3% to -6% on weeks 3-4, week 12, month 11, month 16, month 18); lower bodyweight gain (males -11% after 12 months); lower food intake intake (females -6% on weeks 8 and 11);</td><td>M-513707-01-1</td></tr>
</table>

<table>
<tr><th>Method, guideline, deviations if any, species, strain, sex, no/group</th><th>Test substance (purity), dose levels, duration of exposure</th><th>Results (Effects statistically significantly different unless stated otherwise)</th><th>Reference</th></tr>
<tr><td>recording of food and water consumption, tissue analysis.
GLP
Acceptable</td><td>3.0, 15.1 and 58.6 mg/kg bw/d in females, respectively) for 18 months.</td><td>500 ppm (males 76.3 and females 58.6 mg/kg bw/day):
lower bodyweight (males from -4% to -8% on week 1, month 9, months 11-14, months 16-17; females from-4 to -7% on weeks 1-13, month 4, month 13, months 17-18); lower bodyweight gain (males from -15% to -21% after week 1, afte 9 months, after 12 months; females -39% after week 1 and -12% after week 13); lower food intake (males from -6% to -8% on weeks 1-2; females from-6% to -11% on weeks 1-8, weeks 10-11); lower water intake (males from -22% to -44% on weeks 1-7, weeks 9-10; females from -21% to -52% on weeks 1-52);

Neoplastic findings:
No treatment-related inter-group differences were observed in the incidence of benign, malignant and metastatic/infiltrative tumours and tumour types.
Tumour incidences and number of tumours per tumour bearing mouse for all fates (terminally sacrificed and dead/moribund sacrificed) and combined sex:
<table>
<tr><td></td><td colspan="4">Dose levels (ppm)</td></tr>
<tr><td></td><td>0</td><td>20</td><td>50</td><td>100</td></tr>
<tr><td>No. of mice examined</td><td>100</td><td>87</td><td>88</td><td>100</td></tr>
<tr><td>No. of mice with tumours
No. of tumours per tumour bearing mouse</td><td>37
1.24</td><td>30
1.12</td><td>26
1.15</td><td>41
1.15</td></tr>
<tr><td>No of mice with benign tumours *
No. of tumours per tumour bearing mouse</td><td>21
1.05</td><td>6
1.00</td><td>7
1.00</td><td>15
1.00</td></tr>
<tr><td>No of mice with malignant tumours *
No. of tumours per tumour bearing mouse</td><td>24
1.00</td><td>27
1.04</td><td>21
1.10</td><td>32
1.00</td></tr>
<tr><td>No. of mice with metastatic/infiltrative tumours
No. of tumours per tumour bearing mouse</td><td>0
0.00</td><td>1
1.00</td><td>1
1.00</td><td>1
1.00</td></tr>
</table>
* Analysis based on number of mice with neoplasms</td><td></td></tr>
<tr><td>Chronic toxicity study on rats (two-year feeding experiment)
Wistar rat (40/dose/sex, 80/control/sex)
OECD 453
Deviations regarding dose selection,</td><td>Metribuzin Technical (99.5 %), dietary concentrations of 0, 25, 35, 100 and 300 ppm (mean dose received for males/females 0, 1.30/1.68, 1.87/2.28,</td><td>25 ppm (males 1.3 and females 1.68 mg/kg bw/day):
Relative organ weights:
↓ lung (males -12%); ↑ liver (males +5%); ↓ kidneys (males -11%); ↓ gonads (males -10%);

35 ppm (males 1.87 and females 2.28 mg/kg bw/day):
Relative organ weights:
↑ thyroids (males +10%); ↓ liver (females -9%);

100 ppm (males 5.27 and females 6.53 mg/kg bw/day):
Relative organ weights:
↑ thyroids (males +10%); ↓ kidneys (males -9%); ↓ heart (females</td><td>M-018704-02-1</td></tr>
</table>

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance (purity), dose levels, duration of exposure	Results (Effects statistically significantly different unless stated otherwise)	Reference
number of animals, deficiencies in recording of food and water consumption, insufficient clinical, pathology and organ weights analysis, statistical evaluation. Non-GLP Supplementary	5.27/6.53 and 14.36/20.38 mg/kg bw/day, respectively) for 24 months.	-6%); ↓ liver (females -10%); **300 ppm (males 14.36 and females 20.38 mg/kg bw/day):** Relative organ weights: ↓ kidneys (males -10%); ↓ heart (females -6%); ↓ lung (females -9%); ↓ liver (females -9%); ↓ spleen (females -8%); **Neoplastic findings:** No treatment-related changes in neoplastic findings at any dose level.	
Oncogenicity study in mice CD1 outbred strain Albino (50/dose/sex) Pre-OECD 451 Deviations regarding dose selection, animal housing, no clinico-chemical analyses were performed. GLP Supplementary	Metribuzin (92.9 %), Via diet at 0, 200, 800 and 3200 ppm (equal to 0, 28, 111, 438 mg/kg bw/day for males and 0, 35, 139, 567 mg/kg bw/day for females) for 24 months.	**200 ppm (males 28 and females 35 mg/kg bw/day):** ↓ food consumption (males -46% on week 1; females -38% on week 1 and -7% on week 48); Relative organ weights: ↑ liver (females +19%); **800 ppm (males 111 and females 139 mg/kg bw/day):** ↓ food consumption (males -37% on week 1 and -5% on week 36; females -36% on week 1); Hematology: ↓ Hct (females -13% at 24 months); Relative organ weights: ↑ liver (males +17%; females +20%); **3200 ppm (males 438 and females 567 mg/kg bw/day):** ↓ food consumption (males -37% on week 1; -7% on weeks 36 and 72, -5% on week 84; females -41% on week 1); Hematology: ↓ RBC (males -13% at 18 months; females -12% at 18 months); ↓ Hct (females -10% at 18 months, -13% at 24 months); Relative organ weights: ↑ liver (females +50%); ↑ spleen (females +55%); ↑ kidneys(females +12%); **Neoplastic findings:** No treatment-related changes in neoplastic findings at any dose level.	M-018690-01-1

104-week feeding carcinogenicity study in Fischer 344 rats (M-017948-02-1; RAR B.6.5.), performed as a combined chronic toxicity/oncogenicity study, was in compliance with OECD 453 guideline and according to the principles of GLP. Groups of 50 male and female Fischer 344 rats were dosed with 92.1-93.0 % metribuzin dissolved in ethanol and mixed with feed that was coated with corn oil via the diet at concentrations of 30, 300 and 900 ppm (equivalent to 0, 1.3, 13.8 and 42.2 mg/kg bw/d for males and 0, 1.6, 17.7 and 53.6 mg/kg bw/d for females). A control group of 50 males and 50 females received vehicle control. A satellite group of ten additional animals (20 in the control and the high-dose groups) per sex and dose level was included for interim sacrifice after one year. All animals were subject to a complete gross examination, saving all gross lesions, weighing

designated organs, and collecting standard tissue specimens for histopathologic evaluation. Survival rates at study termination were 64/76, 58/68, 66/74, and 62/80 % for males/females at 0, 30, 300, and 900 ppm, respectively.
Micropathologic evidence of metribuzin-induced neoplasia was not found in this study; however, a compound-related increase in the incidence of follicular hyperplasia of the thyroid gland was noted in 300 and 900 ppm males of The 1-Year Sacrifice Group and 900 ppm males of The 2-Year Sacrifice Group. Lesions common to the aging Fischer 344 rat were seen in the male and female rats of this study and included: degeneration and fibrosis in the heart, nephropathy, bile duct hyperplasia in the liver, abscesses in the prostate gland, tail abscesses, vacuolar change in the adrenal glands, mineralization in the eye, chronic inflammation in the Harderian gland, testicular interstitial cell hyperplasia ar.d/or adenoma, pituitary adenomas, uterine endometrial stromal polyps, mononuclear cell leukemia, and mammary gland fibroadenomas.
A supplemental submission to the original report was prepared to address several points for clarifications. According to the remarks of the reviewer: The presentation of the pathology data is unsatisfactory. The pathology table did not enable to distinguish primary tumours from metastases, tumours were not scored under organs of origin or were possibly even improperly diagnosed. Also e.g. the high incidences of abscesses in prostate and tail seems rather unlikely. The conclusion that evidence of test substance-related neoplasia was not found may be correct, but can not easily be verified by the reviewer in the absence of:
1) Descriptive criteria for 'follicular hyperplasia' and 'follicular adenoma'
2) A summary of tumour incidences without doubts with respect to organ of origin and primary tumour versus metastasis, and unequivocal diagnosis of tumour types in some organs. The final conclusion with respect to carcinogenicity can not be drawn from this study.

2-year oral carcinogenicity study in Wistar rats (M-513629-01-1; RAR B.6.5.) was performed as a combined chronic toxicity and carcinogenicity study. Groups of 50 male and 50 female rats were dosed for 2 years with 91.1 % metribuzin via drinking water at dose levels of 0 (control), 20, 100 and 500 ppm (equivalent to 0/0, 1.6/ 2.3, 7.4/ 9.4 and 32.3/ 38.4 mg/kg bw/day for males and females, respectively). In addition, one control interim sacrifice group with 20 rats (10 males and 10 females) and high dose interim sacrifice group with 40 rats (20 males and 20 females) were included for 12th month interim sacrifice to study non-neoplastic histopathological changes. The study was in accordance with OECD 453 guideline and the principles of GLP. Histopathological examination was carried out on all the tissues collected from the rats of the control and high dose groups (interim and main groups); all preterminally dead and moribund sacrificed rats of the low and mid dose groups and on all lesions of the terminally sacrificed rats from the low and mid dose groups (interim sacrifice and main groups). The pre-terminal deaths (including moribund sacrifice) inclusive of those dead after 730 days in the four groups; control, low, mid and high dose groups, respectively were: i. males: 22, 18, 25, 24; ii. females: 19,24,20,20; iii. combined sex: 41, 42, 45, 44.
Upon necropsy of dead and moribund sacrificed rats, a statistically significantly increased incidence of small testes was recorded in mid (9 of 25 examined rats) and high dose males (10 of 24) compared to the control (3 of 22) and low dose group (4 of 18). In terminally sacrificed males the incidence of this change was inconspicuous between the dose groups and there were no statistically significant inter-group differences observed macroscopically. No treatment-related inter-group differences were observed in the incidence of non-neoplastic changes, benign, malignant and metastatic/infiltrative tumours, and tumour types in all fates (terminally sacrificed and dead/moribund sacrificed rats).

Table 20: Summary of histopathological (neoplastic) findings of terminally sacrificed rats

Summary of histopathological (neoplastic) findings of terminally sacrificed rats								
	Males				**Females**			
	Dose (ppm)				**Dose (ppm)**			
	0	20	100	500	0	20	100	500
Number of rats	28	32	25	26	31	26	30	30
Number of rats examined	28	25	18	26	31	26	30	30

Tissue and observation								
Pituitary – number of samples	28	1	1	26	31	2	1	30
Adenoma (Benign)	6	1	1	5	8	2	1	3
Percent	21	100	100	19	26	100	100	10
Adrenals – number of samples	28	2	3	26	31	4	1	30
Pheochromocytoma (Benign)	7	1	3	5	3	2	0	5
Percent	25	50	100	19	10	50		17
Mammary gland – number of samples	-	1	1	-	31	5	5	29
Fibroadenoma (Benign)	-	0	0	-	6	3	5	4
Percent					19	60	100	14
Snout – number of samples	1	-	-	-	2	-	1	-
Squamous cell carcinoma (Malignant)	1	-	-	-	2	-	1	-
Percent	100				50		100	
Uterus – number of samples					31	1	5	30
Polyp(s)					1	0	3	0
Percent					3		60	

Table 21: Summary of histopathological (neoplastic) findings of dead and moribund sacrificed rats

Summary of histopathological (neoplastic) findings of dead and moribund sacrificed rats								
	Males				**Females**			
	Dose (ppm)				**Dose (ppm)**			
	0	20	100	500	0	20	100	500
Number of rats	22	18	25	24	19	24	20	20
Number of rats examined	22	18	25	24	19	24	20	20
Tissue and observation								
Pituitary – number of samples	22	18	24	23	19	24	20	20
Adenoma (Benign)	3	9	8	4	6	3	6	6
Percent	14	50	33	17	32	13	30	30
Adrenals – number of samples	22	18	25	24	19	24	20	20
Pheochromocytoma (Benign)	11	7	15	11	3	4	5	5
Percent	50	39	60	46	16	17	25	25
Pheochromocytomea (Malignant)	0	0	1	0	0	0	0	0
Percent			4					
Mammary gland – number of samples			2	2	18	24	19	20
Fibroadenoma (Benign)			0	0	1	2	1	1
Percent					6	8	5	5
Snout – number of samples	2	1	-	-	3	5	1	2
Squamous cell carcinoma (Malignant)	2	1	-	-	3	4	1	1
Percent	100	100			100	8	100	50
Uterus – number of samples					19	23	20	20
Polyp(s)					2	1	2	1
Percent					11	4	10	5

In a carcinogenicity study in Swiss albino mice (M-513707-01-1; RAR B.6.5.) groups of 50 male and female mice were dosed with 91.1 % metribuzin via drinking water at concentrations of 0, 20, 100 and 500 ppm (equivalent to 0, 3.4, 17.1 and 76.3 mg/kg bw/d in males and to 0, 3.0, 15.1 and 58.6 mg/kg bw/d in females, respectively) for 18 months. The study was performed according to OECD 451 guideline and the principles of GLP. Histopathological examination was carried out on all tissues collected from the mice of the control and high dose groups; all pre-terminally dead and moribund sacrificed mice of all groups and all lesions of the terminally sacrificed mice from the low and mid dose groups. Survival rates of the male goups were 64% (control), 66% (20 ppm), 62% (100 ppm) and 62% (500 ppm). The respective female survival rates were 74%, 68%, 68% and 72%.
A significant increase in the incidence (within historical control) of malignant tumours in high dose males (14/50) in the case where all fates were presented together was considered incidental and not

treatment-related, as there was a coincidental low incidence in the control (7/50) as compared to historical control data (HCD in Swiss Albino mice from 1996-1999), and there was no significant increase in either fate alone and for combined sex.
Malignant neoplasia, common for this strain of mice and also observed in this study, were mainly tumours of the haemolymphoreticular system (malignant lymphoma and histiocytic sarcoma).

Table 22: Summary of Histopathological (Neoplastic) findings of dead and moribund sacrificed mice

Carcinogenicity Study with Metribuzin Technical in Swiss Albino Mice								
Summary of Histopathological (Neoplastic) findings of dead and moribund sacrificed mice								
Dose (ppm)	**0**	**20**	**100**	**500**	**0**	**20**	**100**	**500**
No of mice	**18**	**17**	**19**	**19**	**13**	**16**	**16**	**14**
No of mice examined	**18**	**17**	**19**	**19**	**13**	**16**	**16**	**14**
Tissue(number of tissues) and observation	**Males**				**Females**			
Salivary Gland	18	17	19	19	13	16	16	13
Esophagus	18	17	19	19	13	16	16	14
Stomach	18	17	19	19	13	16	16	14
Endometrial stromal sarcoma – metastatic (MM)	0	0	0	0	0	1 (6%)	0	0
Duodenum	18	16	19	19	13	16	16	14
Endometrial stromal sarcoma – metastatic (MM)	0	0	0	0	0	1 (6%)	0	0
Jejunum	18	17	18	19	13	16	16	14
Ileum	17	17	19	19	13	16	16	14
Endometrial stromal sarcoma – metastatic (MM)	0	0	0	0	0	1 (6%)	0	0
Cecum	18	14	19	19	13	16	16	14
Colon	18	17	19	19	13	16	16	14
Rectum	17	16	18	19	13	16	16	13
Pancreas	17	17	19	19	13	16	16	14
Endometrial stromal sarcoma – metastatic (MM)	0	0	0	0	0	1 (6%)	0	0
Liver	18	17	19	19	13	16	16	14
Endometrial stromal sarcoma – metastatic (MM)	0	0	0	0	0	1 (6%)	0	0
Hepatocellular adenoma (B)	0	0	1 (5%)	0	0	1 (6%)	0	1(7%)
Hepatocellular carcinoma (M)	1(6%)	0	0	0	1(8%)	0	0	0
Gall Bladder	17	15	19	14	10	14	14	13
Lungs	18	17	19	19	13	16	16	14
Mammary adenocarcinoma-metastatic (MM)	0	0	0	0	0	0	0	1 (7%)
Endometrial stromal sarcoma – metastatic (MM)	0	0	0	0	0	1 (6%)	0	0
Bronchiolo-alveolar adenoma(B)	2(11%)	2(12%)	0	1(5%)	0	0	0	0
Trachea	18	17	19	19	13	16	16	14
Heart	18	17	19	19	13	16	16	14
Endometrial stromal sarcoma – metastatic (MM)	0	0	0	0	0	1 (6%)	0	0
Aorta	-	-	-	-	-	-	-	
Spleen	18	17	19	19	13	16	16	14
Mesenteric Lymph Nodes	17	16	18	19	13	15	16	14
Mediastinal Lymph Node	2	1	-	-	2	1	-	2
Mandibular Lymph Node	18	17	19	19	13	16	16	13
Kidneys Renal cell adenoma (B)	18	17	19	19	13	16	16	14

Carcinogenicity Study with Metribuzin Technical in Swiss Albino Mice								
Summary of Histopathological (Neoplastic) findings of dead and moribund sacrificed mice								
Dose (ppm)	**0**	**20**	**100**	**500**	**0**	**20**	**100**	**500**
No of mice	**18**	**17**	**19**	**19**	**13**	**16**	**16**	**14**
No of mice examined	**18**	**17**	**19**	**19**	**13**	**16**	**16**	**14**
Tissue(number of tissues) and observation	**Males**				**Females**			
	1 (6%)	0	0	0	0	0	0	0
Urinary Bladder	18	17	19	19	13	16	16	14
Testes Leydig cell tumor (B)	18	17	19	19				
	1 (6%)	0	0	2(11%)				
Epididymes	17	17	19	19				
Prostate	18	17	19	19				
Seminal Vesicles	18	17	19	19				
Coagulating Glands	18	16	18	18				
Ovaries Endometrial stromal sarcoma – metastatic (MM) Granulosa cell tumor (B) Tubulostromal adenoma (B)					13	16	16	14
					0	1(6%)	0	0
					1 (8%)	0	0	0
					1 (8%)	0	0	0
Uterus Endometrial stromal sarcoma (M)					13	16	16	14
					0	1(6%)	1(6%)	1(7%)
Vagina					1	-	-	-
Thyroids	18	17	19	19	13	16	16	14
Parathyroids	17	17	17	19	11	16	14	13
Pituitary Adenoma (B)	17	17	19	19	13	16	15	14
	0	0	0	0	0	0	1(7%)	0
Adrenals Pheochromocytome (B)	17	17	19	19	13	16	16	14
	0	0	0	0	1(8%)	0	0	0
Eyes	18	17	19	19	12	16	16	14
Bone Marrow (Smear)	18	17	19	19	13	16	16	14
Skin	18	17	19	19	13	16	16	14
Thymus	11	11	15	16	10	15	14	12
Muscle Femoral	18	17	19	19	13	16	16	14
Spinal Cord	18	17	19	19	13	16	16	14
Sciatic Nerves	18	16	19	19	13	16	16	14
Preputial Glands	3	1	-	1				
Mammary Gland Adenocarcinoma (M)					12	16	16	14
					1(8%)	0	0	1(7%)
Tumor/Mass	18	17	19	19	13	16	14	14
Bone (Femur with Joint Osteoma (B)	18	17	19	19	13	16	16	14
	1(6%)	0	0	0	0	1(6%)	0	1(7%)
Mesentery Endometrial stromal sarcoma – metastatic (MM)	1	1	-	1	1	4	2	1
	0	0	-	0	0	1(25%)	0	0
Sternum with Marrow	18	17	19	19	13	16	16	14
Lymph Node (Others)	-	4	2	3	3	4	5	5
Urethra	1	-	-	-	-	-	-	-
Brain - Cerebrum	18	17	19	19	13	16	16	14
Brain - Cerebellum	18	17	19	18	12	15	16	14
Brain - Medulla	18	17	19	18	12	15	16	14
Lesion	-	-	1	-	-	-	-	-
Exorbital Lacrimal Gland	-	-	1	-	-	-	-	-
Superficial Ing. L. Node	18	17	19	19	12	16	15	14
Hemolymphoreticular System	18	17	19	19	13	16	16	14
Histocytic sarcoma (M)	0	0	0	4(21%)	3(23%)	2(13%)	3(19%)	2(14%)
Malignant lymphoma (M)	2(11%)	6(35%)	4(21%)	2(11%)	4(31%)	9(56%)	7(44%)	5(36%)
Myeloid leukemia (M)	0	0	0	0	0	0	1(6%)	0

Carcinogenicity Study with Metribuzin Technical in Swiss Albino Mice								
Summary of Histopathological (Neoplastic) findings of dead and moribund sacrificed mice								
Dose (ppm)	**0**	**20**	**100**	**500**	**0**	**20**	**100**	**500**
No of mice	**18**	**17**	**19**	**19**	**13**	**16**	**16**	**14**
No of mice examined	**18**	**17**	**19**	**19**	**13**	**16**	**16**	**14**
Tissue(number of tissues) and observation	**Males**				**Females**			
Ear	2	3	3	-	-	-	1	-
Bone (Others) Osteoma	-	1	1	-	-	-	-	-
(B) Vertebrae	-	1(100%)	0	-	-	-	-	-

B: Benign; M: Malignant; MM: Metastatic; I: Infiltrative

Table 23: Summary of Histopathological (Neoplastic) findings of terminally sacrificed mice

Carcinogenicity Study with Metribuzin Technical in Swiss Albino Mice								
Summary of Histopathological (Neoplastic) findings of terminally sacrificed mice								
Dose (ppm)	**0**	**20**	**100**	**500**	**0**	**20**	**100**	**500**
No of mice	**32**	**33**	**31**	**31**	**37**	**34**	**34**	**36**
No of mice examined	**32**	**23**	**25**	**31**	**37**	**31**	**28**	**36**
Tissue (number of tissues) and observation	**Males**				**Females**			
Salivary Gland	32	1	2	31	37	-	-	36
Esophagus	32	-	-	31	37	-	-	36
Stomach	32	-	-	31	37	1	-	36
Duodenum	32	-	-	31	37	-	-	36
Jejunum	32	-	-	31	36	-	-	36
Ileum	31	-	-	31	37	1	1	36
Cecum	32	-	-	31	37	-	-	36
Colon	32	-	-	31	37	-	-	36
Rectum	32	-	-	31	37	-	-	35
Pancreas	32	-	-	31	37	-	-	36
Liver	32	2	-	31	37	2	2	36
Hemangioma (B)	0	1(33%)	-	0	0	0	0	0
Endometrial stromal sarcoma – metastatic (MM)	0	0	-	0	0	0	1(50%)	0
Hepatocellular adenoma (B)	4(13%)	0	-	1(3%)	1(3%)	0	0	0
Hepatocellular carcinoma (M)	0	0	-	1(3%)	0	0	0	0
Gall Bladder	31	-	-	30	36	-	-	32
Lungs	32	-	-	31	37	-	-	36
Bronchiolo-alveolar adenoma (B)	3(9%)	-	-	2(6%)	0	-	-	1(3%)
Bronchiolo-alveolar adeno-carcinoma (M)	0	-	-	0	0	-	-	1(3%)
Trachea	32	-	-	31	37	-	-	36
Heart	32	-	-	31	37	-	-	36
Spleen	32	4	5	31	37	9	1	36
Mesenteric Lymph Nodes	32	5	4	31	37	11	3	36
Mediastinal Lymph Node	-	-	-	1	1	-	-	2
Mandibular Lymph Node	31	7	6	31	37	7	1	36
Kidneys Renal	32	6	7	31	37	5	2	36
cell adenoma (B)	0	0	1(14%)	0	0	0	0	0
Urinary Bladder	32	-	-	31	37	-	-	36
Testes	32	-	2	31				
Epididymes	32	-	-	31				
Prostate	31	-	-	31				
Seminal Vesicles	32	-	-	31				
Coagulating Glands	32	-	-	31				

Carcinogenicity Study with Metribuzin Technical in Swiss Albino Mice								
Summary of Histopathological (Neoplastic) findings of terminally sacrificed mice								
Dose (ppm)	**0**	**20**	**100**	**500**	**0**	**20**	**100**	**500**
No of mice	**32**	**33**	**31**	**31**	**37**	**34**	**34**	**36**
No of mice examined	**32**	**23**	**25**	**31**	**37**	**31**	**28**	**36**
Tissue (number of tissues) and observation	**Males**				**Females**			
Ovaries					37	21	20	36
Tubulostromal adenoma (B)					0	0	0	1(3%)
Endometrial stromal sarcoma – infiltrative (I)					0	0	1(5%)	0
Granulosa cell tumor (B)					0	0	1(5%)	1(3%)
Uterus Hemangioma					37	9	12	36
(B) Leiomyoma (B)					1(3%)	0	0	0
Endometrial stroma					1(3%)	0	0	1(3%)
sarcoma (M)					0	0	1(8%)	0
Vagina					3	-	-	2
Penis	-	-	-	1				
Thyroids	32	-	-	31	37	-	-	36
Parathyroids	30	-	-	31	37	-	-	33
Pituitary Adenoma	32	-	-	31	37	-	-	36
(B)	0	-	-	0	0	-	-	1(3%)
Adrenals Subcapsular	32	-	1	31	37	-	-	36
cell adenoma (B)	1(3%)	-	0	0	0	-	-	0
Eyes	32	-	-	31	37	1	-	36
Bone Marrow (Smear)	32	-	-	31	37	-	-	36
Skin	32	13	14	31	37	11	4	36
Thymus	29	-	-	28	36	1	-	34
Muscle Femoral	32	-	-	31	37	-	-	36
Spinal Cord	32	-	-	31	37	-	-	36
Sciatic Nerves	32	-	-	31	37	-	-	36
Preputial Glands	-	2	-	1				
Mammary Gland					37	-	-	35
Tumor/Mass Hemangioma	-	-	1	-	1	-	-	-
(B) (Pelvic) Squamous cell	-	-	0	-	1(100%)	-	-	-
carcino-ma (M) (Sacral)	-	-	1(100%)	-	0	-	-	-
Bone (Femur) with Joint	32	-	-	31	37	-	1	36
Osteoma (B)	0	-	-	2(5%)	-	-	1(100%)	1(3%)
Mesentery	-	-	-	1	2	-	-	2
Sternum with Marrow	32	-	-	31	37	-	-	36
Lymph Node (Others)	2	-	1	1	2	2	-	1
Brain - Cerebrum	32	-	-	31	37	-	-	36
Brain - Cerebellum	32	-	-	31	37	-	-	36
Brain - Medulla	32	-	-	31	37	-	-	36
Snout	1	-	-	1	-	-	-	-
Superficial Ing. L. Node	32	1	1	31	37	2	1	36
Hemolymphoreticular	32	3	2	31	37	7	3	36
System Histiocytic	2(6%)	0	0	2(6%)	3(8%)	1(14%)	3(100%)	1(3%)
sarcoma (M) Malignant	2(6%)	3(100%)	2(100%)	5(16%)	4(11%)	6(86%)	0	7(19%)
lymphoma (M) Myeloid leukemia (M)	0	0	0	0	1(3%)	0	0	0
Ear	1	6	5	5	2	5	1	-
Bone (Others) Osteoma	-	-	-	-	-	-	1	2
(B) (Cranium)	-	-	-	-	-	-	1(100%)	2(100%)

B: Benign; M: Malignant; MM: Metastatic; I: Infiltrative

In a two-year chronic toxicity feeding study on Wistar rats (M-018704-02-1; RAR B.6.5.) groups of 40 male and female rats were dosed with 99.5 % metribuzin at doses of 0, 25, 35, 100 and 300 ppm

(mean dose received for males/females 0, 1.30/1.68, 1.87/2.28, 5.27/6.53 and 14.36/20.38 mg/kg bw/day, respectively) for 24 months. A control group of 80 males and 80 females was included. The study was non-GLP but partly compliant to OECD 453 guideline. The following organs were examined from 66 male and 72 female control as well as from 29 male and 35 female Wistar rats treated
with the highest dosage (300 ppm) sacrificed at the end of the treatment period of two years: brain, pituitary gland, eyes, cervical lymphnodes, aorta, trachea, sternum including bone marrow, mammary gland, oesophagus, stomach, 4 intestinal segments, pancreas, epidydimis, prostate, seminal vesicle, urinary bladder, uterus, thyroid, heart, lung, liver, spleen, kidneys, suprarenal glands, testicles, ovaries, skeletal muscle with femur and ischiadic nerve, salivary gland. From the other treatment groups (25, 35, 100 ppm), the thyroid, heart, lung, liver, spleen, kidneys, suprarenal glands, testicles, and ovaries were histologically examined for 10 males and 10 females from each of these treatment groups. In addition the main organs from animals which died during the experiment are examined histopathologically. Mortality rates after 2 years of the male groups were 17.5% (0 ppm), 22.5% (25 ppm), 20.0% (35 ppm), 25.0% (100 ppm), 27.5% (300 ppm). The respective female rates were 10.0%, 12.5%, 22.5%, 17.5%, 12.5%.
The most remarkable findings were numerous adenomata and carcinomata of the pituitary gland, medullary tumours of the suprarenal glands and Leydig-cell tumours of the testicles which were most common among the control animals (spontaneous tumours). Lymphocytic infiltration in the interstitium and occasionally either bronchitis or pneumonia were representative findings for the trachea and lungs and were found in the control as well as in the treated animals.
Even though occasional degenerative or inflammatory alterations were seen in parenchymatous organs, especially in the liver and kidneys, they were with all probability not due to the treatment as similar if not more pronounced changes were seen just as frequently in the control rats.
Also the osseous portions which were examined were nonremarkable. Spermiogenesis and morphology of the ovaries were normal for the respective ages of the animals.
46 slides demonstrated a broad spectrum of neoplasms. Benign and malignant tumours were distributed among all of the various groups, inclusive of the controls. These neoplasms are typical for rats and are regarded as spontaneous.
Collectively, regarding the inflammatory and degenerative alterations of the internal organs, the histological findings were assessed not to be treatment or dose dependent.

Table 24: Overall tumour incidence and incidence of selected tumours

	Concentration BAY 94337 in diet (ppm)									
	Males					Females				
	0	25	35	100	300	0	25	35	100	300
Total incidence	24	17	9	8	14	42	19	20	22	27
Incidence of malignant tumours	7	2	2	2	5	14	4	5	4	9
Pituitary - adenoma	8	6	2	5	5	16	6	9	11	14
adenocarcinoma	2	0	0	0	1	11	1	0	0	7
Thyroid - adenoma	0	2	1	0	2	3	0	2	3	1
adenocarcinoma	0	0	0	0	0	0	1	0	0	0
Ovary granulosa	-	-	-	-	-	0	1	1	0	0
Testis Leydig cell	3	2	1	0	0	-	-	-	-	-
Mammary adenoma	-	-	-	-	-	4	5	2	2	1
fibroadenoma	-	-	-	-	-	1	1	1	2	1

In an oncogenicity study in CD1 albino mice (M-018690-01-1; RAR B.6.5.) 92.9 % metribuzin was administered to 50 male and female mice via diet at doses of 0, 200, 800 and 3200 ppm (equal to 0, 28, 111, 438 mg/kg bw/day for males and 0, 35, 139, 567 mg/kg bw/day for females) for 24 months. The study was performed generally in accordance with OECD 451 guideline and the principles of GLP. Mortality rates after 24 months of the male groups were 58% (0 ppm), 58% (200 ppm), 36%

(800 ppm), 58% (3200 ppm). The respective female rates were 58%, 48%, 56%, 58%. Samples for histopathology were taken from all animals on study and processed for histopathological examination. A microscopic examination was performed on all available tissues from all animals on all study levels.

Amyloidosis was diagnosed in various tissues and organs of both control and treated mice. It occurred with a slightly higher incidence in the male mice fed 3200 ppm metribuzin in the diet. These findings were within expected limits for this strain of mice and were not related to administration of the test material. Glandular hyperplasia and chronic inflammation of the gastric mucosa were diagnosed in approximately equal numbers of control and treated mice. These lesions were spontaneous in nature and were not compound or dose-related.

The most commonly observed neoplasm was malignant lymphoma (lymphoscarcoma). This neoplastic disease was observed in various tissues and organs and was generally seen as multicentric lesion in the affected mice. Malignant lymphoma occurred in approximately equal numbers in the control and treated groups of mice. This observation was not related to the administration of the test material but was a naturally occurring neoplastic process of aging mice.

Hepatocellular neoplasms were observed almost exclusively in male mice in this study (an expected sex predilection). These neoplasms occurred in approximately equal numbers in control and treated males and generally occurred at a slightly lower incidence than expected for this strain of mice.

Alveolar-bronchiolar carcinomas commonly occur in inbred strains of mice. In this study, the incidence was within normal expected limits. In fact, the occurrence was lower than expected in the females fed 3200 ppm and in males fed 800 ppm metribuzin in the diet. Therefore, these neoplasms were naturally occurring and not related to the administration of the test material. Small numbers of other primary or metastatic neoplasms were diagnosed in various tissues and organs. These tumors did not occur in a dose-related manner, were of spontaneous nature, and were within the normal limits of expected tumor incidence for the individual tissue or organ.

Table 25: Incidence of selected non-neoplastic findings

	Dose level (ppm)							
	Males				**Females**			
Non-neoplastic finding	**0**	**200**	**800**	**3200**	**0**	**200**	**800**	**3200**
Liver amyloidosis	0	0	0	2 (4%)	0	0	0	0
Liver clear cell cytoplasmic change	2 (4%)	0	0	1 (2%)	0	0	0	0
Liver multifocal necrosis	5 (10%)	0	0	0	0	5 (10%)	1 (2%)	4 (8%)
Stomach glandular hyperplasia	8 (16%)	10 (20%)	5 (10%)	9 (18%)	7 (14%)	7 (14%)	8 (16%)	8 (16%)
Stomach chronic inflammation	7 (14%)	9 (18%)	12 (24%)	8 (16%)	8 (16%)	9 (18%)	12 (24%)	10 (20%)

Incidences in % in brackets

Table 26: Summary of benign and malignant neoplasms and number of animals with neoplasms

Dose level (ppm)	No.	Sex	No. Animals With Tumours (%)	Total No. Tumours (%)	No. Animals With Malignant Tumours (%)	No. Animals With Benign Tumours (%)	Total No. Malignant Tumours (%)	Total No. Benign Tumours (%)
0	**50**	**Male**	28 (56%)	93 (186%)	23 (46%)	8 (16%)	85 (170%)	8 (16%)
	50	**Female**	19 (38%)	61 (122%)	16 (32%)	4 (8%)	56 (112%)	5 (10%)
200	**50**	**Male**	26 (52%)	48 (96%)	20 (40%)	9 (18%)	39 (78%)	9 (18%)
	50	**Female**	28 (56%)	95 (190%)	24 (48%)	6 (12%)	88 (176%)	7 (14%)
800	**50**	**Male**	22 (44%)	48 (96%)	16 (32%)	7 (14%)	40 (80%)	8 (16%)
	50	**Female**	29 (58%)	75 (150%)	21 (42%)	11 (22%)	61 (122%)	14 (28%)
3200	**50**	**Male**	18 (36%)	39 (78%)	14 (28%)	5 (10%)	33 (66%)	6 (12%)
	50	**Female**	20 (40%)	76 (152%)	15 (30%)	5 (10%)	69 (138%)	7 (14%)

Table 27: Incidence of selected neoplastic findings

	Dose level (ppm)							
	Males				Females			
Neoplastic finding	**0**	**200**	**800**	**3200**	**0**	**200**	**800**	**3200**
Liver haemangiosarcoma	0	0	1 (2%)	0	0	0	0	0
Hepatocellular adenoma	6 (12%)	3 (6%)	1 (2%)	2 (4%)	1 (2%)	0	0	0
Hepatocellular adenocarcinoma	6 (12%)	8 (16%)	6 (12%)	1 (2%)	0	1 (2%)	0	0
Liver malignant lymphoma	7 (14%)	2 (4%)	4 (8%)	3 (6%)	3 (6%)	11 (22%)	5 (10%)	8 (16%)
Alveolar-bronchiolar carcinomas	10 (20%)	8 (16%)	3 (6%)	7 (14%)	7 (14%)	11 (22%)	11 (22%)	1 (2%)

Table 28: Summary table of human data on carcinogenicity

Type of data/report	Relevant information about the study (as applicable)	Observations
A prospective cohort study (From literature review) Delancey *et al.* 2009	The Agricultural Health Study (AHS) is a prospective cohort of 57,310 licensed pesticide applicators and 32,347 of their spouses in Iowa and North Carolina. Participants completed a self-administered questionnaire at enrollment to gather information on demographic variables (including smoking history, alcohol consumption, education, family history of cancer), detailed exposure information on 22 pesticides, ever/never exposure information on 28 additional pesticides, pesticide application methods, use of personal protective equipment, pesticide mixing, and equipment handling and repair. Only men were included in this analysis.	Of the 23,072 eligible applicators who provided detailed metribuzin use information on the take-home questionnaire, 8,504 (37 percent) reported using metribuzin. There were 554 incident cancer cases among those applicators who ever used metribuzin, compared to 1,118 cases of cancer in 14,568 applicators never used metribuzin. An association between metribuzin use and overall cancer incidence was not observed. An increased rate ratio was observed at the highest tertile of exposure using the low exposed referent group for all cancers combined and for several individual cancers, including leukemia, NHL, and all lymphohematopoietic cancers combined, although the point estimates and linear trend tests were not statistically significant.
A population based case	Telephone interviews were conducted with men and women diagnosed with	For two herbicides and three insecticides, use was positively associated with risk among both self and proxy

Type of data/report	Relevant information about the study (as applicable)	Observations
control study to determine if agricultural pesticide exposures were associated with the risk of adult glioma (From literature review) Lee *et al.* 2005	gliomas (n = 251) between 1988 and 1993 and controls (n = 498) randomly selected from the same geographical area. Unconditional logistic regression was used to calculate adjusted odds ratios (ORs) for farming and for use of individual and chemical classes of insecticides and herbicides, including pesticides classified as nitrosatable (able to form N-nitroso compounds upon reaction with nitrite). Non-farmers were used as the reference category for all analyses.	respondents. Based on a small number of exposed cases, ORs were significantly increased for the herbicide metribuzin (OR = 3.4, 95% CI 1.2 to 9.7) among others. The authors found significant associations between some specific agricultural pesticide exposures and the risk of glioma among male farmers but not among female farmers in Nebraska; however, most of the positive associations were limited to proxy respondents. These findings warrant further evaluation in prospective cohort studies where issues of recall bias are not a concern.
Pooled study of multiple agricultural pesticides (From literature review) De Roos *et al.* 2003	The pooled data of the three population based case-control studies conducted by the National Cancer Institute during the 1980s were used to conduct an analysis of exposure to multiple pesticides in farming as risk factors for non-Hodgkin's lymphoma (NHL) among men.The large sample size (n = 3417) allowed analysis of 47 pesticides simultaneously, controlling for potential confounding by other pesticides in the model, and adjusting the estimates based on a prespecified variance to make them more stable.	Reported use of several individual pesticides was associated with increased NHL incidence, however not in the case of metribuzin.
Population-based case-control study (From literature review) Brown *et al.* 1993	173 white men with multiple myeloma (MM) were included in this study. Three sources of controls were used to select a population-based stratified sample of White men without lymphatic or hematopoietic cancer: living controls under age 65; living controls aged 65 or over; and deceased controls. A standardized questionnaire was used to obtain detailed information on general farm activities and the use on the farm of 24 animal insecticides, 34 crop insecticides, 38 herbicides, and 16 fungicides.	No significant associations between MM and the handling of pesticides in general, classes of pesticides, or specific pesticides were found.
Population-based case-control study (From literature review) Zahm *et al.* 1993	206 white women with histologically confirmed cases of non-Hodgkin's lymphoma (NHL) residing in eastern Nebraska were identified. A total of 824 female controls were selected from residents of the same area by 3:l frequency matching by race, sex, vital status, and age (5-y age groups) to the combined age distribution of the four cancer case series. The interview included questions on herbicides and insecticides used; the	A total of 119 women diagnosed with NHL and 471 controls reported ever having lived or worked on a farm (OR = 1.0; CI = 0.7,1.4). Pesticides had been used on the farms of 59 cases and 262 controls, which yielded an OR of 0.8 (CI = 0.5,1.3). Two cases and 1 control had unknown farming status. No class of herbicides, whether used on the farm or personally handled, was associated with a significantly increased risk of NHL. Few women, however, reported personally handling herbicides. With respect to other pesticide classes, it is difficult to determine if the women had an excess risk of NHL

Type of data/report	Relevant information about the study (as applicable)	Observations
	application method used most often; use of protective equipment; duration of time wearing work clothes after handling pesticides; animals raised; and use of fungicides, rodenticides, fumigants, wood preservatives, and fertilizers.	associated with exposure, because the numbers of exposed women were too small to be very informative. For triazine herbicides 12 NHL cases and 38 control cases were reported among women who had ever lived or worked on a farm with triazine use. The risk of non-Hodgkin's lymphoma was not significantly increased (OR=1.2; 95 % CI 0.6-2.6).
Population-based case-control study to clarify whether agricultural use of herbicides and insecticides affects risk of soft-tissue sarcoma (STS), Hodgkin's disease (HD), and non-Hodgkin's lymphoma (NHL) in the United States. (From literature review) Hoar *et al.* 1986	Among white male Kansas residents, aged 21 years or older, men diagnosed with STS, HD, and NHL were identified. 139 STS, 132 HD, and 172 NHL cases were histologically confirmed. The controls were white men from the general population of Kansas. Three controls (N=1005) were matched to each patient on age (± 2 years) and vital status. The questions on telephone interviews on farming practices covered the calendar years working or living on farmland during which crops were grown or livestock was raised, the farm locations and sizes, herbicides and insecticides used, years and acres treated, names and locations of companies where pesticides were purchased, method of application, days per year exposed, and use of protective equipment. Interviews were obtained from 133 patients with STS, 121 with HD, 170 with NHL, and 948 controls, which represented 95% of the eligible subjects (patients, 96%; controls, 94%).	Ninety-five patients with STS, 71 with HD, and 133 with NHL reported having worked or lived on farmland, compared with 662 controls, yielding ORs of 1.0 (95% CI, 0.7, 1.6), 0.8 (95% CI, 0.5, 1.2), and 1.4 (95% CI, 0.9, 2.1), respectively. Farm herbicide use on any of the four specific crops (wheat, corn, sorghumghum, or pasture) was reported by 22 patients with STS, 28 with HD, and 40 with NHL, compared with 192 controls, yielding ORs of 0.9 (95% CI, 0.5, 1.6), 0.9 (95% CI, 0.5, 1.5), and 1.6 (95% CI, 0.9, 2.6), respectively. There was a significant trend (P=.02) in risk of NHL with increasing years of herbicide use and with number of days of herbicide exposure per year (P=.0004). Persons exposed to herbicides more than 20 days per year had an OR of 6.0 (95% CI, 1.9,19.5). Subjects who reported usually mixing or applying the herbicides themselves (OR, 1.9; 95% CI, 1.1, 3.3) had higher risks for NHL than those who reported that someone else performed these functions (OR, 1.1). Farmers who did not use protective equipment, such as rubber gloves or masks, had a higher OR associated with herbicide use (OR, 2.1; 95% CI, 1.0, 4.2) than those who protected themselves (OR), 1.5; 95% CI, 0.7, 3.1). Age and annual days of herbicide exposure were significantly related to NHL risk. Significant excesses were associated with ever use of triazines (eg, atrazine, cyanazine, metribuzin, prometone, propazine, terbutryn) among others. In the absence of phenoxyacetic acid exposure, the NHL risk associated with triazine exposure was reduced to 1.9 (95% CI, 0.4, 8.0).

A population-based case control study conducted on males living in Kansas reported a significant correlation between the use of triazines (including metribuzin) and the risk of non-Hodgkin's lymphoma (Odd ratio (OR) = 2.5; 95 % CI 1.2-5.4), based on 14 cases and 43 controls (Hoar *et al*., 1986). However, when the use of other herbicides (i.e., phenoxyacetic acid herbicides such as 2,4-D) were also controlled for, the risk of non-Hodgkin's lymphoma associated with triazine exposure was no longer significant (OR=1.9; 95 % CI 0.4-8.0). Cases and 3 controls/case were assessed for vital status and age (+ 2 years), but not for other cancer risk factors.

There is also no specific epidemiological data available for the exposure of females to metribuzin. However, in a population case-control study conducted in eastern Nebraska, the risk of non-Hodgkin's lymphoma in women who had lived or worked on farms that had used triazines was not significantly increased (OR=1.2; 95 % CI 0.6-2.6), based on 12 cases and 38 controls, although it is noted that this statement is based on a very small case number (Zahm *et al*., 1993). A population-based case-control study of 173 white men with multiple myeloma (MM) and 650 controls was conducted in Iowa (United States), an area with a large farming population. The association between MM, agricultural risk factors and exposure to individual pesticides was evaluated. The herbicide metribuzin was identified in 7 cases of use of pesticides (mixing, handling or applying). Although, a

slight non-significantly elevated risk for MM was seen among farmers, there was no significant association between MM and the handling either of classes of pesticides or specific pesticides (Brown *et al.*, 1993).
Among 47 investigated pesticides, reported metribuzin use was not linked to increased NHL incidence, when pooled data from three population based case-control studies of NHL in Nebraska, Iowa and Minnesota, and Kansas were analysed (De Roos *et al.* 2003).
In addition, there are two epidemiological studies on metribuzin.
Glioma risks were assessed by analysing, whether glioma patients reported pesticide use. 76 % of replies came from proxies, not from patients, which makes them less reliable. Accordingly for most pesticides assessed the proxy-based risk ratios were higher than self-report-based. One of five exceptions was metribuzin, for which both self and proxy reports a trend, which became significant when the two were added. However, the authors point out, that this is somewhat uncertain, as it is based in only 9 cases.
Due to the low number of cases, no reliable conclusion can be drawn from this (Lee *et al.* 2005).
The association between metribuzin use and cancer risk was evaluated in the Agricultural Health Study, a prospective cohort study of licensed pesticide applicators in Iowa and North Carolina. Never/ever use, lifetime days and intensity-weighted lifetime days were the quantitative exposure metrics evaluated. No adjustment is made for use of other suspected carcinogenic pesticides. Applicators (n=23,072) provided information on metribuzin use on a self-administered questionnaire at enrollment (1993-1997). Among metribuzin users (n=8,504), there were 554 incident cancer cases. Using intensity-weighted lifetime days, the rate ratio (RR) and 95% confidence interval (CI) for the highest exposed tertile for lymphohematopoietic malignancies was 2.09 (95% CI:0.99-4.29), p-trend=0.02 and 2.42 (95% CI: 0.82, 7.19), p-trend=0.08 for leukemia. For Non-Hodgkin lymphoma, the RR was 2.64 (95% CI: 0.76, 9.11), p-trend=0.13 for lifetime days and 2.52 (95% CI: 0.66-9.59), p-trend=0.13 for intensity-weighted lifetime days. Patterns of association were similar for both exposure metrics, but associations were generally weaker than for intensity-weighted days. The results from this study suggest a potential association between metribuzin use and certain lymphohematopoietic malignancies in men; however, having not been observed previously
caution should be used in interpretation (DeLancey *et al.* 2009). Overall, therefore, based on these publications an increased risk for tumour development (i.e. non-Hodgkin´s lymphoma, multiple myeloma, glioma and certain lympho-hematopoietic malignancies) in humans using or being exposed to metribuzin cannot be concluded from these studies.

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

The carcinogenic potential of metribuzin has been studied in rodents in two acceptable 2-year combined chronic toxicity/carcinogenicity studies in rats (M-017948-02-1, M-513629-01-1), and in a carcinogenicity study in mice (M-513707-01-1). In addition, a supplementary chronic toxicity study in rats (M-018704-02-1) and supplementary carcinogenity study in mice (M-018690-01-1) were provided.
No treatment-related changes in neoplastic findings at any dose level or evidence of metribuzin-induced neoplasia was found in any of the provided studies in rats and mice. The neoplastic findings occurred with the same frequency in control and treated animals or were not statistically different from the control animals or in comparison with relevant historical control data and therefore were assessed not to be treatment related.

10.9.2 Comparison with the CLP criteria

According to the CLP Regulation, Category 1A (known to have carcinogenic potential for humans) classification is largely based on human evidence, and Category 1B (presumed to have carcinogenic potential for humans) classification is largely based on animal evidence. The classification in Category 1A and 1B is based on strength of evidence together with additional considerations. Such evidence may be derived from: – human studies that establish a causal relationship between human exposure to a substance and the development of cancer (known human carcinogen); or – animal

experiments for which there is sufficient evidence to demonstrate animal carcinogenicity (presumed human carcinogen). In addition, on a case-by-case basis, scientific judgement may warrant a decision of presumed human carcinogenicity derived from studies showing limited evidence of carcinogenicity in humans together with limited evidence of carcinogenicity in experimental animals.
The placing of a substance in Category 2 is done on the basis of evidence obtained from human and/or animal studies, but which is not sufficiently convincing to place the substance in Category 1A or 1B, based on strength of evidence together with additional considerations. Such evidence may be derived either from limited evidence of carcinogenicity in human studies or from limited evidence of carcinogenicity in animal studies.
Based on epidemiological publications regarding the effects on humans, it can be summarized that speculations about an increased risk for tumour development (i.e. non-Hodgkin´s lymphoma, multiple myeloma, glioma and certain lymphohematopoietic malignancies) in humans allegedly using or being exposed to metribuzin cannot be confirmed.
With regard to animal studies, it can be summarized that in the conducted long-term studies no carcinogenic potential of metribuzin was evident.

10.9.3 Conclusion on classification and labelling for carcinogenicity

Since no carcinogenic potential of metribuzin was evident from the conducted animal studies and also not from the epidemiological publications, the available data does not trigger a carcinogenicity classification according to the CLP Regulation.

10.10 Reproductive toxicity

10.10.1 Adverse effects on sexual function and fertility

Table 29: Summary table of animal studies on adverse effects on sexual function and fertility

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance (purity), dose levels, duration of exposure	Results	Reference
A two generation reproduction study in rats Crl:CD®BR rats (30/group/sex) OECD 416 Deviations regarding deficiencies in test substance analysis and reporting of individual test article intake, no resorption data, insufficient organ and histopathological examination, no evaluation of sperm. GLP	Metribuzin (92.6 %), Oral administration via diet at concentrations of 0, 30, 150, or 750 ppm (equivalent to: P (m/f): 0/0, 1.6/2.2, 7.9/11.1, 39.1/52.6 mg/kg bw/d; F1 (m/f): 0/0, 2.0/2.7, 9.9/13.6, 50.1/67.0 mg/kg bw/d).	**Parental toxicity effects:** **30 ppm**: no effects **150 ppm**: ↑ GGT (F1 females, stat. significant p<0,05 level), hepatocellular hypertrophy; **750 ppm**: significant ↓ food consumption (F0: males -8,9% (stat. significant p<0,05) and females -15,1% (stat. significant p<0,05); F1: males -8,3% (stat. significant p<0,05) and females -10,2% (stat. significant p<0,05) compared to control), significant ↓ body wt. (gain) (F0: males -7,4% (stat. significant p<0,05) and females -12,8% (stat. significant p<0,01); F1: males -9,2% (stat. significant p<0,05) and females -13,2% (stat. significant p<0,01) compared to control). **Reproductive/offspring effects:** **30 ppm**: ↑ total number of dead pups on day 4; **150 ppm**: ↓ litter size of F1 parents -14.9% (compared to control, stat. significant p<0,01) (dose-dependent); ↑ % litters with > 2 dead pups on day 4; **750 ppm**: ↑ % pups dead on PND0-4, ↓ pup wt. (from LD 14 onwards -5 to -11 %, stat. significant p<0,01); a stillborn with multiple malformations and a surviving pup from two different high-dose litters were found to have complete situs inversus; ↓ mean viablity index (F1 pups);.	M-018517-01-1

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance (purity), dose levels, duration of exposure	Results	Reference
Acceptable		**Parental NOAEL**: 30 ppm (equivalent to approximately 2.2 mg/kg bw/d) based on hepatocellular hypertrophy and higher GGT values in F1 females at ≥ 150 ppm and on lower body weight (gain) at 750 ppm in both sexes. **Reproductive NOAEL**: 750 ppm (equivalent to 39.1 – 67 mg/kg bw/d), based on re-evaluation of the study including the results of the three-generation study (M-493110-01-1) in an expert statement (M-420537-03-1) which concluded that there was no effect of metribuzin on sexual function and fertility, and that increased incidences of stillborns were only chance findings rather than compound-related (no dose correlation, findings not reproduced over the generations). The increased pup mortality (% pups dead on PND0-4) as well as the reduced pup weights at the high dose of 750 ppm were covered by the historical control range. **NOAEL of offspring** toxicity at 150 ppm was derived, based on increased post-natal mortality and decreased pup weight at the dose level of 750 ppm.	
Three generation reproduction toxicity study in Wistar rats (30/group/sex (P, F1 and F2)) OECD 416, modified to include a third generation. Deviations regarding examinations of oestrus cyclicity, sperm, sexual maturation, organ weights, histopathology of pups, ovarian follicle count. GLP Acceptable	Metribuzin Technical (97.6 %), in drinking water at concentrations of 0, 30, 150 and 600 ppm.	**Parental effects:** **30 ppm**: no effects **150 ppm**: ↓ weekly mean bw. (P dams and F2 sires), ↓ net bw. gain (P dams, F1 dams and F2 sires), ↓ water intake (P sires); **600 ppm**: ↓ weekly mean bw. and net bw. gain (except F1 sires and F2 dams) in total -7,6% in males and -12,9% in females, ↓ water intake (P sires). **Reproductive/offspring effects:** **30 ppm**: no effects **150 ppm**: ↑ partial cannibalism of pups (P); ↓ live birth index (F1); ↓ day 4 and 21 survival index (F1); ↑ post-implantation loss (P and F2); ↓ mean litter size (F2); **600 ppm**: ↑ partial cannibalism of pups (P and F1); ↑ post-implantation loss (P and F2); ↓ live birth index (F1); ↓ day 4 (F1), 7 (F1), 14 (F1) and 21 (F1 and F2) survival index; ↓ mean litter size (P and F1) and ↓ pup wt. (F1, F2 and F3: -5 to -16 %); ↓ mean number of corpora lutea (F1). **Parental NOAEL**: 30 ppm (equivalent to 3.2 mg/kg bw/day) based on decreased parental bodyweight at 150 ppm and 600 ppm; **Reproduction NOAEL**: 30 ppm (equivalent to 14.8 mg/kg bw/day) based on post-implantation loss, lower live birth index and number of pups dead at birth (150 ppm); **Offspring NOAEL**: 30 ppm based on PND 4-21 survival index at 600 and 150 ppm and lower pup weights at 600 ppm.	M-493110-01-1
Multigeneration (3-generation)	Metribuzin (99.5 %),	**Parental effects:** **100 ppm** (approximately 6.7 mg/kg bw/day): slightly ↓ bw. and bw.	M-018361-

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance (purity), dose levels, duration of exposure	Results	Reference
study on rats FB 30 strain (Elberfeld breed) (10 male and 20 female rats/group) Study pre-dates test guidelines. Deviations regarding dose level selection and test compound analysis, deficiencies in food and water consumption monitoring, no haematology, clinical chemistry or urinalysis, insufficient histopathological examinations, high offspring mortality. Non-GLP Not acceptable	Oral administration via diet at 0, 35, 100 and 300 ppm.	Gain (not significantly ($p > 0,05$) different from control) **Reproductive effects:** **300 ppm** (approximately 20 mg/kg bw/day): ↓ female fertility rates without dose-relationship; slight ↓ average litter size (not significantly ($p > 0,05$) different from control). Because important parameters (food/water consumption, clinical chemistry/haematology/urinalysis, visceral and skeletal anomalies) were not determined, the data given is considered insufficient for the setting of a NOAEL.	02-1

Table 30: Summary table of other studies relevant for toxicity on sexual function and fertility

Type of study/data	Test substance (purity)	Relevant information about the study (as applicable)	Observations	Reference
Subacute and subchronic toxicity studies	Metribuzin technical		In a 90-day study in dogs and in a 2-year study in dogs, effects in testes and epididymides and prostate were seen. No other evidence of effects on reproductive organs or functions was seen in subacute and subchronic toxicity studies.	See Section 10.12.
Estrogenic effects	Metribuzin	*In vitro* assays: steroidogenesis assay, estrogen receptor (ER) binding, ER transactivation assay (ERTA) and aromatase assay; *In vivo* assays: uterotrophic and female pubertal assay.	Since the *in vivo* studies did not give any evidence for an estrogen-related effect, and although metribuzin was positive in the steroidogenesis assay where there were dose-dependent increases in estradiol production at the two highest concentrations, it is concluded that metribuzin has no effect on the estrogen	US EPA, 2011

Type of study/data	Test substance (purity)	Relevant information about the study (as applicable)	Observations	Reference
			hormone pathway *in vivo*.	
Androgenic effects	Metribuzin	*In vitro* assays: androgen receptor (AR) binding assay, steroidogenesis assay; *In vivo* assays: Hershberger and male pubertal assays.	There was no evidence for androgenic or anti-androgenic effects in the Hershberger and the male pubertal assays, also AR binding assay and steroidogenesis assays were negative.	US EPA, 2011

M-018517-01-1 (Acceptable)

In a two-generation reproduction study in rats (M-018517-01-1) metribuzin was administered in the diet to groups of each 30 male and 30 female Charles River rats over 3 generations (F0, F1, and F2) and 2 delivery phases at dose levels of 0, 30, 150 and 750 ppm daily.

The study was conducted mainly in accordance with OECD 416 and with GLP requirements.

Remarkable untoward effects on survival, development, male or female fertility, or reproductive parameters were not reported. No deaths ocurred for F0 or F1 rats selected for the growth and development phase. There were also no indications of embryotoxic or teratogenic effects and no increase in gross pathologic changes at these doses. The only untoward effects observed in this study were significantly inhibited body weight gain (F0 generation -7,4% and females -12,8%; F1 generation males -9,2% and females -13,2%) and reduced food consumption (F0 generation males -8,9% and females -15,1%; F1generation males -8,3% and females -10,2%) at 750 ppm (approximately 54.8 mg/kg bw/d). At ≥ 150 ppm (approximately 11.3 mg/kg bw/d) doses parental toxicity was observed as significant dose-related increase in gamma glutamyl transferase (GGT) levels (F1 females at mid- and high doses) and mild dose-related hepatocellular hypertrophy in F0 and F1 generation rats.

Histopathologic examination of reproductive organs, pituitary gland, and tissues gave no indication of induced effects in F0 and F1 generations rats. Examination of livers was done due to an apparent dose-related increase in GGT values for mid- and high dose F1 dams (significant at the 0,05 level), the increase may be related to hepatic alterations. Examination revealed mild hepatocellular hypertrophy in 8 male and 44 female high-dose, 1 male and 31 female mid-dose, 0 male and 6 female low-dose and 0 male and 3 female control rats. Hypertrophy is probably due to early enzyme induction and would be expected to be reversible.

Food consumption and body weight gain were consistently inhibited for F0 and F1 animals at a dietary concentration of 750 ppm. Slight, but significant, inhibition of body weight gain was also observed for F1 and F2 neonates in the high-dose group.

In the initial study, reproductive toxicity as increased number of pup deaths after live-birth (days 0-4) was noted at ≥ 30 ppm (approximately 2.2 mg/kg bw/d) doses, as well as decreased litter size of F1 parents (dose-dependent); increased percentage of litters with > 2 dead pups on day 4 and decrease of mean viablity index (F1) at ≥ 150 ppm. At 750 ppm, increased percentage of dead pups on PND0-4, decreased pup weight was observed, both values were covered by the historical control range. In addition, the study reported a stillborn with multiple malformations and a surviving pup from two different high-dose litters were found to have complete situs inversus.

Table 31: Multi-generation study in rats – mean body weight gains of F_0 and F_1 parental animals ([g], percent gain in parentheses)

Group	Dose level [ppm]			
	0	**30**	**150**	**750**
F_0 males	339.6 (156.0)	319.7 (148.0)	344.0 (159.5)	301.5 (140.3)*
F_0 females pre-mating	93.8 (53.1)	91.3 (52.4)	86.9(50.5)	61.1 (35.0)$
F_0 females during pregnancy	106.8 (39.6)	96.8 (36.2)	99.0 (37.8)	105.2 (45.0)
F_0 females during lactation	3.4 (1.1)	8.6 (2.9)	20.7 (7.2)&	37.7 (14.2)§
F_1 males	345.8 (177.8)	345.1 (184.5)	353.9 (195.3)	313.8 (177.4)*
F_1 females pre-mating	127.0 (88.9)	118.6 (84.9)	117.1 (85.4)	98.7 (72.8)$
F_1 females during pregnancy	110.8 (40.7)	101.7 (39.0)	95.7 (37.7)$	98.9 (42.4)*
F_1 females during lactation	9.8 (3.3)	13.7 (4.8)	20.5 (7.3)&	31.7 (12.2)§

* significantly lower than control ($p < 0.05$); $ significantly lower than control ($p < 0.01$)
& significantly higher than control ($p < 0.05$); § significantly higher than control ($p < 0.01$)

Table 32: Multi-generation study in rats – average daily food intake [g/d] of F_0 and F_1 parental animals

Group	Dose level [ppm]			
	0	**30**	**150**	**750**
F_0 males	23.7	23.2	23.4	21.6*
F_0 females pre-mating	17.2	16.7	16.5	14.6*
F_0 females during pregnancy	20.7	19.8	19.9	17.8
F_1 males	27.6	26.8	27.2	25.3*
F_1 females pre-mating	19.7	19.3	19.3	17.7*
F_1 females during pregnancy	22.6	21.7	21.3	19.6

* significantly lower than control ($p < 0.05$)

Table 33: F_0 Dam Reproductive Efficiency

F_0 Dam Reproductive Efficiency and F_1 Neonatal Data					
Dose (ppm)		**Control**	**30**	**150**	**750**
Copulation Index[a]		100	100	96.7	100
Fertility Index[b]		96.7	96.7	93.1	100
Gestation Index[c]		100	100	100	100
Gestation length (Days)	Mean	21.8	21.9	21.9	21.7
	Median	22	22	22	22
	Range	21-22	21-23	21-23	21-22
Number of Litters		29	29	27	30
Number of Death among dams		0	0	0	0
Total Number of Pups born		371	358	329	370
Litter Size	Mean	12.8	12.3	12.2	12.3
	Median	13	13	13	12
	Range	5-14	5-17	1-16	7-16
Stillborn Pups	Number	8	6	13	6
	%	2.2	1.7	4.0	1.6
	Mean	0.3	0.2	0.5	0.2
	Median	0	0	0	0
	Range	0-3	0-3	0-5	0-3
Total Number of Dead Pups	Number	16	20	23	29+
(Stillborns + Death)	%	4.3	5.6	7.0	7.8
	Mean	0.6	0.7	0.9	1.0
	Median	0	0	0	0

F_0 Dam Reproductive Efficiency and F_1 Neonatal Data					
Dose (ppm)		**Control**	**30**	**150**	**750**
	Range	0-4	0-3	0-5	0-10
Pup Deaths after Livebirth	Day 0-4	8	14	10	23
Viability Index[d]	Mean	98.0	96.2	97.0	92.4
	Median	100	100	100	100
	Range	84.6-100	81.8-100	76.9-100	0-100
Pup Deaths	Days 5-21	0	0	0	0
Weaning Index[e]	Mean	100	100	100	100
	Median	100	100	100	100
	Range	100-100	100-100	100-100	100-100
Total number of Implantations		393	381	363	395
	Mean	13.6	13.1	13.4	13.2
	Median	14	14	14	13
	Range	6-17	5-17	1-17	8-16
Birth Index[f]	Mean	92.1	91.8	87.4	92.1
	Median	92.3	92.3	92.9	93.1
	Range	78.6-100	66.7-100	50.0-100	58.3-100
Median weight viable Pups (g)	Birth	6.2	6.0	6.0	6.0
	Day 4	9.5	9.7	9.6	9.2
	Day 7	15.4	15.8	15.3	14.6
	Day 14	32.4	31.4	31.3	30.7
	Day 21	52.6	51.5	51.6	48.5**
	Gain	46.8	45.3	45.8	42.5**
Percent Male Fetuses	Median	42.9	45.5	53.8	50.0
	Mean	45.5	47.4	53.1	48.4

+: Includes 10 death in one litter; **: Significantly different from control at the 0.01 level using Kruskal-Wallis test; [a]: No. of animals with successful copulation/ No. of mated animals x 100; [b]: No. of pregnant animals/no. of animals with successful copulation x 100; [c]: No of animals with liveborn/ no of pregnant dams x 100; [d]: No. of neonates viable on day 4/ no. of viable neonates at birth x 100; e: No. of viable neonates on day 21/ no. of viable neonates an day 4 following culling x 100; [f]: No. of liveborn/ no. of implantations x 100

Table 34: F_1 Dam Reproductive Efficiency

F_1 Dam Reproductive Efficiency and F_2 Neonatal Data					
Dose (ppm)		**Control**	**30**	**150**	**750**
Copulation Index[a]		100	100	96.7	100
Fertility Index[b]		83.3	96.7	96.7	93.3
Gestation Index[c]		100	100	100	100
Gestation length (Days)	Mean	21.7	21.6	21.9	21.6
	Median	22	22	22	22
	Range	21-22	21-23	21-23	21-22
Number of Litters		25	29	29	28
Number of Death among dams		0	0	0	0
Total Number of Pups born		353	386	347	345
Litter Size	Mean	14.1	13.3	12.0	12.3
	Median	14	14	13**	12**
	Range	11-17	6-16	3-18	4-16
Stillborn Pups	Number	5	4	3	3
	%	1.4	1.0	0.9	0.9
	Mean	0.2	0.1	0.1	0.1
	Median	0	0	0	0
	Range	0-2	0-1	0-1	0-1
Total Number of Dead Pups	Number	11	17	11	6
(Stillborns + Death)	%	3.1	4.4	.32	1.7
	Mean	0.4	0.6	0.4	0.2
	Median	0	0	0	0
	Range	0-3	0-3	0-3	0-1
Pup Deaths after Livebirth	Day 0-4	6	12	8	3
Viability Index[d]	Mean	98.3	96.9	97.9	99.2
	Median	100	100	100	100

F_1 Dam Reproductive Efficiency and F_2 Neonatal Data					
Dose (ppm)		**Control**	**30**	**150**	**750**
	Range	92.9-100	85.7-100	83.3-100	91.7-100
Pup Deaths	Days 5-21	0	1	0	0
Weaning Index[e]	Mean	100	99.6	100	100
	Median	100	100	100	100
	Range	100-100	87.5-100	100-100	100-100
Total number of Implantations		375	407	378	379
	Mean	15.0	14.0	13.0	13.5
	Median	15	14	14*	14*
	Range	12-18	7-17	3-18	6-16
Birth Index[f]	Mean	93.1	93.7	90.5	89.7
	Median	93.3	93.3	92.3	82.3
	Range	81.2-100	66.7-100	42.9-100	66.7-100
Median weight viable Pups (g)	Birth	5.9	5.9	5.9	6.0
	Day 4	9.4	9.2	9.5	9.2
	Day 7	15.2	15.0	14.9	15.2
	Day 14	31.7	30.5	30.2*	30.0**
	Day 21	52.3	49.1*	49.2*	46.8**
	Gain	46.5	43.1*	43.0*	41.0**
Percent Male Fetuses	Median	50.0	50.0	42.9	42.3
	Mean	48.6	52.1	45.7	44.7

+: Includes 10 death in one litter; *: Significantly different at the 0.05 level using Kruskal-Wallis, **: Significantly different from control at the 0.01 level using Kruskal-Wallis test; [a]: No. of animals with successful copulation/ No. of mated animals x 100; [b]: No. of pregnant animals/no. of animals with successful copulation x 100; [c]: No of animals with liveborn/ no of pregnant dams x 100; [d]: No. of neonates viable on day 4/ no. of viable neonates at birth x 100; e: No. of viable neonates on day 21/ no. of viable neonates an day 4 following culling x 100; [f]: No. of liveborn/ no. of implantations x 100

Table 35: Multi-generation study in rats – F_1 and F_2 pup data (percentage in parentheses, if not stated otherwise)

		Dose level [ppm]			
		0	**30**	**150**	**750**
F_1 pups					
No. of stillborn pups		8 (2.2)	6 (1.7)	13 (4.0)	6 (1.6)
No. of pup deaths after live-birth (days 0-4)		8 (2.2)	14 (3.9)	10 (3.0)	23 (6.2)
No. of pup deaths after culling (days 5-21)		0	0	0	0
Total no. of dead pups on day 4		16 (4.3)	20 (5.6)	23 (7.0)	29 (7.8)
% litters with > 2 dead pups on day 4		3.4	3.4	11.1	10.0
Mean viability index§ [%] (range)		98.0 (84.6-100)	96.2 (81.8-100)	97.0 (76.9-100)	92.4 (0-100)
Median weight of viable pups	**at birth**	6.2	6.0	6.0	6.0
	on day 4	9.5	9.7	9.6	9.2
	on day 7	15.4	15.8	15.3	14.6
	on day 14	32.4	31.4	31.3	30.7
	on day 21	52.6	51.5	51.6	48.5*
	gain (%)	46.8	45.3	45.8	42.5*
F_2 pups					
No. of stillborn pups		5 (1.4)	4 (1.0)	3 (0.9)	3 (0.9)
No. of pup deaths after live-birth (days 0-4)		6 (1.7)	12 (3.1)	8 (2.3)	3 (0.9)
No. of pup deaths after culling (days 5-21)		0	1 (0.3)	0	0
Total no. of dead pups on day 4		11 (3.1)	17 (4.4)	11 (3.2)	6 (1.7)
% litters with > 2 dead pups on day 4		4.0	3.4	3.4	0
Mean viability index§ [%] (range)		98.3 (92.9-100)	96.9 (85.7-100)	97.9 (83.3-100)	99.2 (91.7-100)
		Dose level [ppm]			
		0	**30**	**150**	**750**
Median weight of viable pups	**at birth**	5.9	5.9	5.9	6.0
	on day 4	9.4	9.2	9.5	9.2
	on day 7	15.2	15.0	14.9	15.2
	on day 14	31.7	30.5	30.2*	30.0$
	on day 21	52.3	49.1*	49.2*	46.8$
	gain	46.5	43.1*	43.0*	41.0$

* significantly different from control ($p < 0.05$, Kruskal-Wallis test); $ significantly different from control ($p < 0.01$, Kruskal-Wallis test);§ (no. of viable pups on day 4/number of viable pups at birth)

Table 36: Multi-generation study in rats – F_1 generation GGT levels (possibly in U/L, no units specified in study)

Sex	**Dose level [ppm]**			
	0 ppm	**30 ppm**	**150 ppm**	**750 ppm**
Males	9.71	11.22	7.39	4.79$
Females	5.96	8.56	10.15*	11.81*

* significantly different from control ($p < 0.05$, two-tailed Dunnett's t-test); $ significantly different from control ($p < 0.01$, Bartlett test)

However, a re-evaluation of the study in 2016 with additional information and in a weight of evidence approach together with the three-generation study by (M-493110-01-1) in an expert statement (M-420537-03-1) provided by the submitter, concluded there was no effect of metribuzin on sexual function and fertility. The increased incidences of stillborns were considered chance findings rather than substance-related effect (no dose correlation, findings not reproduced over the generations). The number of F1 pup deaths after live-birth (days 0-4) in the 0, 30, 150 and 750 ppm dose groups were 8, 14, 10 and 23 respectively in the initial study report, but after the exclusion of

one dam and her pups in the high dose group due to severe dystocia (caused by cephalopelvic disproportion between the big pups and the small dam), the respective numbers were calculated as 8, 14, 10 and 16 F1 pup deaths. It was demonstrated that the increased pup mortality (% pups dead on PND0-4) as well as the reduced pup weights at the high dose of 750 ppm were covered by the historical control range.

Table 37: Body weight [g] of dam 8015 (750 ppm, F0) suffering severe dystocia, and mean body weight [g] of the 750 ppm and control group animals on GD 0 and 20 in the two-generation study on metribuzin conducted by M-018517-01-1.

	Body weight (bw) / mean bw [g] Gestation day 0	Body weight (bw) / mean bw [g] Gestation day 20
Dam 8015, 750 ppm, F0	218	313
750 ppm group, F0	233.5** (2.9)	338.7** (4.6)
Control group, F0	269.7 (4.1)	376.4 (5.8)

**: statistically significantly reduced in comprison to controls (p< 0.01)

Table 38: Pup weight at birth [g] of 3 pups of dam 8015 (750 ppm) suffering severe dystocia, median pup weight at birth [g] of the 750 ppm and control group pups, and historical control range for pup weight at birth in the two-generation study on metribuzin conducted by M-018517-01-1.

	Pup weight at birth [g]
Pup weight of the 3 pups of dam 8015 found dead after birth on GD 23	6.6 (female) 6.6 (female) 6.8 (male)
Median pup weight 750 ppm group at birth	6.0
Median pup weight control group at birth	6.2
Historical control range for pup weight at birth	5.6 – 6.4

Exclusion of F0 dam 8015 and her F1 pups (750 ppm) from the evaluation changes the no. of stillborn F1 pups and the no. of pup deaths after live-birth (days 0-4) as follows:

Table 39: Nos. and percentages of pups born, stillborn pups and pup deaths after live birth (days 0-4) in the two-generation study on metribuzin conducted by Porter et al. (1988) after exclusion of F0 dam 8015 and her F1 pups (750 ppm) due to severe dystocia

	Dose [ppm]				
	0	30	150	750	
F1 pups					
Total no. of pups born	371	358	329	~~370~~*	**360**
Stillborn pups	8	6	13	~~6~~*	**3**
Stillborn pups [%]	2.2	1.7	4.0	~~1.6~~*	**0.8**
No. of pup deaths after live-birth (days 0-4)	8	14	10	~~23~~*	**16**
No. of pup deaths after live-birth (days 0-4) [%]	2.2	3.9	3.0	~~6.2~~*	**4.4**

* Former data crossed out

M-493110-01-1 (Acceptable)

In a 3-generation (P, F1 and F2) reproduction study (M-493110-01-1), metribuzin mixed in drinking water at concentrations of 30, 150 and 600 ppm was provided. Parental effects as decreased body weight were observed at 150 ppm (approximately 14.8 and 22.1 mg/kg bw/d for males and females) and 600 ppm (49.6 and 77.1 mg/kg bw/d). Reproductive effects were observed at ≥ 150 ppm: increased incidences of partial cannibalism and percentage of post-implantation loss and decreased live birth and survival indices. At the high dose of 600 ppm decreased pup weight (-5 to -16 %) was reported, the mean number of corpora lutea in the F1 generation was lower, the percentage of post-implantation loss was higher in the P generation, the mean litter size was lower in P and F1 generations, the mean weight of pups on some occasions or throughout the pre-weaning period was reduced in all three generations, the pup survival index (4-21 days of age) during the pre-weaning period in the F1 litter were lower. Administration of metribuzin at a concentration of 600 ppm resulted in significant reduction in body weights, growth, food and water consumption prior to cohabitation of males and females for breeding, during gestation and lactation periods in all three generations. The effects on the reproductive performance and on the litter were more prominent in the P generation than in the subsequent generations. In conclusion, in this study a concentration of 30 ppm in drinking water had no effect on growth and development and reproductive performance in the three successive generations studied. Treatment with metribuzin technical at a concentration of 150 ppm in drinking water resulted in effects on body weights of parental animals, litter data and reproductive parameters. Administration of metribuzin technical at 600 ppm resulted in significant reduction in body weights, growth, food and water consumption in all three generations and also some effects on reproduction parameters.

Table 40: Three-generation study in rats - mean net body weight gains [g] of parental animals

Dose level [ppm]	Parental generation P		Parental generation F_1		Parental generation F_2	
	Male wk 1-16	Female wk 1-10	Male wk 1-15	Female wk 1-13	Male wk 1-16	Female wk 1-13
0	208 ± 41.3	80 ± 14.4	330 ± 35.9	188 ± 21.1	364 ± 37.8	185 ± 13.2
30	211 ± 38.1	74 ± 11.8	348 ± 35.4	195 ± 16.6	357 ± 34.6	192 ± 15.9
150	190 ± 31.9	68 ± 13.8*	313 ± 28.5	173 ± 14.6*	336 ± 34.1*	181 ± 17.2
600	177 ± 37.6*	50 ± 13.0*	313 ± 35.4	174 ± 17.5*	342 ± 31.5*	179 ± 13.7

* significantly ($p \leq 0.05$) different from control

Table 41: Three-generation study in rats - mean food, water and test compound intake

Parameter	Generation	Sex	Dose level [ppm]			
			0	30	150	600
Food intake [g/kg bw/d]	P	male	67.1	67.0	66.5	66.5
		female	100.5	101.9	100.8	101.4
	F_1	male	74.7	73.2	75.2	76.6
		female	114.9	112.3	114.3	119.6
	F_2	male	77.9	76.1	77.8	80.0
		female	112.8	110.8	112.3	114.4
Water intake [g/kg bw/d]	P	male	109.3	111.2	98.5*	82.7*
		female	165.2	171.0	147.5	128.5
	F_1	male	108.0	106.8	113.4	94.8
		female	171.6	159.8	162.8	138.0
	F_2	male	116.5	99.6	94.9	92.7
		female	168.9	155.6	144.6	128.0
Test compound intake [mg/kg bw/d]	P	male	0	3.3	14.8	49.6
		female	0	5.1	22.1	77.1
	F_1	male	0	3.2	17.0	56.9
		female	0	4.8	24.4	82.8
	F_2	male	0	3.0	14.2	55.6
		female	0	4.7	21.7	76.8

* significantly ($p \leq 0.05$) different from control

Table 42: Three-generation study in rats - summary of statistically significant effects on fertility indices

Dose level [ppm]	Mean no, of corpora lutea $^{\$}$	Implantations [%] $^{\#}$	Live pups born [%] $^{\&,\#}$	Pre-implantation loss [%] $^{\#}$	Post-implantation loss [%] $^{\#}$
Generation P / Litter F_1					
0	14.6	85.8	92.3	14.2	7.7
30	14.5	91.9*	90.2	8.1*	9.8
150	15.1	90.9*	85.0*	9.1*	15.0*
600	14.1	90.5*	84.9*	9.5*	15.1*
Generation F_1 / Litter F_2					
0	15.7	85.2	86.6	14.8	13.6
30	16.0	88.3	84.7	11.7	15.3
150	15.2	88.4	93.1	11.6	6.9
600	14.5*	92.2*	78.6	7.8*	21.4
Generation F_2 / Litter F_3					
0	15.4	85.7	77.8	14.3	22.2
30	16.1	83.3	81.2	16.7	18.8
150	15.4	87.0	85.3*	13.0	14.7*
600	14.0	85.7	85.4*	14.3	14.6*

* significantly ($p \leq 0.05$) different from control, $^{\#}$ Z-test, $^{\$}$ t-test, $^{\&}$ calculated as (no. of live pups born / no. of implantations) x 100 %

Table 43: Three-generation study in rats - summary of statistically significant effects on litter and pup survival data

Parameter	Dose level [ppm]											
	Generation P / Litter F_1				Generation F_1 / Litter F_2				Generation F_2 / Litter F_3			
	0	30	150	600	0	30	150	600	0	30	150	600
No. of pregnancies	27	29	30	29	27	30	29	29	30	29	29	29
No. of live litters #	25	29	30	29	26	30	29	28	28	28	29	27
Total no. of pups born	319	352	379	333	337	380	365	328	320	325	336	305
Mean litter size $	12.8	12.1	12.6	11.5*	13.0	12.7	12.6*	11.7*	11.4	11.6	11.6	11.3
Mean viable litter size $	12.5	12.0	11.7	10.8*	12.5	12.0	12.5	10.9	11.0	11.3	11.4	11.0
Live birth index (%) @#	98.1	98.9	92.3*	94.3*	96.1	94.5	99.5*	93.0	96.6	97.2	98.8	97.7
No. of pups dead at birth #	6	4	29*	19*	13	21	2*	23	11	9	4	7
No. of live pups on day 1 #	313	348	350*	314*	324	359	363*	305	309	316	332	298
No. of live pups on day 2 #	313	348	349*	314*	324	359	363*	305	309	316	332	298
No. of pups dead/cannibalised up to day 4 # (including pups dead at birth)	8	22*	41*	37*	23	45*	8*	30	14	24	12	15
No. of live pups on day 4 #	311	330*	338*	296*	314	335*	357*	298	306	301	324	290
No. of pups left after standardisation	200	219	224	221	198	222	232	208	207	206	217	198
No. of pups dead/cannibalised on day 5-7 #	1	1	1	9*	0	0	0	0	2	0	2	1
No. of live pups on day 7 # (after standardisation on day 4)	199	218	223	212*	198	222	232	208	205	206	215	197
No. of live pups on day 14 #	199	216	223	208*	196	222	232	206	204	205	214	196
No. of pups dead/cannibalised on day 15-21 #	0	2	8*	5*	1	2	0	9*	2	1	1	0
No. of live pups on day 21 #	199	214	215*	203*	195	220	232	197*	202	204	213	196
Rate of pups dead or cannibalised (%) § (days 0-21, corrected for culled animals)	3.0	7.6	14.9	17.4	7.4	12.7	2.1	13.4	6.2	7.9	5.0	5.4
Day 0-4 survival index& (%) #	99.4	94.8*	96.6*	94.3*	96.9	93.3*	98.3	97.7	99.0	95.3*	97.6	97.3
Day 4-21 survival index& (%) #	99.5	97.7	96.0*	91.9*	98.5	99.1	100	94.7*	97.6	99.0	98.2	99.0

* significantly ($p \leq 0.05$) different from control; # Z-test; $ t-test; @ calculated as (no. of viable / total no. of pups born) x 100 %, based on first observation of pups after birth

Table 44: Three-generation study in rats - summary of pup weights (mean and standard deviations) [g]

Dose level [ppm]	Male pups on day					Female pups on day					Combined sexes on day				
	1	4	7	14	21	1	4	7	14	21	1	4	7	14	21
Generation P / Litter F_1 (means and standard deviations)															
0	5.7 0.5	7.7 0.7	11.9 1.1	22.2 2.4	30.8 4.7	5.4 0.4	7.4 0.6	11.3 1.1	21.4 2.6	30.3 4.7	5.5 0.5	7.6 0.7	11.6 1.1	21.8 2.4	30.6 4.6
30	5.7 0.4	7.8 0.8	11.3 1.2	19.9 2.0*	29.4 3.1	5.3 0.4	7.3 0.7	10.7 1.1	19.2 2.2*	28.6 3.5	5.5 0.4	7.6 0.7	11.0 1.1	19.6 1.9*	29.0 3.1
150	5.4 0.5	7.3 0.9	11.1 1.2*	20.7 2.6	31.4 3.8	5.1 0.6	7.1 0.9	10.8 1.5	20.0 3.3	30.4 4.6	5.3 0.6	7.2 0.9	11.0 1.2	20.4 2.8	31.0 4.0
600	5.3 0.6*	7.0 0.9*	10.1 1.2*	18.8 2.1*	27.7 3.5*	5.0 0.6*	6.7 0.8*	9.7 1.2*	18.0 2.7*	26.7 4.2*	5.2 0.6	6.8 0.9*	9.9 1.2*	18.4 2.3*	27.1 3.7*
Generation F_1 / Litter F_2															
0	5.6 0.7	7.9 1.1	12.3 1.4	23.9 1.9	36.6 4.7	5.2 0.8	7.5 1.2	11.8 1.5	23.0 1.9	36.5 2.6	5.4 0.7	7.7 1.1	12.0 1.4	23.4 1.8	36.4 3.5
30	5.6 0.7	7.8 1.4	12.0 1.6	23.2 2.2	35.6 4.0	5.2 0.7	7.3 1.3	11.3 1.5	22.0 1.9	34.2 3.1	5.3 0.4	7.5 0.8	11.6 1.0	22.6 1.6	34.9 2.4
150	5.6 0.6	7.7 1.1	11.6 1.3	22.6 2.3	35.1 4.1	5.4 0.5	7.4 1.1	11.1 1.3	21.7 2.2	34.1 4.2	5.5 0.6	7.6 1.1	11.4 1.3	22.2 2.2	34.6 4.0
600	5.5 0.6	7.5 1.2	11.3 1.6	21.1 3.9*	31.8 5.4*	5.3 0.7	7.2 1.2	10.9 1.6	20.7 3.6*	31.1 5.6*	5.4 0.6	7.3 1.2	11.1 1.5*	20.8 3.5*	31.4 5.4*
Generation F_2 / Litter F_3															
0	5.7 0.7	7.8 1.0	11.7 1.1	23.0 2.4	34.0 3.2	5.5 0.6	7.5 1.0	11.4 1.1	22.5 2.3	33.8 3.4	5.6 0.6	7.6 1.0	11.6 1.1	22.8 2.3	34.1 3.3
30	5.7 0.8	8.0 1.6	12.3 2.3	22.8 3.0	32.0 4.6	5.4 0.8	7.6 1.5	11.7 2.0	22.1 2.9	31.6 5.2	5.6 0.8	7.8 1.5	12.0 2.0	22.4 2.8	31.7 4.6
150	5.5 0.6	7.6 0.8	11.7 1.3	22.3 2.8	31.4 4.3	5.3 0.4	7.4 0.7	11.3 0.9	21.4 2.6	30.4 4.3*	5.4 0.5	7.5 0.7	11.5 1.2	21.9 2.7	31.0 4.2*
600	5.5 0.5	7.5 0.9	11.5 1.3	21.5 2.3	30.2 5.1*	5.2 0.5	7.2 1.0	11.1 1.3	20.4 2.3*	28.4 4.1*	5.3 0.5	7.4 0.9	11.3 1.3	21.0 2.0*	29.3 4.1*

* significantly ($p \leq 0.05$) different from control

Also this study was re-evaluated in an expert statement (M-420537-03-1), with additional historical control data and in a weight of evidence approach together with the two-generation study (M-018517-01-1). It was concluded in the re-evaluation that there was no effect of metribuzin on sexual function and fertility.
The re-evaluation revealed as an overall conclusion that:

- The increased incidences of stillborns in single dose groups of the F1 and F2 generation of the reproductive toxicity studies on metribuzin are considered to be chance findings and not compound related effects, since there was no dose-relationship in any generation and the findings were not reproduced over the generations. This is supported by the fact that no increase in post-implantation losses were seen in the new developmental toxicity studies in rat and rabbit at comparable doses and no compound related effects on the birth process were observed in any generation study.
- The same applies for other parameters influenced by the number of stillborns, such as the decreases of the live birth index and pups dead at birth as well as for the increased no. of pups dead or cannibalized on PND 0-4 in the three-generation study (M-493110-01-1). Due to lack of dose dependency and the fact that the findings are not seen consistently in the three generations, they are not considered to be treatment related.
- The incidences of % pups dead on postnatal day 0-4 showed no dose-correlation and were all covered by the historical controls, so that there is also no compound related adverse effect.
- Regarding the partly statistically significant trend towards reduced pup weights in high dose F1 and F2 pups of the two-generation study (M-018517-01-1) all median pup weights were well in the range of historical controls. Statistically significantly reduced pup weights (-5 to -16 % of control pup weights) were also observed in the high dose of the three-generation study (M-493110-01-1). Historical control data for pup weights are not available for this study. The slight reductions observed at birth and shortly after are considered to be secondary to the markedly reduced body weights in the dams. The more pronounced decreases on lactation day 14 and 21, when the pups are already taking up metribuzin in the diet on their own, are considered to be a consequence of the systemic toxicity of the substance (observed in both studies in the high dose) and not as a specific toxic effect on offspring.

M-018361-02-1 (Not acceptable)

In a 3-generation reproduction study (M-018361-02-1), rats were exposed to 0, 35, 100 and 300 ppm of metribuzin via diet. Dietary concentrations of up to 300 ppm did not affect the behaviour, mortality, body weight development orthe reproductive performance of the treated rats. In dietary concentrations up to 100 ppm no significant difference was observed between the treated groups and the control animals as regards fertility, litter size, average pup weight shortly after birth and during the rearing phase. The lactation performance of the dams was also unaffected. Metribuzin administered to rats in dietary concentrations of up to 100 ppm thus had no effect on reproduction. At the high dose of 300 ppm slight reduction of female fertility was observed although without a dose-relationship, and a small decrease of average litter size was noted, which both were not significantly ($p > 0,05$) different from those of the controls. In parental animals slightly reduced body weights and body weight gain were observed commencing at 100 ppm (approximately 6.7 mg/kg bw/d). This study was considered not acceptable due to the lack of important parameters and insufficient for the establishment of NOAEL.

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

The data of three multigeneration studies in rats are available.
Based on the available data, metribuzin did not cause clear treatment related effects on sexual function and fertility. This is further supported by the results of the conducted studies on an endocrine potential of metribuzin which demonstrated that treatment with metribuzin did not lead to

endocrine findings on the male and female reproductive tract, nor to endocrine-related effects on reproduction or development.

10.10.3 Comparison with the CLP criteria

According to the criteria in the CLP Regulation, reproductive toxicity includes adverse effects on sexual function and fertility in adult males and females, as well as developmental toxicity in the offspring. In this classification system, reproductive toxicity is subdivided under two main headings:

- Adverse effects on sexual function and fertility;
- Adverse effects on development of the offspring.

Some reproductive toxic effects cannot be clearly assigned to either impairment of sexual function and fertility or to developmental toxicity. According to the CLP criteria, a 'Suspected human reproductive toxicant' is a substance which is classified in Category 2 for reproductive toxicity when there is some evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on sexual function and fertility, or on development, and where the evidence is not sufficiently convincing to place the substance in Category 1. If deficiencies in the study make the quality of evidence less convincing, Category 2 could be the more appropriate classification.

Such effects shall have been observed in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of the other toxic effects.

As described before, and based on the re-evaluation, the three rat dietary multigeneration reproductive toxicity studies with oral administration of metribuzin via diet or drinking water at concentrations up to 750 ppm (which corresponded to approximately 39-67 mg/kg bw/daily), resulted in some uncertain decrease of litter size and live birth and survival indices from ≥ 150 ppm doses and a decrease of pup weight at the high doses of 600-750 ppm. However, all these findings were re-evaluated as unsignificant as falling in the range of expanded historical control data. Therefore it can be concluded that metribuzin did not effect reproductive parameters, like fertility, mating, days between pairing and mating, gestation, parturition, lactation, litter size, sex ratios, pup mortality, neonatal toxicity (body weights and clinical condition), or markers of endocrine function (oestrous cycling, balano-preputial separation, vaginal opening, spermatogenetic function and capacity). The data regarding effects on sexual function and fertility are conclusive and do not warrant classification.

10.10.4 Adverse effects on development

Table 45: Summary table of animal studies on adverse effects on development

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance (purity), dose levels, duration of exposure	Results (effects statistically significantly different unless stated otherwise)	Reference
Dose-range finding study preceding a developmental toxicity study in rats Wistar: Rcc Han™: WIST (4 females/ group) Non-GLP Acceptable	Metribuzin (91.9 %), Formulation in 0.5 % Methyl-cellulose + 0.4 % TWEEN8O in tap water, by gavage at 0, 10, 50, 100 mg/kg bw/day on gestation days	**Maternal toxicity:** **10 mg/kg bw/d:** ↓ food consumption (GD 16-18) (-20% in comparison to controls, statistically significant $p < 0.05$), ↑ thyroid wt (+8% in comparison to controls; dose dependent, but not statistically significant); **50 mg/kg bw/d:** signs of weakness, hypoactivity, ↓ body temperature (GD 5, 4 h post-dose, statistically significant $p <$ 0,01), discharge from eyes or nose, ↓ bw. gain (statistically significant $p < 0.01$; -109% in comparison to controls); **100 mg/kg bw/d:** ↓ food consumption (GD10-12) (-22% in	M-525369-02-1

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance (purity), dose levels, duration of exposure	Results (effects statistically significantly different unless stated otherwise)	Reference
	(GD) 5 through 21. Caesarean section and necropsy on GD 21.	comparison to controls, statistically significant p< 0.01) irregular breathing (2/4), ↑ thyroid wt. (statistically significant p < 0.05;+55% in comparison to controls); ↑ relative thyroid (statistically significant p < 0.05;+66% in comparison to controls); ↓ bw. gain (statistically significant p < 0.05; -87% in comparison to controls) **Developmental toxicity:** **10 mg/kg bw/d:** slightly ↓ fetal weight (-13% in comparison to controls, not statistically significant); **100 mg/kg bw/d:** slightly ↓ fetal weight (-15% in comparison to controls, not statistically significant); one malformation (absent mandible) and a small eye bulge in one fetus. **NOAEL**: 10 mg/kg bw/d (maternal toxicity), 100 mg/kg bw/d (developmental toxicity). On the basis of this dose range finding study, dose levels of 0, 3, 15 and 75 mg metribuzin/kg bw/day were selected for the subsequent prenatal developmental toxicity study.	
Prenatal oral developmental toxicity study in rats Wistar Outbred rats RccHan™:WIST (24 mated females per group) OECD 414 GLP Acceptable	Metribuzin technical (91.9 %), Oral gavage as a suspension in 0.5 % methylcellulose and 0.4 % TWEEN®80 in tap water at levels of 0, 3, 15 and 75 mg/kg bw/d from gestation day (GD) 5 up to and including GD 20. Caesarean section: GD 21.	**Maternal toxicity**: **15 mg/kg bw/d:** ↓ food intake (GD 5-14, -7% to -15%, statistically significant p < 0.05), ↓ body temperature (GD 5, 2 h post-dose, statistically signifiant p < 0,05) ; hypoactivity (1 female) and piloerection (3 females) on GD 5 **75 mg/kg bw/d:** hunched posture, piloerection (GD 5-6 in all) and/or dyspnoea (one rat), hypoactivity (GD 5 23 out of 24 rats), motor activity: ↓ total distance moved; GD 8 ↓ bw loss (17/24 rats) or ↓ bw growth (7/24 rats) (-5,7% in comparison to controls, statistically significant p < 0.05), ↑ liver wt.(+12% in comparison to control, statisticaly significant p < 0.01); ↓ body temperature (GD 5, statistically significant p < 0,01) ↓ bw gain (not statistically significant); ↓ food consumption (GD 5-14, -8% to -43%, statistically significant p < 0.01) **Developmental toxicity:** **15 mg/kg bw/d:** retardations (slight): absence of ossification of digits in the proximal hind limb phalanges (statistically significant p < 0.05) (within the historical control data); **75 mg/kg bw/d:** slightly ↓ fetal weight (statistically significant (total fetal weight: 5.0 (p<0.05) *vs.*. 5.2 g (-3.8% of controls); female fetal weight: 4.8 (p<0.05) vs. 5.1 g (-5.9% of controls)). retardations in ossification of the forelimbs, hindlimbs, vertebrae and skull (statistically significant p < 0.05, in the range of historical controls). No evidence for teratogenicity. **NOAEL**: 3 mg/kg bw/d (maternal and developmental toxicity).	M-530086-01-1
Prenatal oral Developmental dose range-	Metribuzin (91.9 %), Oral gavage of	**Maternal toxicity:** **10 mg/kg bw/d:** no effects	M-537597-01-1

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance (purity), dose levels, duration of exposure	Results (effects statistically significantly different unless stated otherwise)	Reference
finding study in New Zealand White rabbits Hra:(NZW)SPF (groups of 6 females) No applicable guideline. GLP Acceptable	0, 10, 30, 100 mg/kg bw/day during gestation (GD) days 7-28. Caesarean section: GD 29.	**30 mg/kg bw/d:** ↓ food intake (GD 7-8, stat. sign. p<0.05), ↓ bw gain (GD 7-10, not statistically significant); **100 mg/kg bw/d:** ↓ defecation (GD 12-21), wet clear material around mouth/nose (2-4 h post-dose), ↓ respiration rate (4-8 h postdose, GD 7), slightly ↓ body temperature (2-4 h post-dose, GD 7, -2% in comparison to control, not stat. significant), bw loss (GD 7-8, statistically significant p<0.05) and ↓ bw gain (GD 7-10, -9,2% in comparison to controls, not statistically significant), ↓ food intake (GD 7-29, -34% in comparison to control, statistically significant p<0.01), ↑ mean absolute liver weight (+6,9% in comparison to controls, not stat. sign.); ↓ net bw change (significantly different from the control group p<0.05) **Developmental toxicity:** **100 mg/kg bw/d:** ↓ fetal weight (-18,4% in comparison to controls, just below the lower limit of HCD), ↓ mean litter proportion of viable fetuses (due primarily to only 1 dam with total litter loss), ↑ post implantation losses (not stat. sign.). **NOAEL**: 10 mg/kg bw/d (maternal) based on decrease in food consumption and body weight at 30 mg/kg bw/d; and 30 mg/kg bw/d (developmental) based on decreased fetal weight, increased post-implantation losses and increased % of dead fetuses per litter at 100 mg/kg bw/day. Doses of 10, 30, and 100 mg/kg/day were suggested for a definitive prenatal developmental toxicity study in timemated New Zealand White rabbits.	
Prenatal oral developmental toxicity study in New Zealand White Rabbits Hra:(NZW)SPF (groups of 25 time-mated females) OECD 414 GLP Acceptable	Metribuzin (91.9 %) suspended in 0.5 % aqueous methylcellulose (400 cps) and 0.4 % Tween® 80. Oral gavage of 0, 10, 30, 100 mg/kg bw/day gestation days (GD) 7-28; caesarean section: GD 29.	**Maternal toxicity:** **10 mg/kg bw/d:** no effects **30 mg/kg bw/d:** ↓ bw gain (transient), ↓ food intake (-13% on GDs 7-29); **100 mg/kg bw/d:** ↓ defecation, wet clear material around mouth (2-4 h post-dose); lower body weights (from -5% to -9% on GDs 8-29); ↓ food intake (-34% on GDs 7-29). 1 animal sacrificed in extremis on GD 12 (due to ↓ defecation, extensive body weight loss (9.7% of GD 7 body weight), minimal food intake (≤ 20 g/anmal/day)). **Reproductive toxicity**: no effects **Developmental toxicity:** **10 mg/kg bw/d:** no effects **30 mg/kg bw/d:** no effects **100 mg/kg bw/d:** ↓ fetal weight (males -11%; combined sex -11%).	M-537608-01-1

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance (purity), dose levels, duration of exposure	Results (effects statistically significantly different unless stated otherwise)	Reference
		NOAEL: 10 mg/kg bw/d (maternal toxicity) and 30 mg/kg bw/d (developmental toxicity).	
Prenatal oral developmental toxicity study in American Dutch rabbits, artificially inseminated (groups of 17 females) OECD 414 Deviations regarding the test substance batch used was more than seven years old, no purity but homogeneity and stability were analysed. GLP Acceptable	Metribuzin (92.7 %) suspended in 0,5 % carboxymethyl cellulose, 0,4 % Tween 80 and distilled water. Oral gavage to 3 groups of rabbits at doses of 0 (vehicle control), 10, 30, or 85 mg/kg bw/day from day 6 to day 18 of gestation. Caesarean section: GD 28.	**Maternal toxicity:** **10 mg/kg bw/d:** no effects **30 mg/kg bw/d:** no effects **85 mg/kg bw/d:** lower bodyweight (-6% on day 14); lower bodyweight gain (-58% on days 6-18); ↓ food consumption (from -13% to -51% on days 7-19); increase in the incidence of stool changes (soft (in 1/0/1/2 animals of 17 in the 0/10/30/85 mg/kg bw/d dose groups) or diminished quantity (in 0/4/4/10 animals of 17 in the 0/10/30/85 mg/kg bw/d dose groups)); **Developmental toxicity:** **10 mg/kg bw/d:** no effects **30 mg/kg bw/d:** Significantly reduced fetal and placental weights at 30 mg/kg bw/d were related to the higher average litter size of this group and were not seen as a test substance effect **85 mg/kg bw/d:** sex ratio of fetuses was shifted towards the female side (% male foetuses 50.0/50.0/47.7/33.3 in the 0/10/30/85 mg/kg bw/d dose groups). **NOAEL:** 30 mg/kg bw/d (maternal toxicity), ≥ 85 mg/kg bw/d (developmental toxicity).	M-018201-01-1
Rat oral developmental toxicity study Charles River Crl:CD® BR rats (Groups of 33 dams each) No specific guidline GLP Supplementary	Metribuzin (92.6 %), By gavage at doses of 0, 25, 70 or 200 mg/kg bw/day from gestation day 6-15. Each group of 33 dams was subdivided into 2 termination phases: Phase I: 5 dams on Day 16 of gestation, and Phase II: 28 dams on Day 20 of gestation.	**Maternal toxicity:** **25 mg/kg bw/d:** hypoactivity and ptosis in 25/28 dams; ↓ body wt. (-5% at day 15); ↓ food consumption (-17% on day 8, -16% on day 12, -10% on day 15 of gestation); **70 mg/kg bw/d:** hypoactivity and ptosis in all animals, ataxia in all dams; ↓ body weight (-6%...-8% on days 8...20 of gestation); ↓ body weight gain (8%); ↓ food consumption (-37% on day 8; -16% on day 12, -12% on day 15 of gestation) Thyroid hormone levels: ↓ T4 (-52% on day 16) **200 mg/kg bw/d:** hypoactivity and ptosis in all animals, ataxia in all dams; ↓ body weight (-8...-10% on days 8...20 of gestation); ↓ body weight gain (-13%); ↓ food consumption (-41% on day 8; -22% on day 12, -17% on day 15 of gestation), Thyroid hormone levels and organ weights: ↓ T4 (-85% on day 16); ↑ absolute thyroid weight (+57% on day 16; +33% on day 20) **Reproductive effects:** dose-related increase in the number of dams with more than one resorption (6/8/10/11 at 0/25/70/200 mg/kg bw/d, respectively).	M-018676-01-1

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance (purity), dose levels, duration of exposure	Results (effects statistically significantly different unless stated otherwise)	Reference
		Develomental toxicity: **25 mg/kg bw/d:** ↓ median fetal weight (females -5%) **70 mg/kg bw/d:** ↓ median fetal weight (females -5%) **200 mg/kg bw/d:** ↓ median fetal weight (males -5%, females -6%, combined -8%); increased incidence of reversible rib deformations (wavy, curved and/or bulbous ribs) and of retarded ossification in the areas of the skull, ribs, spine, sternum and limbs; ↓ median placental wt. (-8%).	
Oral developmental toxicity study in Wistar rats (groups of 27-35 dams each) OECD 414 Deviations regarding Insufficient analysis of test item, maternal effects were seen already at mid-dose while embryo-/fetotoxicity was noted even at the lowest dose tested. GLP Supplementary	Metribuzin (97.6 %), Formulation in refined groundnut oil, gavage at 0, 10, 40 and 150 mg/kg bw/day from day 6 through day 15 of gestation. Caesarean sections were performed on day 20 of gestation.	**Maternal toxicity:** **10 mg/kg bw/d:** lower feed consumption (-24% on treatment days 6-15 of gestation; -14% on gestation days 0-20); **40 mg/kg bw/d:** lower feed consumption (-19% on treatment days 6-15 of gestation; -16% on post-treatment days 15-20; -18% on gestation days 0-20). **150 mg/kg bw/d:** lower feed consumption (-33% on treatment days 6-15 of gestation; -20% on post-treatment days 15-20; -23% on gestation days 0-20) **Developmental toxicity:** There was no statistical difference in the mean litter size, abnormal foetuses, total number of live foetuses, male and female foetuses, foetal weights and the sex ratio in the treatment groups compared to the respective control values. **10 mg/kg bw/d:** ↑ incidences of slight dilatation of renal pelvis, slightly tortuous ureter, delayed ossification of hyoid, rudimentary rib no. 14, incomplete/poor ossification of parietal/interparietal, pubis, fore limb metacarpals: ¼, dumb-bell shaped thoracic vertebrae; **40 mg/kg bw/d:** ↑ incidences of slight dilatation of renal pelvis, slightly tortuous ureter, delayed ossification of hyoid, incomplete/poor ossification of parietal/interparietal, pubis, fore limb metacarpals: 1/4, dumb-bell shaped thoracic vertebrae; **150 mg/kg bw/d:** ↑ incidences of small fetuses, slight dilatation of renal pelvis, hydronephrosis, delayed ossification of hyoid, , rudimentary rib no. 14, incomplete/poor ossification of frontal, parietal/interparietal, ischium, pubis, fore limb metacarpals: 1/4, dumb-bell shaped thoracic vertebrae; **NOAEL:** 40 mg/kg bw/d (maternal effects).	M-493058-02-1
Teratogenicity study in rabbits (amendmended final report)	Metribuzin (97.8 %), Oral gavage at 10, 30 and 100	**Maternal toxicity:** 10 mg/kg bw/d: no effects 30 mg/kg bw/d: no effects	M-493061-02-1

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance (purity), dose levels, duration of exposure	Results (effects statistically significantly different unless stated otherwise)	Reference
New Zealand White rabbits (15 pregnant animals/group) OECD 414 Deviations regarding insufficient data of test item analysis, no maternal toxicity was seen at the highest dose tested, no extended dosing period until the day prior to termination, number of animals per group was less than the recommended 20. GLP Supplementary	mg/kg bw/d in 0.5 % aqueous carboxymethyl cellulose during days 6-18 of gestation; cesarean section day 28.	100 mg/kg bw/d: no effects **Developmental toxicity:** **External malformations:** 100 mg/kg bw/d: membranous frontals **Visceral malformations**: 30 mg/kg bw/d: hypoplastic 4th right lung lobe; seal heart 100 mg/kg bw/d: hyperplastic thymus; hypoplastic 4th right lung lobe; dilated ventricles of heart **Skeletal malformations:** 10 mg/kg bw/d: ↑ incidence of hypoplastic pubis 30 mg/kg bw/d: ↑ incidence of hypoplastic pubis; ↑ incidence of hypoplastic sternebra no. 2 100 mg/kg bw/d: ↑ incidence of hypoplastic pubis; extra rib no. 13 **NOAEL:** 100 mg/kg bw/d (maternal toxicity); 10 mg/kg bw/d (developmental toxicity).	
Prenatal oral developmental toxicity study in rabbits New Zealand White rabbits (groups of 19-21 mated females) Non-guideline study. Insufficient analyses of test item and administered doses, inappropriate mating scheme, no precise knowledge about day 1 of gestation, full coverage by dosing of the period of organogenesis was not guaranteed,	Metribuzin technical (93.0 %), Oral gavage at 0, 15, 45 and 135 mg/kg bw/day from day 6 through day 18 of gestation. Caesarean section: GD 30.	**Maternal toxicity:** 15 mg/kg bw/day: no effects 45 mg/kg bw/day: no effects **135 mg/kg bw/d:** lower bodyweight (statistical significance on days 13, 18 and 20); lower bodyweight gain (statistically significant during days 6-18 of gestation); ↓ food consumption, ↑ abortion rate. **Developmental toxicity:** **≥ 15 mg/kg bw/d:** ↑ incidences of extra ribs and rib buds; **135 mg/kg bw/d:** ↓ percentage of viable fetuses (clear but not statistically significant), ↑ rate of resorptions, ↑ number of non-viable implants per doe; ↓ percentage of live litters, ↓ fetal wt. **NOAEL:** 45 mg/kg bw/d (maternal toxicity), 45 mg/kg bw (developmental toxicity).	M-018495-01-1

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance (purity), dose levels, duration of exposure	Results (effects statistically significantly different unless stated otherwise)	Reference
insufficient inspection of soft tissue and skeletal anomalies, high mortality among does, number of evaluable animals low. GLP Not acceptable			
Rat oral developmental toxicity study FB 30 rats (groups of 21-22 inseminated female rats) Non-guideline study. No analyses of test item, insufficient reporting of clinical and soft tissue examination, no individual data on food and body weight and fetal malformation. Non-GLP Not acceptable	Metribuzin (99.5 %), Gavage doses of 0 , 5, 15, 50 and 100 mg/kg bw/day from day 6 through day 15 of gestation. Caesarean sections were performed on day 20 of gestation.	**Maternal toxicity:** 5 mg/kg bw/day: no effects 15 mg/kg bw/day: no effects **≥ 50 mg/kg bw/d:** reduced activity, piloerection and dyspnea in one or two animals; **100 mg/kg bw/d:** ↓ bw. gain (nonsignificant) **Developmental toxicity:** **100 mg/kg bw/d:** ↓ placental wt., 'osseous alterations'. **NOAEL:** 15 mg/kg bw/d (maternal), but data insufficient for further use in risk assessment; 50 mg/kg bw/d (developmental toxicity).	M-018346-01-1

Studies in rats

The new oral prenatal developmental toxicity study in Wistar rats (M-530086-01-1; RAR B.6.6.2.) was performed according to the OECD 414 guideline and the requirements of GLP. Metribuzin (91.9 %) was administered via oral gavage as a suspension in 0.5 % methylcellulose and 0.4 % TWEEN®80 in tap water at levels of 0, 3, 15 and 75 mg/kg bw/d from gestation day (GD) 5 up to and including GD 20.The dose levels were selected on the basis of the dose range-finding study (M-525369-02-1; RAR B.6.6.2.). In the dose-range finding study (0, 10, 50, 100 mg/kg bw/day), maternal toxicity was observed as clinical effects, reduced body temperature and reduced body weight development starting at 50 mg/kg bw/day; absolute and relative weights of the thyroid were statistically significantly increased in the high-dose group at 100 mg/kg bw/d. Fetal weight was relatively low in all test groups, although without statistical significance. One malformation (absent mandible) and a small eye bulge were observed in a fetus in the high-dose group. Due to the single occurrence of these findings in one fetus, they cannot clearly be ascribed to treatment. In the main study, maternal toxicity was observed as effects on body temperature, food consumption and slight effect on piloerection at the mid dose of 15 mg/kg bw/day. At the high dose of 75 mg/kg bw/d also

bodyweight loss or reduced bodyweight growth was observed. A number of 23, 20, 21 and 22 out of 24 females were pregnant in group 1 through 4, (resulting in fertility indices of 96%, 83%, 88% and 92%, respectively). Mean total fetal weight and female fetal weight were slightly, but statistically significantly decreased in the high-dose group in comparison to controls (total fetal weight: 5.0 ($p < 0.05$) vs. 5.2 g (-3.8% of controls); female fetal weight: 4.8 ($p < 0.05$) vs. 5.1 g (-5.9% of controls)). However, the individual fetal weights were well within the range of control weights obtained in a recent OECD 414 study conducted with the same strain of rats (control range for total fetal weight and female fetal weight: 3.389 – 6.008 g). There were no treatment-related fetal external observations. External observations did not reveal any malformed foetuses. Gross evaluation of placentas did not reveal any abnormalities. Malformations were not observed in any group. Variations were observed in heart, eyes, ureters, kidneys and urinary bladder. These variations occurred randomly among the groups or occurred in a single fetus per group only. Based on the incidences and distribution of the observations, it was concluded that visceral examination did not reveal any treatment-related effects. Skeletal malformations were not observed in any group. Skeletal variations consisted of retardation in ossification of the forelimbs (phalanges-front proximal, metacarpals), hindlimbs (phalanges-hind proximal and distal, metatarsals), sternebrae, vertebrae (lumbar and cervical bodies) and skull (supraoccipital) in the high-dose group. Differences in the incidences of observations in forelimbs, hindlimbs, vertebrae and skull occasionally reached the level of statistical significance in this group.
A number of the above retardations were, to a lesser extent, also noted in the mid-dose group, but only an increase in incidence of absence of ossification of digits in the proximal hind limb phalanges was statistically significant in this group. The latter change was, however, within the range of historical control data.
Generally, all skeletal observations in this study were within the range of historical control data obtained in previous studies, with the exception of the retarded ossification in sternebrae (Three or more sternebrae incompletely ossified) in high dose fetuses. It was concluded that retardations in ossification occurred in the high-dose group and, to a slight extent, in the mid-dose group, but no skeletal malformations were found. These retardations in ossification are considered transient developmental effects, and are considered to be related to the lower fetus weight.

The oral developmental toxicity study in Charles River Crl:CD® BR rats (M-018676-01-1; RAR B.6.6.2.) was performed in accordance with the requirements of GLP. Groups of 33 dams each were administered metribuzin (92.6 %) via gavage at doses of 0, 25, 70 or 200 mg/kg bw/day from gestation day 6-15. A group of 33 dams received the vehicle alone and served as the control. Each group of 33 dams was subdivided into 2 termination phases: Phase I: 5 dams on Day 16 of gestation, and Phase II: 28 dams on Day 20 of gestation. On Day 16 of gestation, approximately 24 hours after the tenth and final dose of the test or control article, Phase I dams were weighed and sacrificed. Intact thyroids were removed and weighed, and blood samples obtained for determination of maternal serum T3'and T4 levels. On Day 20 of gestation, 5 days after the final dose of the test or control article, Phase II dams were sacrificed. Parameters measured included body weight; food consumption; thyroid weight; T3 and T4 levels; pregnancy rates; dams with live progeny; corpora lutea; implantations; resorptions; litter size; foetal weights and viability; placental weights; uterine weights; foetal sex ratios; and pre- and post-implantation loss. Each dam was examined for gross external and visceral changes. Foetuses were sexed and examined for gross external, visceral, and skeletal dysmorphogenesis. Maternal toxicity was noted as clinical signs (hypoactivity and ptosis), reduced body weight gain and food consumption from the lowest dose level of 25 mg/kg bw/d. At the mid and high dose (70 and 200 mg/kg bw/d) also decreased T4 hormone levels were measured and ataxia was seen in all mid- and high-dose dams; and at the high dose also higher mean absolute thyroid weight and lower mean placental weight in dams.
Median fetal weight was significantly lower in all treated groups, but lay within the historical control range for the low- and mid-dose group. Mean fetal weights were neither specified nor compared to historical control.

Table 46: Median fetal weight [g]

Sex	Dose level [mg/kg bw/d]			
	0	**25**	**70**	**200**
Male	3.9	3.7	3.7	3.3[&]
Female	3.7	3.5[*]	3.5[*]	3.1[&]
Combined	3.8	3.6[&]	3.6[&]	3.1[&]

[*] significantly different from control (p< 0.05, Dunnett's test); [&] significantly different from control (p< 0.01, Dunnett's test)

No treatment-related changes were observed during external or visceral fetal examinations up to and including 200 mg/kg bw/d. Skeletal examinations showed an increased incidence of reversible rib deformations (wavy, curved and/or bulbous ribs) and of retarded ossification in the areas of the skull, ribs, spine, sternum and limbs at 200 mg/kg bw/d. These changes were considered to represent secondary fetotoxicity resulting from marked maternal toxicity at this dose level.

Table 47: Fetuses with minor skeletal variations (incidences of relevant findings only, percentage given in parentheses)

Variation and/or abnormality		Dose level [mg/kg bw/d]			
		0	25	70	200
Skull	bones incompletely ossified	106 (55.5)	102 (55.7)	109 (60.6)	160 (89.9)$
	sutures enlarged	17 (8.9)	5 (2.7)*	13 (7.2)	54 (30.3)$
	fontanelle enlarged	28 (14.7)	12 (6.6)*	21 (11.7)	70 (39.3)$
	variations of hyoid body or arch	41 (21.5)	32 (17.5)	23 (12.8)	63 (35.4)$
Ribs	incompletely ossified	1 (0.5)	0	3 (1.7)	13 (7.3)$
	wavy or curved	2 (1.0)	0	4 (2.2)	12 (6.7)*
Vertebrae	cervical arches incompletely ossified	4 (2.1)	14 (7.7)*	6 (3.3)	41 (23.0)$
	sacral arches incompletely ossified	82 (42.9)	110 (60.1)$	99 (55.0)	149 (83.7)$
	sacral arches unossified	2 (1.0)	1 (0.5)	2 (1.1)	17 (9.6)$
	caudal arches unossified	8 (4.2)	4 (2.2)	7 (3.9)	34 (19.1)$
Pelvis	pubis incompletely ossified	7 (3.7)	4 (2.2)	2 (1.1)	18 (10.1)*
Sternebrae	1st unossified	0	0	0	3 (1.7)
	1st incompletely ossified	12 (6.3)	22 (12.1)	7 (3.9)	33 (18.8)$
	1st bipartite	0	0	0	1 (0.6)
	2nd unossified	2 (1.1)	4 (2.2)	2 (1.1)	11 (6.3)*
	2nd incompletely ossified	28 (14.7)	50 (27.6)$	58 (32.4)$	112 (63.6)$
	3rd unossified	0	0	0	2 (1.1)
	3rd incompletely ossified	9 (4.7)	19 (10.5)	10 (5.6)	45 (25.4)$
	3rd bipartite	0	0	0	1
	4th unossified	0	0	1 (0.6)	7 (4.0)*
	4th incompletely ossified	137 (71.7)	141 (77.9)	146 (82.0)	149 (84.2)*
	5th unossified	41 (21.5)	43 (23.8)	38 (21.2)	76 (42.9)$
	5th incompletely ossified	150 (78.5)	136 (75.1)	138 (77.1)	98 (55.4)$
	5th bipartite	0	2 (1.1)	2 (1.1)	0
	6th unossified	3 (1.6)	7 (3.9)	6 (3.4)	27 (15.3)$
Appendages	anterior – incompletely ossified radius	0	0	0	1 (0.6)
	anterior – unossified metacarpals	0	0	0	11 (6.2)$
	anterior – incompletely ossified metacarpals	4 (2.1)	2 (1.1)	1 (0.6)	16 (9.0)*
	posterior – unossified metatarsals	0	2 (1.1)	0	15 (8.5)$
	posterior – incompletely ossified metatarsals	2 (1.0)	1 (0.5)	0	11 (6.2)*

* significantly different from control ($p < 0.05$); $ significantly different from control ($p < 0.01$)

The oral developmental toxicity study in Wistar rats (M-493058-02-1; RAR 6.6.2.) was performed according to the OECD 414 guideline and the principles of GLP. Groups of 27-35 dams each were administered metribuzin (97.6 %) formulation in refined groundnut oil via oral gavage at 10, 40 and 150 mg/kg bw/day from day 6 through day 15 of gestation along with a vehicle control group. Caesarean sections were performed on day 20 of gestation.

Table 48: Animal numbers and treatment groups

	Dose level (mg/kg bw/day)			
	0 (control)	10	40	150

No. of day 0 mated females	35	27	31	32
No. of pregnant females at term	22	20	22	22

At the mid and high dose levels signs of mild maternal toxicity were noted as lower bodyweight gain (no statistical significance) and lower feed intake.

Table 49: Feed consumption (in g/rat/day) and body weight gain (in g as compared to day 0) in dams.

Dose [mg/kg bw/d]	Pre-treatment (days 0-6)	Treatment (days 6-15)	Post-treatment (days 15-20)	Throughout gestation (days 0-20)
Feed consumption				
0	20 ± 5.9	21 ± 3.4	25 ± 5.8	22 ± 3.3
10	18 ± 3.4	16[1] ± 3.3	27 ± 7.4	19[1] ± 2.4
40	17 ± 5.1	17[1] ± 4.1	21[1] ± 5.7	18[1] ± 4.4
150	19 ± 5.0	14[1] ± 4.2	20[1] ± 4.6	17[1] ± 4.0
Body weight gain				
0	19 ± 7.7	23 ± 9.5	49 ± 18.3	91 ± 20.3
10	25 ± 8.3	26 ± 11.4	39 ± 21.1	89 ± 28.1
40	25 ± 9.2	21 ± 7.7	44 ± 15.6	90 ± 20.5
150	25 ± 12.8	17 ± 8.1	41 ± 16.2	83 ± 25.1

[1] Significantly lower than control (Dunnett's test)

Maternal gross necropsy revealed occasional incidences of petechiae in the lungs of 0/2/3/1 (pregnant and non-pregnant) dams at 0/10/40/100 mg/kg bw/d. The mean number of corpora lutea, implantations and the number and percentage of early resorptions, post -implantation loss and the number of dams with any resorption in the treatment groups did not statistically differ from their respective control group values.There was no statistical difference in the mean litter size, abnormal foetuses, total number of live foetuses, male and female foetuses, foetal weights and the sex ratio in the treatment groups compared to the respective control values.

Developmental findings are summarized in the Table 50 below. There were 1 (0.5%) and 7 (3.4%; statistically significant) fetuses in the mid and high dose groups respectively which were small as compared to their litter mates. The incidences of other malformations did not statistically differ from their respective control values.

There was a significant increase in the incidence of partial ossification (PRO) of skull bones and dumb-bell shaped thoracic centra 2/13 in most of the treatment groups. Split thoracic centra 1/13; dumb-bell shaped stern # 2 and # 5, TV centra 3/13, 4/13; asymmetrical dumb-bell shaped stern # 5, TV centra 1/13 and rudimentary ribs have been observed in one or more treatment groups. However, none of these incidences have shown dose correlation. The incidences of malformed sternebra:#l in low dose, malformed scapula at mid dose and wavy (+++) 10/13 ribs in the high dose group though observed, were not statistically significant. Though these types of malformations (malformed scapula and wavy ribs) were not seen in the concurrent control group, they have been recorded in historical control data. The number of fetuses with major skeletal malformations and the number of dams with major malformed fetuses were 0, 1, 1 and 1 for both in control, low, mid and high dose groups respectively which did not show statistical significance.

Table 50: Summary of developmental toxicity findings (in %).

Parameter		Dose level [mg/kg bw/d]				Historical control		
		0	10	40	150	1990-1992[3]	1990-1998	
						mean	mean	range
Small fetuses [%]		0.0	0.0	0.5	3.4[1,2]	0.43 ± 0.36	0.59	0 - 6.7
Displaced umbilical artery		3.3	10.1[1]	14.6[1]	13.5[1]	6.97 ± 6.29	no data	
Slight dilatation of renal pelvis		0.8	11.1[1]	14.6[1]	15.4[1]	6.15 ± 7.81	3.83	0.0-19.4
Moderate dilatation of renal pelvis		1.6	1.0	2.7	1.0	4.58 ± 6.10	2.50	1.6-16.2
Slightly tortuous ureter		1.6	12.1[1]	22.7[1]	21.1[1]	22.48 ± 15.30	no data	
Hydronephrosis		0.0	0.0	0.0	3.9[1,2,4]	0.4 ± 0.98	0.24	0-2.4
Delayed ossification of hyoid		0.0	4.1[1]	4.5[1]	4.8[1]	0.98 ± 1.08	no data submitted	
Rudimentary rib no. 14		13.0	24.5[1]	14.6	36.2[1]	7.6 ± 3.63		
Incomplete/poor ossification	**frontal**	5.7	10.2	7.3	14.3[1]	0.67 ± 0.94		
	parietal/inter parietal	11.4	21.4[1]	23.6[1]	26.7[1]	34.92 ± 42.69		
	ischium	0.8	1.0	2.7	9.5[1,2]	0.23 ± 0.57		
	pubis	0.8	7.1[1]	5.5[1]	10.5[1]	0.17 ± 0.41		
	fore limb: metacarpals: 1/4	4.1	20.4[1]	10.9[1]	18.1[1]	11.27 ± 10.32		
Dumb-bell shaped thoracic vertebra:	**centra: 1/13**	17.1	24.5	24.6	21.9	only data on single vertebrae given		
	centra: 2/13	4.1	15.3[1]	15.4[1]	20.9[1]			
	centra: 3/13	0.8	8.2[1]	4.5	6.7[1]			
	centra: 4/13	0.0	7.1[1]	4.5[1]	2.9			
Asymmetrically dumb-bell shaped thoracic vertebra centra: 1/13		0.0	10.2[1]	8.2[1]	2.9			

[1] significantly different from control (contingency test);[2] significant dose correlation;[3] based on 6 studies with 141 litters/676 fetuses examined; [4] four fetuses from the same litter

In the original study report, hydronephrosis was regarded as a major malformation, however, in an amendment initiated by the study sponsor, it was stated, that the respective findings were seen in 4 fetuses from the same litter, and that in these individual fetuses both ureters and bladders were inconspicuous. Thus, the effect was reclassified as a developmental retardation, and was not interpreted as a sign of teratogenicity. The diagnostic criteria for 'hydronephrosis' were not specified any further.

Signs of developmental retardation in the form of delayed or poor ossification of frontal and (inter)parietal skull bones, ischium and pubis, as well as dumb-bell shaped thoracic vertebrae and a rudimentary 14th rib were noted. In part, these findings were seen already at the lowest dose tested. Additionally, in the high dose group, a significantly high incidence of small fetuses was observed. The value of the historical control data used in this study was compromised by the fact that in three out of the six studies used a different vehicle had been administered (water instead of peanut oil).

The authors of the original final study report came to the conclusion that embryotoxic effects were seen at 40 mg/kg bw/d and above. This was argued against in an amendment demanded by the sponsor of the study with a reference to an extended set of historical control data (1990-1998).

However, the use of historical control data over a range of eight years is not in accordance with general recommendations according to which control data are only recommended for a period of ± 2 years around a study. By using longer time frames, the range of statistical distributions is enlarged, thereby overestimating real biological variance at the time of the actual experiment and reducing the statistical significance of observed effects. The inclusion of the current study's control group into the historical control data appears inappropriate. Furthermore, no data on the vehicle used in these

historical studies was given and only malformation incidences averaged over all studies were presented.

In a not acceptable prenatal developmental toxicity study in FB 30 rats (M-018346-01-1; RAR 6.6.2.) metribuzin (99.5%) was administered by oral gavage to groups of 21-22 inseminated female rats at 0 (vehicle control), 5, 15, 50 and 100 mg/kg bw/day from day 6 through day 15 of gestation. The rats were observed for clinical signs, mortality and body weight gain. Caesarean sections were performed on day 20 of gestation. The dams were observed for maternal and litter data. The foetuses were examined for external, visceral and skeletal malformations.

There were several deviations to this study: No analyses of the test item for stability, homogeneity, and/or actual concentration were performed. Neither the frequency of clinical examination performed on the dams nor the parameters included into the examination procedure were documented. No individual food or absolute body weight data were given. No individual fetal malformation data was provided. Inspection for soft tissue changes is clearly insufficient.

Following dosing with 50 and 100 mg/kg bw/day, 1 or 2 animals displayed clinical signs of ruffled coats, dyspnoea and reduced general activity. The females at 100 mg/kg bw/day showed a slight, nonsignificant reduction of weight gain compared to controls during the treatment period. The average placenta weight at 100 mg/kg bw/day was significantly less than that in the control group. There were no treatment-related skeletal malformations. The following malformations were seen during the external and internal inspections of the foetuses (Wilson Technique and Alizarin Red Staining): One control foetus had micrognathia (mandible) and one 5 mg/kg foetus had hypoplasia of the mandible. The dams treated with dose levels of 15, 50 and 100 mg/kg bw/day had no malformed foetuses. Several fetuses of all dose-groups displayed skeletal changes termed "osseous alterations", but not further specified on an individual litter basis. If calculated on an individual litter basis, a slight increase was seen in the rate of fetuses with osseous alterations per litter in the upper two dose groups.

Studies in rabbits

The new oral prenatal developmental toxicity study in New Zealand White rabbits (M-537608-01-1; RAR B.6.6.2.) was performed according to the OECD 414 guideline and the requirements of GLP, the dose levels were selected on the basis of the dose range-finding study (M-537597-01-1; RAR B.6.6.2.). Maternal toxicity was observed as decreased defecation, lower bodyweights and lower food intake primarily at 100 mg/kg bw/d, but slightly lower food intake was also observed at 30 mg/kg bw/d; no effects were observed at 10 mg/kg bw/d.

Mean fetal weights in the 100 mg/kg/day group (37.6 g, 36.5 g, and 36.9 g for male, female, and combined fetal weights) were lower (10.7%, 10.3%, and 10.9%) than in the control group (42.1 g, 40.7 g, and 41.4 g for male, female, and combined fetal weights); differences in male and combined weights were significant ($p<0.01$). These results were considered test substance-related. However, these values were within the ranges of values in the WIL Research historical control data. Mean fetal weights in the 10 and 30 mg/kg/day groups were comparable to the control group.

Intrauterine survival was unaffected by test substance administration at dosage levels of 10, 30, and 100 mg/kg/day. Parameters evaluated included postimplantation loss, live litter size, and fetal sex ratios. Mean numbers of corpora lutea and implantation sites and the mean litter proportions of pre-implantation loss were comparable across all groups. Differences from the control group were slight and not statistically significant.

In the dose-range finding study, no external developmental variations were noted at any dosage level.

In the main satudy, the numbers of fetuses (litters) available for morphological evaluation were 186(22), 195(21), 219(24), and 189(22) in the control, 10, 30, and 100 mg/kg/day groups, respectively. Malformations were observed in 7(6), 7(4), 7(6), and 9(8) fetuses (litters) in these same respective dosage groups and were considered spontaneous in origin.

External malformations observed in single fetuses, without statistical significance and not considered test substance-related, were: cleft palate in the 100 mg/kg bw/day group (1 fetus); open eyelid (bilateral) (1 fetus) and microphthalmia (bilateral) (1 fetus) in the 30 mg/kg bw/day group.

Table 51: Summary of fetal visceral malformations

Dose group (mg/kg bw/day)		**0**	**10**	**30**	**100**	**HCD range** (Min–Max)
	Litters evaluated (n)	*22*	*21*	*24*	*22*	*2354*
	Fetuses evaluated (n)	*186*	*195*	*219*	*189*	*20582*
Total visceral malform.	Litters affected (n)	1	6	2	5	
	Fetuses affected (n)	2	11	2	5	
	% per litter (mean ±SD)	1.0 ± 4.74	3.5 ± 7.91	1.0 ± 3.53	2.1 ± 4.53	0.0 - 4.3
Lungs – Lobular agenesis [absent]	Litters affected (n)	1	3	1	3	
	Fetuses affected (n)	2	5	1	3	
	% per litter (mean ±SD)	1.0 ± 4.74	2.5 ± 6.97	0.5 ± 2.55	1.5 ± 3.90	0.0 – 3.5
Stenotic pulmonary trunk [narrow]	Litters affected (n)	0	0	0	1	
	Fetuses affected (n)	0	0	0	1	
	% per litter (mean ±SD)	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.6 ± 2.67	0.0 – 0.8
Inter-ventricular septal defect	Litters affected (n)	0	1	0	0	
	Fetuses affected (n)	0	2	0	0	
	% per litter (mean ±SD)	0.0 ± 0.00	1.0 ± 4.36	0.0 ± 0.00	0.0 ± 0.00	0.0 – 0.9
Vestigial pulmonary trunk	Litters affected (n)	0	1	0	0	
	Fetuses affected (n)	0	2	0	0	
	% per litter (mean ±SD)	0.0 ± 0.00	1.0 ± 4.36	0.0 ± 0.00	0.0 ± 0.00	0.0 – 0.0
Dose group (mg/kg bw/day)		**0**	**10**	**30**	**100**	**HCD range (Min–Max)**
Bulbous aortic arch [dilated]	Litters affected (n)	0	1	0	1	
	Fetuses affected (n)	0	2	0	1	
	% per litter (mean ±SD)	0.0 ± 0.00	1.0 ± 4.36	0.0 ± 0.00	0.6 ± 2.67	0.0 – 0.9
Dia-phragmatic hernia	Litters affected (n)	0	0	1	0	
	Fetuses affected (n)	0	0	1	0	
	% per litter (mean ±SD)	0.0 ± 0.00	0.0 ± 0.00	0.5 ± 2.55	0.0 ± 0.00	0.0 – 1.0

HCD: Historical control data (Study date range: Aug 2006 – Nov 2013)
[ ]: designates equivalent DEVTOX.ORG nomenclature
Modified statistics used. None significantly different from control group.

The visceral malformations observed in the test substance-treated groups occurred infrequently, in a manner that was not dose-related, and/or at similar frequencies in the control group. In addition, the mean litter proportions of these findings were not statistically significantly different from the control group and/or were within the ranges of values in the WIL Research historical control data. Therefore, these findings were not considered test substance-related.

Visceral developmental variations, including accessory spleen(s), major blood vessel variation, absent or small gallbladder, retrocaval ureter, extra papillary muscle in the heart, and only 2 papillary muscles of the heart present, occurred at similar frequencies in the control group and/or in a manner that was not dose-related. Other visceral developmental variations occurred infrequently. The mean litter proportions of these findings were not statistically significantly different from the control group

and/or were within the ranges of values in the WIL Research historical control data. Therefore, these findings were not considered test substance-related.

Cystic oviducts were noted in 3 fetuses in the 10 mg/kg/day group and 1 fetus in the 30 mg/kg/day group. A distended, gas filled stomach was noted for 1 fetus in the 10 mg/kg/day group. Renal papilla not fully developed (Woo and Hoar Grade 1) was noted for 1 fetus in the control group and 1 fetus in the 30 mg/kg/day group. These findings were not classified as either a malformation or developmental variation, were not included in the summary table 52, and were not considered to be test substance-related because they occurred infrequently, in a manner that was not dose-related, and/or at similar frequencies in the control group.

Skeletal malformations observed in the test substance-treated groups occurred infrequently, at similar frequencies in the control group, and/or in a manner that was not dose-related. In addition, the mean litter proportions of these findings were not statistically significantly different from the control

group and/or were within the ranges of values in the WIL Research historical control data. Therefore, these findings were not considered test substance-related.

Table 52: Summary of selected fetal skeletal malformations

Dose group (mg/kg bw/day)		**0**	**10**	**30**	**100**	**HCD range** (Min–Max)
	Litters evaluated (n)	*22*	*21*	*24*	*22*	*2354*
	Fetuses evaluated (n)	*186*	*195*	*219*	*189*	*20582*
Total skeletal malformations	Litters affected (n)	4	0	5	3	
	Fetuses affected (n)	6	0	5	4	
	% per litter (mean ±SD)	2.3±5.28	0.0±0.00	2.5±5.08	1.7±4.56	0 – 5.1
Sternebrae fused	Litters affected (n)	0	0	1	0	
	Fetuses affected (n)	0	0	1	0	
	% per litter (mean ±SD)	0.0±0.00	0.0±0.00	0.7±3.40	0.0±0.00	0.0 – 2.1
Rib anomaly	Litters affected (n)	1	0	0	1	
	Fetuses affected (n)	1	0	0	2	
	% per litter (mean ±SD)	0.9±4.26	0.0±0.00	0.0±0.00	0.8±3.55	0.0 – 1.5
Vertebral anomaly w/o associated rib anomaly	Litters affected (n)	2	0	3	1	
	Fetuses affected (n)	2	0	3	1	
	% per litter (mean ±SD)	1.4±4.68	0.0±0.00	1.4±3.72	0.5±2.13	0.0 – 2.1
Sternebra(e) malaligned (severe)	Litters affected (n)	2	0	1	0	
	Fetuses affected (n)	2	0	1	0	
	% per litter (mean ±SD)	1.4±4.68	0.0±0.00	0.4±2.04	0.0±0.00	0.0 – 1.1
Costal cartilage anomaly	Litters affected (n)	1	0	0	1	
	Fetuses affected (n)	1	0	0	1	
	% per litter (mean ±SD)	0.5±2.13	0.0±0.00	0.0±0.00	0.5±2.37	0.0 – 1.7

HCD: Historical control data (Study date range: Aug 2006 – Nov 2013)
w/o: with or without
Modified statistics used. None significantly different from control group.

Skeletal variations observed in the test substance-treated groups, including 27 presacral vertebrae, 13th rudimentary rib(s), 13th full rib(s), unossified sternebra(e) nos. 5 and/or 6, occurred at similar frequencies in the control group and/or in a manner that was not dose-related. Other skeletal variations were noted infrequently. The mean litter proportions of these findings were not statistically significantly different from the control group and/or were within the ranges of values in the WIL Research historical control data. Therefore, these findings were not considered test substance-related.

The prenatal oral developmental toxicity study in American Dutch rabbits (M-018201-01-1; RAR B.6.6.2.) was performed in accordance with OECD 414 guideline and the principles of GLP. Metribuzin (92.7%) was administered orally by gavage to groups of 17 rabbits at dose levels of 0

(vehicle control), 10, 30 and 85 mg/kg bw/d from day 6 to day 18 of presumed pregnancy. The does had been impregnated by artificial insemination (day 0 of pregnancy). There were three deaths – one in each of the treated groups – which were attributed to either systemic infections or dosing trauma. One abortion occurred in the high-dose group. Maternal toxicity was observed at 85 mg/kg bw/d as lower bodyweight and bodyweight gain, lower food consumption and increase in the incidence of soft stool or diminished quantity of stool.
Significantly reduced fetal and placental weights at 30 mg/kg bw/d were related to the higher average litter size of this group and were not seen as a test substance effect.

Table 53: Litter and fetus data

Observation	Dose level (mg/kg bw/day)			
	0 (control)	**10**	**30**	**85**
Animals Assigned (Inseminated)	17	17	17	17
% male foetuses	50.0	50.0	47.7	33.3
Mean foetal weight (g)	39.9	38.6	35.0**	39.4
Mean male foetal weight (g)	40.5	38.6	33.6**	40.3
Mean female foetal weight (g)	39.2	37.6	35.5**	38.7
Median placental weight (g)	5.5	5.5	5.1*	5.8

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

In this study, no treatment-related external or visceral malformations in fetuses were observed. Assessment of fetal skeletons revealed a slight, but statistically significant increase in delayed ossification and/or variation of a few skeletal elements in the treatment groups. However, in general, values were not increased in a dose-dependent manner and were inside the historical control range; they were therefore considered as incidental. Furthermore, significant values were found mostly at 30 mg/kg bw/d, and these findings were seen as connected with reduced fetal weights observed in this group.

Table 54: Summary of External, Visceral, and Skeletal Malformations

Dose, mg/kg bw/d	Observation	No. fetuses
0	Caudal -centra, unaligned	1
	Cervical -arch, abnormal, unaligned	1
	Umbilical hernia	1
10	Brain, dilated ventricles; sutures, fused	1
	Major vessel anomaly, vena cava displaced to the left	1
30	Caudal -centra, fused	1
	Thoracic-arch, missing; thoracic-centra, missing	1
85	Appendage, anterior, phalange missing	2
	Sternebrae, fused; caudal -centra, unaligned	1

The teratogenicity study in New Zealand White rabbits (M-493061-02-1; RAR 6.6.2.) was performed according to the OECD 414 guideline and the principles of GLP. Metribuzin (97.8 %)

was administered to 15 pregnant animals/group via oral gavage at 10, 30 and 100 mg/kg bw/d in 0.5 % aqueous carboxymethyl cellulose during days 6-18 of gestation; cesarean section was performed on day 28.

No treatment-related effects on maternal parameters (body weights, weight gain, food consumption) were observed. One dam each in low, mid and high dose died on day 20, 24 and 20, respectively.

There were no significant changes in the mean number of implantations, incidence of early and late resorptions, pre- and post-implantation losses and dams with any resorptions. There were 2 dams with complete resorptions in the control group and two abortions (partial/ complete) in the low dose group.

There were no significant changes in the mean number of implantations, incidence of early and late resorptions, pre- and post-implantation losses and dams with any resorptions. There were 2 dams with complete resorption in the control group and two abortions (partial/complete) in the low dose group.

There were no intergroup differences in the mean litter size, number of dead and abnormal foetuses, number and weight of live male or female foetuses or sex ratio.

There were no treatment-related findings on visceral examinations. The incidence of thymus hyperplasia was 5.1% for the high dose group versus 0% in the control, and the incidence of this finding was above the current historical control range of 0-1.9%. However, the incidence of heart ventricle dilated (10.3% in the high dose) is comparable to the current historical data of 0.0-15.2%. These findings reflect observed physiological changes and do not represent teratogenic defects. Incomplete development of lung lobes is an indication of immaturity in development / growth retardation and not a teratogenic effect. The increase in the incidence with 16.3% and 15.4% in the mid and high dose groups were above the historical control range of 0-9.3%, but did not show dose-correlation and are, therefore, regarded as incidental finding. The statistically significant increase in the incidence of seal heart in the mid dose group (11.3%) versus the control value (0.0%) was above the historical control range of 0-2.3%. However, this observation was not dose-related (2.6% in the high dose). Therefore, this change was only an incidental finding and is interpreted as a physiological change observed and not a teratological effect.

There were no treatment-related skeletal findings. The increase in the incidence of hypoplastic sternebrae #2 in the mid dose (7.5%) above the control (0%) and the historical control (0-2.3%) without dose correlation was only an incidental finding. The incidence of hypoplastic pubis was 14.9, 26.3 and 24.4% for low, mid and high dose groups, respectively, versus 2.5% in the control group and 0-24.1% for the historical control range. Since there was no dose correlation, and the incidences for the low and high dose groups were comparable to the historical control range, this finding was regarded as incidental. The findings of accessory rib (Rt/Lt/B) #13 in the low and high dose groups (6% and 3.9%) and extra rib (Rt/LT/B) #13 in the high dose group (5.1%) were well within the historical control ranges (0-11.8% and 0-9.3%) and therefore, without any toxicological relevance. Bent femur was recorded only in the mid dose group with an incidence of 7.5% versus 0% in the control and high dose group and was not regarded as a related effect but only as an incidental finding. Moreover, this observation was recorded only in all 6 foetuses of 1 dam.

Table 55: Teratogenicity study in rabbits - summary of developmental toxicity findings [%]

Parameter	Dose level (mg/kg bw/day)				Historical control (1991-1994)
	0	10	30	100	
Membranous frontal	0.0	0.0	0.0	5.1[1]	no data
Slight dilatation of renal pelvis	0.0	0.0	3.8	3.9	no data
Moderate dilatation of renal pelvis	1.2	1.5	1.3	0.0	0.16 ± 0.35 (0.0-0.7)
Slightly tortuous ureter	1.2	1.5	3.8	5.1	no data
Moderately tortuous ureter	0.0	0.0	2.5	1.3	no data
Thymus hyperplasia	0.0	0.0	0.0	5.1[1]	0.48 ± 0.95 (0.0-1.9)
Enlarged heart ventricle	0.0	0.0	0.0	1.3	no data
Dilated heart ventricle(s)	1.2	0.0	1.3	10.3[1,2]	1.78 ± 2.14 (0.0-4.3)
Lung (Right-4th) hypoplasia	0.0	3.0	16.3[1]	15.4[1]	3.23 ± 4.23 (0.0-9.3)
Lung (Right-4th) agenesis	2.5	6.0	6.3	9.0	1.00 ± 0.83 (0.0-1.9)
Extra or unusual lobation of the liver	0.0	0.0	1.3	3.9	0.33 ± 0.65 (0.0-1.3)

Parameter		Dose level (mg/kg bw/day)				Historical control (1991-1994)
		0	10	30	100	
Seal heart		0.0	0.0	11.3[1]	2.6	0.16 ± 0.35 (0.0-0.7)
Delayed, incomplete or poor ossification of	**sternebra no. 5**	4.9	7.5	16.3[1]	14.1[1]	no data
	one or more caudal vertebrae (central)	11.1	4.5	15.1	16.7	
	one or more caudal vertebrae (central)	0.0	1.5	2.5	5.1[1,2]	
	one or more proximal phalanges of the front limbs	11.1	13.4	15.1	30.8[1,2]	
Hypoplastic sternebra no. 2		0.0	0.0	7.5[1]	2.6	0.33 ± 0.65 (0.0-1.3)
Hypoplastic pubis		2.5	14.9[1]	26.3[1]	24.4[1]	10.3 ± 10.6 (1.4-24.1)
Extra rib no. 13		0.0	3.0	3.8	5.1[1]	2.33 ± 4.65 (0.0-9.3)
Bent femur		0.0	0.0	7.5[1]	0.0	no data

[1] significantly different ($p \leq 0.05$) from control by Contingency test; [2] significant dose correlation

Unfortunately, 'individual' data on malformations was presented only litterwise, making the reconstruction of malformation patterns for individual fetuses impossible. While some of the findings shown in Table 55 were either only slight, did not show a clear dose-response, or lay within corresponding historical ranges, nevertheless, effects on lung and heart development were seen at both the mid- and high-dose as well as signs indicative of delayed development of the skeletal system. Also, effects on the renal pelvis and ureter were seen, that were of low significance in this study, but were also noted in the rat teratogenicity study performed with metribuzin by the same laboratory (see elsewhere in this chapter).

The cardial finding of 'seal heart', appearing with high incidences and a strong dose correlation in the mid- and high-dose groups (Table 55), was reclassified from a major malformation to a minor visceral anomaly by the contract laboratory on request of the sponsor. Despite the fact, that 'seal heart' is not a standard term used in the evaluation of visceral abnormalities, unfortunately neither a picture or a description of the effect was provided.

Taking together the observed effects on lung and heart development as well as a noticeable retardation in skeletal development, it was concluded that substance-related fetotoxic effects were present at dose-levels, where no maternal toxicity was seen. However, as no major malformation was found, no evidence of teratogenicity was found.

The authors of the original final study report came to the conclusion that embryotoxic effects were seen at 30 mg/kg bw/d and above. This was argued against in an amendment demanded by the sponsor of the study with reference to an extended set of historical control data (1990-1998). However, the use of historical control data over a range of eight years is not in accordance with general recommendations following which control data should only be used for a period of ± 2 years around a study. By using longer time frames, the range of statistical distributions is enlarged, thereby overestimating real biological variance at the time of the actual experiment and reducing statistical significance of observed effects. The inclusion of the current study's control group into the historical control data appears inappropriate. Furthermore, no data on the vehicle used in these historical studies was given and only malformation incidences averaged over all studies were presented.

In a not acceptable prenatal developmental toxicity study in New Zealand White rabbits (M-018495-01-1; RAR 6.6.6.), metribuzin (93.0%), was administered by oral gavage to groups of 19-21 mated females at 0, 15, 45 and 135 mg/kg bw/day from day 6 through day 18 of gestation. The rabbits were observed for mortality, clinical signs and body weight change. Caesarean sections were performed on day 30 of gestation for the collection of litter data. The foetuses were examined for external, visceral and skeletal malformations.

There were severe limitations in the conduct of this study: No analyses were performed with regard to test item stability, homogeneity or the verification of administered doses. Because an

inappropriate mating scheme was used with no exact proof of the onset of pregnancy, no precise knowledge about day 1 of gestation was obtained. Hence, the time chosen for the beginning of dosing was arbitrary and full coverage of the period of organogenesis was not guaranteed. All fetuses should have been inspected for both soft tissue and skeletal anomalies. High mortality among does due to infection or misdosing. Number of evaluable animals low.
There was a high mortality among the does: Of the does which had died, 1/1/0/1 animals in the 0/15/45/135 mg/kg bw/d dose groups were killed by lung punctuation as a result of accidental introduction of the stomach tube into the lung. 2/1/0/1 animals in the 0/15/45/135 mg/kg bw/d dose groups had died of pneumonitis or pleuritis. For one doe each in the control and low-dose groups, the cause of death was not determined. Due to a miscalculation of the onset of pregnancy as a consequence of the selected mating schedule, two does, one each in the mid- and high-dose group, delivered 'early' and had to be sacrificed post partum. Although not reported as statistically significant, a higher rate of animals had to be sacrificed in the high-dose group after having aborted. As a consequence, in this group, the absolute number and rate of pregnant does having survived until term without aborting was lower than in the other groups. This effect was seen as substance-related.
Maternal toxicity evidenced by lower body weights during the dosing period was observed only at the highest dose level, 135 mg/kg bw/day. Instances of reduced feed consumption were seen in 1/0/2/7 of all animals from the 0/15/45/135 mg/kg bw/d groups and in 0/0/2/2 does from the same respective groups of those pregnant animals surviving until the scheduled cesarean section.
Although not labelled statistically significant, at 135 mg/kg bw/d the percentage of viable fetuses was clearly reduced. Also, the rate of early resorptions and the number of non-viable implants per doe were both increased in this group, while the percentage of live litters and the average fetal weight were lower than in the other groups. At 45 mg/kg bw/d, the number of late resorptions was slightly higher than in the other groups and average fetal weight was also reduced. However, this latter finding was seen as a consequence of the higher mean litter size in this group and not considered to be a toxic effect.
At 45 mg/kg bw/day, undersized pups were observed in 9/15 litters (13.2%); the incidence of undersized pups was 4.4%, 4.5%, and 4.3% in the control, 15 and 135 mg/kg bw/day groups, respectively. The differences from control were not statistically significant.
There were no treatment-related findings in visceral examinations of the foetuses.
Skeletal examinations did not reveal any treatment-related findings. Most of the lesions involved the sternebrae which included incomplete ossification, fused, malaligned, or split bones; these anomalies occurred in control and treated animals and appeared to be more prominent but not statistically significant at the high dose, 135 mg/kg bw/day. Rib structure anomalies, extra ribs and/or rib buds, showed no apparent differences in incidence between control and chemically-treated animals.
There was no evidence at any dose level of a statistically significant adverse effect of the chemical on maternal reproduction parameters, foetal weights, or on the foetuses based on gross, soft tissue, or skeletal examination.

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

In the developmental rat and rabbit toxicity studies maternal toxicity was mainly characterized by a reduction in body weight and body weight gain and in food/water consumption. In rats, lower feed consumption in dams was noted from the 10 mg/kg bw/d dose levels, and clinical signs such as hypoactivity were noted from doses 25 mg/kg bw/d and above. In rabbits, no maternal effects were noted at the low dose level of 10 mg/kg bw/d, but lower feed intake and bodyweight gains were observed from the dose 30 mg/kg bw/d and higher in the prenatal developmental studies. At higher doses in rabbits (from 85 mg/kg bw/d), reduced food intake and body weight development was more pronounced leading to reduced defecation and even moribundity (one high dose rabbit in the new developmental toxicity study had to be euthanised in extremis). Clinical symptoms such as hypoactivity, ptosis, ataxia, piloerection, discharge from nose or eyes as well as reduced body temperature on GD 5 (2h post-dose), reduction in total distance moved in the motor activity assessment and effects on the liver and thyroid (increased liver and thyroid weight, hepatocellular

hypertrophy, increased γ-GT levels) were seen in the rat, and wet clear material around mouth/nose (2-4 h post-dose) in the rabbit in accordance with the results of acute and subchronic toxicity tests.
In the two newly conducted developmental toxicity studies (M-530086-01-1, M-537608-01-1) the only developmental findings were slightly reduced fetal weights at the high dose of 75 or 100 mg/kg bw/day in rat or rabbit as well as a slightly increased incidence of retardations in ossification in high dose rats (75 mg/kg bw/day). In the mid dose group (15 mg/kg bw/d) increase in incidence of absence of ossification of digits in the proximal hind limb phalanges in rats was statistically significant, but was within the range of historical control data. Differences in the incidences of observations in forelimbs, hindlimbs, vertebrae and skull occasionally reached the level of statistical significance in the high dose group, but only in the case of “three or more sternebrae unossifed” slightly exceeded the historical control range. It was concluded that retardations in ossification in rats occurred in the high-dose group and, to a slight extent, in the mid-dose group, but no skeletal malformations were found. These retardations in ossification in rats were considered transient developmental effects, and were considered to be related to the lower fetus weight. Thereby the results of the new studies indicate no developmental toxicity of metribuzin, consisting of slightly reduced fetal weights/small pups and minor skeletel retardations in rats at high doses and, thus at maternally toxic doses. The NOAELs for maternal and developmental toxicity in the new developmental toxicity studies were 3 mg/kg bw/day in the rat and 10 mg/kg bw/d (maternal toxicity) and 30 mg/kg bw/d (developmental toxicity) in the rabbit. No evidence of a teratogenic potential of metribuzin was found.
The findings from the older studies - i.e., slight to moderate abnormalities of ureter and kidney in rat as well as “seal heart” (no internationally agreed terminus; the meaning of this finding is not clear) and effects on lung and heart development in rabbit – were not observed in any other studies. This applies also to the new developmental toxicity studies. The older studies contained many important deviations from accepted test guidelines and therefore are considered to have less weight of evidence compared to the new rat and rabbit developmental toxicity studies (from 2015) which were conducted and analyzed according to relevant standards.
In the supplementary rat developmental toxicity study (M-493058-02-1), the incidence of the finding ‘small fetus’ was 3.4 % in the high dose group versus 0 % in the concurrent control group at the time of performance of the study, and also higher than the historical range of 0.0 - 0.8 % at that time. However, this incidence was comparable to the extended (1990 - 1998) historical data range of 0.0 - 6.7 % (compiled from 22 studies comprising a total of 516 litters). Due to the low incidence of this observation, and in view of the historical control data and the lack of any other signs of embryo- or fetotoxicity, this finding can be regarded as incidental. The incidence of hydronephrosis was 3.9 % in the high dose group vs. 0 % in the control group, but regarded as incidental and being without teratogenic relevance in the study amendment. However, the use of extended historical control data over a range of eight years overestimates biological variance and reduces the statistical significance of observed effects. As these findings of abnormalities of renal pelvis and urether, and hydronephrosis were not confirmed in the newly conducted guidline based studies, they are therefore considered as not reliable.
In the rabbit developmental toxicity study (M-493061-02-1), a few findings which were originally diagnosed as developmental effects were evaluated again under modern nomenclature and historical background aspects. This re-evaluation concluded that the occurrences of ‘small foetuses’, thymus hyperplasia and heart ventricle (Rt/Lt/B) dilated, lung (Rt-4th) hypoplasia, seal heart, minor skeletal anomalies, like hypoplastic sternebra #2 and hypoplastic pubis, and bent femur, can be regarded as physiological, incidental or within the historical background range. Therefore, in conclusion, there were no teratogenic or developmental toxic effects observed in this study.

10.10.6 Comparison with the CLP criteria

According to the CLP Regulation, developmental toxicity includes any effect which interferes with normal development of the conceptus, either before or after birth, and resulting from exposure of either parent prior to conception, or exposure of the developing offspring during prenatal development, or postnatally, to the time of sexual maturation.

With regard to developmental toxicity, reduced fetal weights or pup weights were observed in the developmental toxicity studies with metribuzin in rats and rabbits and in the generation studies in rats, respectively. Reduced fetal weights or pup weights occurred in combination with markedly reduced maternal body weights and are considered to be secondary, non-specific consequences of this finding.
Furthermore, metribuzin treatment did not lead to increased incidences of structural malformations in the reproductive and developmental toxicity studies. Retardations in ossification (only in case of "three or more sternebrae unossifed" slightly exceeding the historical control range) were observed in the new developmental toxicity study in rats (M-530086-01-1) at the high dose of 75 mg/kg bw/day. This confirms the results of earlier developmental toxicity studies in rats, in which similar findings (reduced fetal weights/small pups and skeletel retardations) were observed. Retardations in ossification are considered transient developmental effects, and are regarded as secondary, non-specific consequences of the lower fetus weight in the affected animals.
Therefore, the data regarding adverse effects on development do not warrant a developmental toxicity classification.

10.10.7 Adverse effects on or via lactation

Table 56: Summary table of animal studies on effects on or via lactation

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance (purity), dose levels, duration of exposure	Results	Reference
No special studies on lactation available			
Multigeneration (3-generation) study on rats FB 30 strain (Elberfeld breed) (10 male and 20 female rats/group) Study pre-dates test guidelines Non-GLP Not acceptable	M-018361-02-1, See Section 10.10.1	At the tested dietary concentrations of up to 300 ppm (approximately 20 mg/kg bw/day, food consumption not monitored) were tolerated by rats without any significant effects on lactation.	M-018361-02-1, See Section 10.10.1
2-generation reproduction study in rats. Crl:CD®BR rats (30 male and 30 female rats/group) CFR 40 Part 158.135, EPA Guideline No. 83-4. GLP Acceptable	M-018517-01-1, See Section 10.10.1	No effects on lactation, since all pup parameters were within historical control ranges (M-420537-03-1).	M-018517-01-1, See Section 10.10.1

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance (purity), dose levels, duration of exposure	Results	Reference
Three generation reproduction toxicity study with metribuzin technical in Wistar rats (30 males and 30 females/group (P, F1 and F2)) OECD 416, modified to include a third generation. GLP Acceptable	M-493110-01-1, See Section 10.10.1	No effects on lactation, since all pup parameters were within historical control ranges or the changes were considered to be a consequence of the systemic toxicity of the substance (re-evaluation study: M-420537-03-1).	M-493110-01-1, See Section 10.10.1

10.10.8 Short summary and overall relevance of the provided information on effects on or via lactation

In the rat dietary multigeneration reproductive toxicity studies, no effects on the lactation phases of the studies or any negative subsequent effects were seen. As explained in a paper about a re-evaluation of all reproduction data (M-420537-03-1), no effects on lactation are derived from the three multigeneration studies, since all pup parameters were within historical control ranges or the changes were considered to be a consequence of the systemic toxicity of the substance.
Therefore, it can be summarized that based on these results no effects on lactation occurred.

10.10.9 Comparison with the CLP criteria

According to CLP Regulation this classification is intended to indicate when a substance may cause harm due to its effects on or via lactation. This can be due to the substance being absorbed by women and adversely affecting milk production or quality, or due to the substance (or its metabolites) being present in breast milk in amounts sufficient to cause concern for the health of a breastfed child.
In the rat multigeneration reproductive toxicity studies, no treatment-related findings of reproductive parameters or on the lactation phases of this study were seen.
Therefore, no parameter indicated a negative effect on or via lactation, so that it can be summarized that based on these results no effects on lactation occurred.

10.10.10 Conclusion on classification and labelling for reproductive toxicity

The rat and rabbit dietary multigeneration reproductive toxicity studies with metribuzin did not reveal effects on reproductive parameters, or neonatal toxicity. Therefore, the data regarding effects on sexual function and fertility do not warrant classification.
In the conducted developmental toxicity studies in rats and rabbits, no compound-related effects of metribuzin were demonstrated on developmental parameters or on the incidences of malformations. Therefore, the data regarding adverse effects on development do not warrant a developmental toxicity classification.

Also none of the parameters indicated a negative effect on or via lactation, so that it can be summarized that based on these results no effects on lactation occurred.
Thus, based on the results of the multigeneration and the developmental toxicity studies, the data do not warrant reproductive toxicity classification.

10.11 Specific target organ toxicity-single exposure

Table 57: Summary table of animal studies on STOT SE

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance (purity), route of exposure, dose levels, duration of exposure	Results	Reference
Acute oral (gavage) neurotoxicity screening study in Fischer 344 rats CDF(F-344)/BR (12/sex/dose) OECD 424 GLP Acceptable	Metribuzin techn. (94.2-95.3 %), suspended in 0.5 % methylcellulose/ 0.4 % Tween 80 in deionised water. Single oral gavage administration of 0, 2.0, 5.2, 20 and 106 mg/kg bw	No effects related to specific neurotoxicity up to 106 mg/kg bw. ≥ 5.2 mg/kg bw: decreased motor and locomotor activity, ptosis, oral staining and reduced body temperature as signs of general toxicity. NOAEL: 2.0 mg/kg bw for neurotoxicity	M-009782-01-1

Table 58: Summary table of other studies relevant for STOT SE

Type of study/data	Test substance (purity)	Relevant information about the study (as applicable)	Observations	Reference
Acute oral, dermal and inhalation toxicity studies.	See under 10.1-10.3	No specific target organ toxicity, also no narcotic effects, which would fall under any STOT-SE criteria were noted in the acute toxicity studies.	See Section 10.1-10.3	See under 10.1-10.3

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

Studies which can be used to evaluate potential STOT-SE effects were already discussed under 10.1-10.3. These studies on acute oral, dermal and inhalation toxicity demonstrated a low acute toxic potential of metribuzin with oral LD50 values of 322 mg/kg bw and > 2000 mg/kg bw in separate studies, and all dermal LD50 and inhalation LC50 values above the classification criteria. From these studies no specific, nonlethal target organ toxicity is obvious.
In addition, an acute neurotoxicity study is available in which no acute neurotoxicity or narcotic effects were observed at the dose levels of 2-106 mg/kg bw.

10.11.2 Comparison with the CLP criteria

According to the CLP criteria, a classification for STOT-SE needs to be considered if the substance causes specific non-lethal target organ toxicity after a single exposure (i.e. significant health effects that can impair function, both reversible and irreversible, immediate and/or delayed and not covered by acute toxicity, skin corrosion / irritation, eye damage / irritation, respiratory or skin sensitisation, genotoxicity, carcinogenicity and reproductive toxicity should be taken into consideration).
Categories 1 and 2 for non lethal 'significant and/or severe toxic effects' are the basis for classification with the Category reflecting the dose level required to cause the effect. Guidance value ranges for oral single-dose exposures are ≤ 300 mg/kg bw for Category 1 and > 300 and ≤ 2000 mg/kg bw for Category 2.
Category 3 covers 'transient effects' occurring after single exposure, specifically respiratory tract irritation and narcotic effects. Under Category 3, evaluation for respiratory tract irritation will be primarily based on human data, but useful information may be obtained from single and repeated inhalation toxicity tests in animals. The criteria for classifying substances as Category 3 for narcotic effects in animal studies are: lethargy, lack of coordination, loss of righting reflex, and ataxia. If these effects are not transient in nature, then they shall be considered to support classification for Category 1 or 2 specific target organ toxicity single exposure.
Based on the results after acute exposure to metribuzin in toxicological studies, no significant toxic effects on specific target organs were observed at non-lethal dose levels. Thus, classification of metribuzin for STOT-SE Category 1 or 2 is not warranted.
There is also no clear indication of transient effects like respiratory tract irritation and narcotic effects after single exposure to metribuzin. Classification of metribuzin for STOT-SE Category 3 is therefore also not warrented.

10.11.3 Conclusion on classification and labelling for STOT SE

Based on the results of acute oral, dermal and inhalation toxicity studies in test animals with metribuzin, according to the CLP Regulation, classification for STOT SE is not warranted.

10.12 Specific target organ toxicity-repeated exposure

Table 59: Summary table of animal studies on STOT RE

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance (purity), route of exposure, dose levels, duration of exposure	Results (effects statistically significantly different unless stated otherwise)	Reference

28-day study for subacute oral toxicity in rats (four-week treatment and two-week recovery period) Wistar rat (Hsd Win:WU) 10/dose/sex OECD 407 Deviations: TSH-determinations were not conducted in compliance with the Principles of GLP. GLP Acceptable	Metribuzin (94.7 %), Gavage administration of 0, 5, 30, and 150 mg/kg bw over a period of 28 days. Additional groups of 10 male and 10 female rats were observed for 14 days post treatment of reversibility persistence or delayed occurrence of toxic effects.	No animal died throghout the entire study (including recovery) period. **30 mg/kg bw:** clinical signs, ↑ water intake (males +14% on day 14) Clinical chemistry: ↑ ALAT (males +32%); ↓ triglyceride (males -32%) Relative organ weights: ↑ kidneys (females +6%) **150 mg/kg bw:** clinical signs, ↑ water intake (males +27% on day 14, +29% on day 21, +23% on day 28; females +25% on day 14), ↓ body weight (males and females -8% on day 7), Clinical chemistry: serum levels: ↑ ALAT (males +51%); ↓ triglyceride (males -52%); ↓ creatinine (males 13%); liver tissue: ↑ N-demethylase (males +46%) and ↑ O-demethylase activities (males ~2-fold); ↓ triglyceride (females -24%); ↓ T4 (males -55% and females -67%), ↑ TBC (males +15% and females +11%) and ↑ TSH conc. (males ~8-fold and females ~5-fold) Haematology: ↓ Hct (males -5%), ↓ Hb (males -5%) Relative organ weights: ↑ liver (males +10%); ↑ adrenals (males +19%); ↑ kidneys (females +13%) and ↑ thyroid (females +33%); Histopathology: changes in thyroid gland: increase in incidence and severity of colloidal vacuoles in the thyroid gland (average grades of tissues in dose groups 0/5/30/150 mg/kg bw/d were 1.0/1.0/2.0/2.8 for males and 0/0/0/2.0 for females) and severely altered colloidal staining (average grades of tissues in dose groups 0/5/30/150 mg/kg bw/d were 1.6/2.2/2.4/4.8 for males and 1.3/1.0/2.3/4.2 for females) in both sexes. After the recovery period these effects were reversible.	M-018443-01-1

28-day oral toxicity study in rats (four-week treatment and two-week recovery period) Wistar rats (5/sex/group) OECD 407 Deviations regarding insufficient ophthalmic examinations, functional observations, reticulocytes, cholesterol, albumin and bile acids and organ weights measurement and histopathology, results were not reported sexes separately. GLP Acceptable	Metribuzin technical (97.8 %), In drinking water at concentrations of 0, 50, 200 and 800 ppm (equivalent to 0, 7.0, 28.7 and 89.5 mg/kg bw/day). A second 800 ppm (95.3 mg/kg bw/day) group served as a recovery group.	No mortality, no clinical signs **50 ppm (**7.0 mg/kg bw/d)**:** ↓ water consumption (-26%, combined sex); Haematology: ↓ neutrophils (-13%, combined sex); Relative organ weights: ↓ liver (-6%, combined sex) **200 ppm (**28.7 mg/kg bw/d)**:** Clinical chemistry: ↑ BUN levels (+30%, combined sex); ↓ creatinine (-5%, combined sex) Relative organ weights: ↓ liver (-14%, combined sex); ↑ kidney (+6%, combined sex) **800 ppm** (89.5 mg/kg bw/d)**:** ↓ water consumption (-41%); Clinical chemistry: ↑ BUN levels (+40%, combined sex); ↓ K (-18%, combined sex); ↓ Ca (-5%, combined sex) Haematology: ↑ Hct (+12%, combined sex), ↓ MCH (-11%, combined sex), ↓ MCHC (-12%, combined sex), ↓ clotting time (-7%, combined sex), ↓ neutrophils (-13%, combined sex) Relative organ weights: ↑ kidney (+7%, combined sex); ↑ liver (+6%, combined sex)	M-513577-01-1
Rat 5-day dietary repeat dose study Hsd Cpb:WU Wistar rats (5/dose/sex) OECD 205 Deviations from the Avian dietary toxicity test guidelines regarding 5 rats/group were used instead of 10 birds/group, the recommended highest treatment level of 5000 ppm was exceeded, there was only one control group, the animals were not observed twice on day 1. GLP Supplementary	DIC 1468 / Metribuzin Technical (94.4 %), Diet administration of 0, 3000, 4800, 7800, 12500 or 20000 ppm for 5 consecutive days (equivalent to doses of 0, 204, 445, 716, 1281, 2384 mg/kg bw/day for males and doses of 0, 242, 376, 744, 1393, 1355 mg/kg bw/day for females), followed by a 3 day recovery period without treatment.	No mortalities up to the highest dose tested. **3000 ppm:** ↓ bodyweight (males -24% on day 5, -15% on day 8; females -16% on day 5); ↓ food consumption (males -69%, females -61%); **4800 ppm:** ↓ bodyweight (males -29% on day 5, -19% on day 8; females -23% on day 5); ↓ food consumption (males -62%, females -65%); Clinical signs in one female consisting of piloerection, increased motility, decreased feces excretion and discoloured feces **7800 ppm:** ↓ bodyweight (males -37% on day 5, -28% on day 8; females -25% on day 5, -11% on day 8); ↓ food consumption (males -66%, females -59%); Clinical signs in one male and 4 females consisting of piloerection, increased motility, decreased feces excretion and discoloured feces **12500 ppm:** ↓ bodyweight (males -38% on day 5, -26% on day 8; females -28% on day 5, -8% on day 8); ↓ food consumption (males -63%, females -54%); Clinical signs in 2 males and 3 females consisting of piloerection, increased motility, decreased feces excretion and discoloured feces; **20000 ppm:** ↓ bodyweight (males -44% on day 5, -33% on day 8; females -28% on day 5, -9% on day 8);); ↓ food consumption (males -62%, females -72%); Clinical signs in 3 males and 4 females consisting of piloerection, increased motility, decreased feces excretion and discoloured feces. No gross pathologic changes in animals sacrificed at the end of the recovery period.	M-032527-01-1

90-day oral toxicity study in Wistar rats (10/sex/group) OECD 408 Deviations regarding insufficient clinical and organ weights and histopathology analysis and functional observations. GLP Supplementary	Metribuzin technical, (97.8 %) Via drinking water at concentrations of 0, 20, 100 and 500 ppm (equivalent to 0, 2.1, 9.9 and 46.8 mg/kg bw/day for males and 0, 2.6, 12.1 and 45.0 mg/kg bw/day for females), 2 recovery groups at concentrations of 0 and 500 ppm.	No compound-related clinical signs, and no mortality was observed. **100 ppm:** Clinical chemistry: ↑ Na (males +4%) **500 ppm:** ↓ water intake (females -30%) Clinical chemistry: ↑ BUN levels (males +18%); ↑ Na (males +4%)	M-513580-01-1

Subchronic toxicological study to establish the dose-time-effect relationship for the thyroid (nine-week feeding study) in male Wistar rats TNO/W 74 (60 males/dose) Non-guideline investigative study Deviations from recommended guidelines regarding missing data on the test item analysis, only male rats were used in the study. Non-GLP Supplementary	Metribuzin (93.3 %), Dietary concentrations of 0, 35, 100, 300 and 900 ppm (equivalent to 0, 2.41, 7.43, 21.69 and 65.06 mg/kg bw/day) for up to 9 weeks, Post-observation: 3 weeks (300 and 900 ppm). Additional satellite groups of 20 rats per group were also fed diet at concentrations of 0, 300 and 900 ppm and used for radioimmunoassays at the end of the treatment period.	**35 ppm (**2.41 mg/kg bw/d)**:** Clinical chemistry: Day 7: ↑ T4 (+22%); ↓ T3 (-19%); Day 21: ↑ T4 (+45%); ↑ iodine concentration (+33%) Day 63: ↑ T4 (+50%); ↑ iodine quantity (+64%); ↑ iodine concentration (+46%); Relative organ weights: Day 7: ↓ thyroid (-11%); Day 63: ↑ liver (+13%); **100 ppm (**7.43 mg/kg bw/d)**:** Clinical chemistry: Day 7: ↑ T4 (+30%); ↓ T3 (-15%); ↑ iodine quantity (+38%), ↑ iodine concentration (+60%) Day 21: ↑ T4 (+58%); ↑ iodine concentration (+19%) Day 63: ↑ T4 (+60%); ↑ iodine quantity (+37%), ↑ iodine concentration (+24%) Relative organ weights Day 63: ↑ liver (+18%) **300 ppm (**21.69 mg/kg bw/d)**:** ↓ body weight (-5% at week 1, -4% at week 5); Clinical chemistry: Day 7: ↑ T4 (+34%); ↓ T3 (-19%); ↑ iodine quantity (+40%), ↑ iodine concentration (+93%) Day 21: ↑ T4 (+51%); Day 63: ↑ T4 (+55%); ↑ iodine quantity (+51%); Relative organ weights: Day 7: ↓ thyroid (-22%); **900 ppm (**65.06 mg/kg bw/d)**:** ↓ body weight (-11% at week 1, -10% at week 5, -7% at week 9); Clinical chemistry: Day 7: ↓ T3 (-20%); ↑ iodine quantity (+31%), ↑ iodine concentration (+83%); Day 21: ↑ T3 (+39%); Day 63: ↑ T4 (+24%); ↓ T3 (-13%); ↑ iodine quantity (+37%); Relative organ weights: Day 7: ↓ thyroid (-11%); Day 21: ↑ liver (+7%); Histopathology: Histopathological changes (Change in stainability of thyroid colloid; colloid mostly with loose and netlike structure) in 10/10 animals (on day 63) at all doses tested (0/10 in controls). The thyroid colloid of the controls was mostly stained homogeneously and bright red by the haemalum eosin and azan, whereas in the case of the treated animals it was usually pale red (haemalum eosin), with a loose to netlike structure, and the azan stained blue. This feature of the thyroid secretion was apparent in most of the treated animals in all the dose groups after 21 and 63 days, however it was also noted in some animals in all the groups even after seven days.	M-018468-01-1

Subacute inhalation toxicity studies in rats Wistar TNO/W 74 albino rats (10/dose/sex) OECD 412 Deviations regarding insufficient analysis and data on test material, reporting of age, acclimatisation and housing of rats, mode of exposure, insufficient duration of exposure, reporting of food availability and water consumption, statistical evaluations and histopathological findings, data for in-life observations and macroscopic changes are missing. Non-GLP Supplementary	Metribuzin (98.2 % first study, 93.1 % second study), Exposure to aerosols with analytical concentrations of 0, 93, 219 and 720 mg/m^3 in the first study, and 0, 31 and 90 mg/m^3 in the second study. 15 consecutive working days (6 hours/5 times per week for 3 weeks).	Statistical analysis was missing in these reports **Fist study:** No toxicologically (statistically) significant changes in hematology, clinicalchemistry and urinalysis **93 mg/m3:** ↓ bw gains (males 40%, females 75% by week 3) Clinical chemistry: ↑ O-demethylase (males 30%), ↓ O-demethylase (females 14%); ↑ T4 (males 63%, females 39%); Relative organ weights: ↑ thyroid (females 25%); **219 mg/m3:** ↓ bw (females 2% week 1-2, 1% week 3); ↓ bw gains (males 24% by week 3) Clinical chemistry: ↑ N-demethylase (males 42%, females 31%); ↑ O-demethylase (males 32%); ↓ O-demethylase (females 10%); ↑ P450 (males 18%), ↓ P450 (females 5%); ↑ T4 (males 24%, females 24%); Relative organ weights: ↑ thyroid (males and females 25%); **720 mg/m3:** ↓ bw (females 1% week 1); ↓ bw gains (males 38%, females 75% by week 3); disturbed behaviour Clinical chemistry: ↑ N-demethylase (males 42%, females 80%); ↑ O-demethylase (males 75%, females 41%); ↑ P450 (males 42%, females 10%); ↑ T4 (males 8%), ↓ T4 (females 9%) Relative organ weights: ↑ liver (males 15%, females 11%); ↑ thyroid (males 25% and females 50%); ↑ ovaries (females 18%) In the 219 and 720 mg/m3 dose groups substance-related alterations in the bone marrow (vacuoles in the nucleoplasm of the myelocytes and polymorphonuclear neutrophile leucocytes, instances of nuclear anomalies in myelocytes, myeloblasts and erythroblasts, high incidence of megacaryocytes). **Second study:** **31 mg/m³:** ↓ bw gains (females 33% by week 3) Clinical chemistry: ↑ T4 (males 50%, females 23%); **93 mg/m³ air:** ↓ bw gains (males 41%, females 33% by week 3) Clinical chemistry: ↑ N-demethylase (males 30%, females 24%); ↑ O-demethylase (females 53%); ↑ P450 (males 19%, females 10%); ↑ T4 (males 56%, females 37%) Relative organ weights: ↑ thyroid (males 25%, females 20%); ↑ testicles (males 13%), ↑ ovaries (11%); Damage to the haematopoietic system in the bone marrow was observed in female rats in the high concentration group (vacuoles in the nucleoplasm, and nuclear anomalies in myelocytes and leucocytes).	M-018391-01-1

Subacute Inhalation Study With Rats (two separately conducted study series, supplementary studies to Report M-018391-01-1) Wistar rats (10/dose/ sex) OECD 412 Deviations regarding insufficient test material analysis, duration of exposure, reporting of mode of exposure, insufficient reporting of age, acclimatisation and housing of rats, food availability and water consumption. Non-GLP Supplemental	Metribuzin (93.0 %), Formulated in ethanol-Lutrol® (1:1). Exposure to aerosols in a dynamic inhalation system 6 hours/5 times per week for 3 weeks, at target concentrations of 0 and 3000 mg/m^3 (recorded concentration of DIC 1468 in the inhalation air 674 mg/m^3) in the first study and 0 and 3000 mg/m^3 (745 mg/m^3) in the second study.	These supplementary studies were carried out in order to establish the toxicological relevance of the bone marrow findings described in report No. M-018391-01-1. **First study:** **674 mg/m^3:** ↓ bw (females -4% by week 3) **Second study:** **745 mg/m^3:** ↓ bw (males -6% by week 1; -8% by week 2; -7% by week 3) The animals in both treatment groups, particularly in the first study, showed non-specific disturbed behaviour in the form of apathy, ungroomed coats, and signs of incipient ir ritation of the visible eye and nose mucosa. Examination of the bone marrow: There were no treatment-related differences between treated and control animals. There were in particular no morphological alterations.Since the morphological assessment of the rats in the control and treatment groups did not detect any pathological alterations in the bone marrow, and in particular no vacuoles in the nucleoplasm of myelocytes and leucocytes and no nuclear anomalies in myelocytes, myeloblasts and erythroblasts occurred, the bone marrow findings in report No. M-018391-01-1 were not reproducible.	M-018475-01-1
Subacute Dermal Toxicity Study in New Zealand rabbits (HC:NZW strain) 5/sex/dose OECD 410 Deviations regarding duration of exposure; no data on the stability of test substance; histopathologically examined liver specimen in females at 1000 mg/kg bw/d (main group) was 4 instead of 5. GLP Acceptable	Metribuzin (94.0 %), Mixed with Cremophor EL (2 % v/v) in physiological saline solution applied to the back and flanks of the animals at dose levels of 0, 40, 200 or 1000 mg/kg bw/day for 15 consecutive days, recovery group: 0, 1000 mg/kg bw observed for a further 14 days.	**40 mg/kg bw/d:** no effects **200 mg/kg bw/d:** Clinical chemistry: ↑ T4 (females +45%) Relative organ weights: ↓ brain (males -12%); ↓ heart (-19%); ↓ kidney (males -13%) **1000 mg/kg bw/d:** Clinical chemistry: ↑ T4 (males +33%, females +53%); ↓ T3 (males -23%); ↑ Cholesterol (males +31%; females +32%); ↑ N- demethylase (males +45%); ↑ P450 (males +33% Relative organ weights:, ↓ heart (males -10%); ↓ liver (males -10%); ↓ spleen (females -43%)	M-018488-01-1

Subchronic Toxicity Feeding Study in the Beagle Dog (4/dose/sex) OECD 409 Additional parameters measured/performed: Intraocular pressure and corneal thickness, electrocardiogram and blood pressure assessments, T3, T4 and TSH. GLP Acceptable	Metribuzin technical (94.3 %), Via diet at 0, 75, 300 and 1200 ppm (equivalent to 0, 2.0, 8.4 and 26.2 mg/kg bw/day (males), and to 0, 1.9, 8.6 and 30.2 mg/kg bw/day (females) for 3 months.	**75 ppm (**2.0/1.9 mg/kg bw/day m/f)**:** ↓ bodyweight by day 84 (males -3%); ↓ bodyweight gain by day 84 (males -6%, females -39%) (no statistics performed on bw gain data) **300 ppm (**8.4/8.6 mg/kg bw/d m/f)**:** ↓ bw by day 84 (males -13%, females -15%); ↓ bw gain by day 84 (males -46%, females -66%) Clinical chemistry: ↑ UDP-GT (females +29%); Pathology: Macroscopic findings: discolored zone in spleen (1/4 males; 0/4 in controls); Microscopic findings: chronic inflammation of the liver (1/4 males; 0/4 in controls); Kupffer cell aggregates in liver (1/4 males; 0/4 in controls); **1200 ppm (**26.2/30.2 mg/kg bw/d m/f)**:** Two animals were sacrificed prior to study termination (one female at day 58 and one male at day 59) due to marked body weight decrease, attributed to treatment, animals showed severe anemia and platelet counts were very low in the female dog. ↓ bw by day 84 (females -10%); ↓ bw gain by day 84 (females +44%) Clinical signs: thin, unthrifty appearance and with pale yellow gingiva in two sensitive high-dose animals, one female dog who was sacrificed at day 58 was reluctant to walk and stand which was considered a secondary manifestation of the moribund condition. Clinical chemistry: ↑ UDP-GT (females +43%); Haematology: ↓ RBC (males -22%); ↓ Hb (males -25%); ↓ Hct (males -24%). There was substantial evidence of anemia in both animals sacrificed on days 58 and 59. The trend toward anemia was not pronounced except in these animals. Pathology: Macroscopic findings: Enlarged spleen (1/4 males; 0/4 in controls), raised zone in spleen (1/4 males; 0/4 in controls); Microscopic findings: Discoloration of the liver (2/4 males; 0/4 in controls); chronic inflammation of the liver (1/4 males; 0/4 in controls); Kupffer cell aggregates in liver (1/4 males; 0/4 in controls); congestion of the liver (1/4 males; 0/4 in controls); pigmentation of the liver (1/4 males; 2/4 females; 0/4 in controls); lymphocytic infiltrates in the gall bladder (3/4 males, 1/4 in controls; 3/4 females, 2/3 in controls); abnormal spermatozoa in testes (3/4 males; 1/4 in controls); abnormal spermatozoa in epididymides (4/4 males; 2/4 in controls) Lesions specific to the two dogs sacrificed in moribund condition included: bone marrow hypoplasia and hyperplasia, myocardial degeneration and hemorrhage, inflammation of liver, kidney and small intestine, unusual extramedullary hematopoiesis in liver and spleen, unusual pigmentation in the liver, pronounced involution of the thymus, and renal hemorrhage. In addition, at 1200 ppm effects in testes and epididymides were observed.	M-038758-01-1

Subchronic (90 Day) Oral Toxicity Study with Metribuzin Technical in Beagle Dogs (4/dose/sex) OECD 409 Deviations regarding insufficient reporting of results of ophthalmic examinations, water intake, observations for mortality and moribundity not performed with sufficient frequency, insufficient clinical, organ weights and histopathology analysis and urinalysis. GLP Acceptable with reservations	Metribuzin technical (> 91 %), By capsule at doses of 0, 5, 15 and 50/30 mg/kg bw, for 90 days. The dose level for the high dose group was reduced from 50 to 30 mg/kg bw/d from day 35 onwards, due to occurrence of severe signs of toxicity in dogs of either sex.	**5 mg/kg bw/d:** ↓ bodyweight gain (females -36% on weeks 0-13); Haematology: ↑ WBC (males +25% on day 90); ↓ WBC (females -24% on day 45); ↑ clotting time (females +23% on day 90); Clinical chemistry: ↓ inorganic phosphorus (males -18% on day 90); ↑ chloride (females +3% on day 45); **15 mg/kg bw/d:** Clinical signs in one female (dullness, weakness, blood tinged faeces); ↓ bodyweight gain (females -64% on weeks 0-13); Haematology:↑ WBC (males +23% on day 90); ↓ WBC (females -22% on day 45); ↑ MCV (males +13% on day 90); ↑ MCH (males +6% on day 90); ↓ RBC (females -19% on day 45); ↓ Hb (females -17% on day 45); ↓ Hct (females -19% on day 45); ↓ eosinophiles (females -70% on day 90); Clinical chemistry: ↑ total bilirubin (males +61% on day 45, +70% on day 90; females +27% on day 90); ↓ inorganic phosphorus (males -22% on day 90); ↑ chloride (males +3% on day 45); ↑ GGT (females +57% on day 45); **50/30 mg/kg bw/d:** 50 mg/kg bw was reduced to 30 mg/kg bw from day 35 onwards since one male dog died on day 33 and the other males and one female showed toxic signs (dullness, weakness, blood tinged faeces, eye discharge); body weight loss (males and females weeks 1-13; males -12% and females -3% on week 13); lower food intake (males at weeks 1-8, -40% at week 8; females at weeks 1-5, -38% at week 5) Haematology: ↓ WBC (females -25% on day 45); ↑ RBC (males 16% on day 90); ↓ RBC (males -24% on day 45; females -17% on day 45); ↑ Hb (males +21% on day 90); ↓ Hb (males -26% on day 45; females -17% on day 45, -14% on day 90); ↓ Hct (males -28% on day 45; females -16% on day 45, -10% on day 90); ↑ MCHC (males +3% on day 45); ↓ neutrophils (males -34% on day 45); ↑ reticulocytes (females ~5-fold on day 90); ↑ clotting time (females +34% on day 90); ↓ eosinophiles (females -65% on day 90); Clinical chemistry: ↓ ASAT (males -20% on day 90); ↑ total bilirubin (males +93% on day 90; females +52% on day 90); ↓ creatinine (males -36% on day 45, -19% on day 90); ↓ inorganic phosphorus (males -22% on day 90); ↑ GGT (females +57% on day 45); ↓ albumine (males -11% on day 45); ↑ chloride (males +4% on day 45; females +3% on day 45); ↓ Calcium (females -9% on day 90); Relative organ weights: ↑ liver (males +42%; females +26%); ↑ kidneys (males +30%); ↑ adrenal glands (males +27%); ↑ thyroid gland (females +43%); Emaciation and enlarged spleen was seen in high dose animals (in one high dose male and three females)	M-513582-01-1

<table>
<tr>
<td>90-day toxicity study in rats
Wistar rats (15/sex/group)
Pre-OECD 408
Deviations regarding no analysis of the test substance, no ophthalmological examinations, no hormone measures, reporting of body weight development, in-life observations, mortality, macroscopic changes, histopathological findings, blood collection method.
Non-GLP
Not acceptable</td>
<td>Metribuzin (purity not reported),
Via the diet at dose levels of 0, 50, 150, 500 and 1500 ppm (equal to 0, 5, 15, 50 and 150 mg/kg bw/d) for 3 months.</td>
<td>
≥ 50 ppm: ↑ liver wt. (f), histopathol. changes in thyroid, pituitary gland and liver;

500 ppm: ↓ Hct (males -6%; females -8%), Hb (males -7%; females -6%), RBC (males -12%; females -11%);
↑ reticulocyte count and cholesterol conc.;

1500 ppm: ↓ mean terminal bw (males -4%; females -10%) and bw gain, ↓ RBC (males -13%; females -5%), ↓ Hb (males -12%; females -8%); ↓ Hct (males -4%; females -8%) and platelet count, ↑ reticulocyte count, ↑ cholesterol conc., ↑ ALP, ↑ total bilirubin, BUN and creatinine conc., ↑ ALAT and ASAT act., ↑ thyroid (absolute males +30%; females +62%, both statistically significant; relative males +39%; females +78%, both statistically significant), spleen, liver wt., ↓ heart wt., histopathological changes in the liver (hepatocytes containing fat, some variation in hepatocyte size, fatty change of peroportal region), thyroid (loss of colloid, variations in follicular size, desquamation of epithelial cells) and pituitary gland (enlarged and “castrated” cells).

Histopathological findings in the thyroid:

<table>
<tr><td></td><td></td><td colspan="3">Thyroid gland</td></tr>
<tr><td>Dose</td><td>Total no examined</td><td>Loss of colloid</td><td>Follicular hypertrophy</td><td>Desquamation</td></tr>
<tr><td></td><td></td><td colspan="3">Male</td></tr>
<tr><td>0</td><td>5</td><td>-</td><td>1</td><td>4</td></tr>
<tr><td>50</td><td>5</td><td>1/1a</td><td>-</td><td>5/2b</td></tr>
<tr><td>150</td><td>5</td><td>0</td><td>0</td><td>5/3b</td></tr>
<tr><td>500</td><td>5</td><td>0</td><td>0</td><td>5/3b</td></tr>
<tr><td>1500</td><td>5</td><td>3/1b</td><td>5</td><td>5/4b</td></tr>
<tr><td></td><td></td><td colspan="3">Female</td></tr>
<tr><td>0</td><td>5</td><td>-</td><td>-</td><td>-</td></tr>
<tr><td>50</td><td>5</td><td>-</td><td>-</td><td>2</td></tr>
<tr><td>150</td><td>5</td><td>1/1b</td><td>-</td><td>5/1b</td></tr>
<tr><td>500</td><td>5</td><td>-</td><td>-</td><td>4</td></tr>
<tr><td>1500</td><td>5</td><td>2/1b</td><td>1</td><td>3/2b</td></tr>
</table>
a: moderate
b: much/complete

NOAEL: < 50 ppm (equivalent to < 5 mg/kg bw/day) based on higher liver weights in females and on histopathological findings in the liver, thyroid and pituitary gland at 50 ppm.
</td>
<td>M-018365-01-1</td>
</tr>
</table>

90-day feeding toxicity study in rats Wistar SPF rats (15/sex/group) Pre-OECD 408 Deviations regarding no analysis of the test substance, no ophthalmological examinations, no hormone measures, insufficient reporting of in-life observations, macroscopic changes, histopathological examinations. Non-GLP Not acceptable	Metribuzin (purity not reported), Via diet at dose levels of 0, 10, 25 and 60 ppm (equivalent to males: 0, 0.23, 0.56 and 1.31 mg/animal/day, females: 0, 0.20, 0.46 and 1.06 mg/animal/day) for 3 months.	**10 ppm:** no effects **25 ppm:** no effects **60 ppm:** ↑ liver wt. (females absolute +5% and relative +7%, statistically significant). NOAEL: 25 ppm (equivalent to 0.46 mg/animal/day) based on significantly higher liver weights (absolute and relative) in female rats at 60 ppm (equivalent to 1.06 mg/animal/day).	M-018356-01-1
90-Day Subacute Oral Toxicity in Beagle Dogs (4/dose/sex) Non-guideline study Deviations regarding no analysis of the test substance, or statistical analysis, no data for in-life observations, no ophthalmogical examinations, insufficient examinations of hematological and clinical chemistry parameters. Non-GLP Not acceptable	BAY 94337 technical (purity not reported), Via diet at 0, 50, 150 and 500 ppm (equivalent to 0, 1.25, 3.75 and 12.5 mg/kg bw/day) for 90 days.	**50 ppm:** ↑ relative liver wt. (males +2%; females +3%, no statistical significance); 150 ppm: ↑ relative liver wt. (males +7%; females +15%, no statistical significance); **500 ppm:** ↓ bw gain in males (overall weight gain, weeks 0-13, groups 1 to 4: male dogs 2.8/2.8/2.8/2.2 kg ; female dogs: 1.9/2.8/2.3/2.2 kg), ↓ Hb (males -6%, females -8%, no statistical significance), Hct, ↑ ALAT, ASAT act. (f), ↑ relative liver wt. (males +29%; females +45%, no statistical significance); ↑ relative ↑ spleen wt. (males +24%, females +14%, no statistical significance). No fatalities occurred, no significant abnormalities and no untoward behavioral reactions were recorded during the investigation. NOAEL: < 50 ppm (equivalent to 1.25 mg/kg bw/day), based on increased liver weights at 150 and 500 ppm.	M-019874-01-1

Chronic toxicity study on Dogs (2-year feeding experiment). Beagle dogs. (4/dose/sex) Pre-OECD 452 Deviations regarding the highest dose level was too high, resulting in 75 % mortality, the analysis of test substance was not performed, insufficient statistical analysis, insufficient measurements of haematology, clinical biochemistry and urinalysis parameters, organ weights. Non-GLP Not acceptable	Metribuzin (99.5 %), Via diet at concentrations of 0, 25, 100 and 1500 ppm (equivalent to 0, 0.8, 3.4, 55.7 mg/kg bw/day for males and 0, 0.8, 3.6, 55.3 mg/kg bw/day for females, respectively) for a period of two years.	**25 ppm (0.8/0.8 mg/kg bw/d m/f):** 1 female died after 1 year, 1 female died after 2 years **100 ppm (3.4/3.6 mg/kg bw/d m/f):** no effects **1500 ppm (55.7/55.3 mg/kg bw/d m/f):** ↑ mortality (mortality in 0, 25, 100, 1500 ppm groups after 1 yr in males: 0/4, 0/4, 0/4, 2/4 and in females: 0/4, 1/4, 0/4, 2/4; after 2 yrs in males: : 0/4, 0/4, 0/4, 3/4 and in females: 0/4, 1/4, 0/4, 5/4), ↓ food consumption (males -6%, females -5%), ↓ bw (m), ↑ liver (relative (m)) wt., ↑ thyroid (absolute (m) and relative (m and f)) wt., ↑ spleen (absolute (m) and relative (m)) wt., clinical-chemistry changes (fluctuating bilirubin, ALT, AST increases), haemolytical anemia (↓ RBC counts (males – 11%, females -10%), Hb (males -13%, females -1%), Hb and Hct (males -19%, females -11%) values and ↑ reticulocyte counts), changes in liver (fat-less cytoplasm vacuoles, nucleus wall hyperchromatosis, karyopyknosis, necrobiosis and siderosis, increased iron storage in the Kupffer's cells) and spleen (siderosis, extramedullary haematopoiesis, increased numbers of megakaryocytes), necroses of the tubular cells and iron deposition in the kidneys, the testes and prostate of some animals in this group showed signs of immaturity, adaptive changes in the adrenal glands. NOEL: 100 ppm (equivalent to 3.4 mg/kg bw/day in males and 3.6 mg/kg bw/day in females). At 1500 ppm, there were mortalities, reduced body weight gain and food consumption and haematological and blood clinical-chemistry changes together with histopathological changes.	M-018381-01-1

Table 60: Summary table of other studies relevant for STOT RE

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
Chronic toxicity and carcinogenicity studies in rats and mice	See Section 10.9	See Section 10.9	See Section 10.9	See Section 10.9
Reproduction and developmental toxicity studies in rats and rabbits	See Section 10.10	See Section 10.10	See Section 10.10	See Section 10.10
Subchronic 13-week dietary neurotoxicity screening study in Fischer 344 rats CDF (F-344) (12/sex/dose) US-EPA-FIFRA, Pesticide Assessment Guidelines, Subdivision F, Hazard Evaluation: Human and	Metribuzin techn (94.2 % (January 1997); 95.3 % (September 1997)) Dietary administration of 0, 30, 300 and 900 ppm (M/F: 0/0, 1.92/2.19, 21.2/23.9, 62.3/70.1	This study type is useful for assessment of STOT-RE effects.	No effects related to neurotoxicity up to the highest dose. General toxicity: ≥ 300 pm: ↓ food consumption and body weight / body weight gain in females. NOAEL for neurotoxicity and systemic toxicity is 900 ppm (62 mg/kg bw in males and 70 mg/kg bw in females).	M-009781-01-1

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
Domestic Animals, Guideline Addendum 10, Neurotoxicity; NTIS, 1991 GLP Acceptable	mg/kg bw/d)			
Assessment of pubertal development and thyroid function in juvenile/peripubertal male and female rats. US E.P.A., OPPTS Series 890 GLP Acceptable	Metribuzin technical (purity 94.4 %), Dose levels 0, 60, 120 mg metribuzin /kg bw/day by oral gavage		Signs of general toxicity: 60 mg/kg bw/d: ↓ serum T4 (males 43%); ↑ TSH (changes were marked and statistically significant in males 109%; in females non-significant decrease by 29%); ↑ (relative) liver wt (males 12%; females 10%); 120 mg/kg bw/d: ↓ T4 (males 72%; females 44%), ↑ TSH (changes were marked and statistically significant in males 236%; in females non-significant decrease by 33%); ↑ thyroid wt. (45-46% in males; 15% in females); enlarged thyroids (6/15 males; 1/15 females) and minimal to slight follicular cell hyprtrophy, colloid alteration, and an increased number of mitoses in thyroid (2/15 males); enlarged livers (11/15 males; 5/15 females); increased number of mitoses in livers (7/11 males; 4/5 females); minimal centrilobular hepatocellular hypertrophy in the liver (males 4/11); ↑ ALAT (females).	US EPA, 2011

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

The short-term toxicity of metribuzin was investigated in a subacute 5-day oral study in rats, in two oral 28-day studies in rats and six 3-month studies in rats and dogs via diet, drinking water and capsules. In addition, the short-term toxicity following dermal exposure was determined in rabbits. The toxicity of metribuzin via inhalative exposure was tested in two 21-day studies in rats. In a 9-week mechanistic investigative feeding study conducted in rats the effects of metribuzin on the thyroid gland and circulating hormones were studied. In addition, a not acceptable 2-year chronic toxicity feeding study on dogs was presented (studies in Table 59). Relevant information on the

repeated-dose exposure is also available from the Combined Chronic Toxicity/ Oncogenicity Feeding Toxicity Study in Fischer rats (M-017948-02-1, Table 19), oral developmental toxicity study in Charles River rats (M -018676-01-1, Table 45), and from the Assessment of pubertal development and thyroid function in juvenile/peripubertal male and female rats from the Tier 1 test battery of US EPA's Endocrine Disrupter Screening Program (referred as US EPA, 2011, available at *https://www.epa.gov/endocrine-disruption/endocrine-disruptor-screening-program-tier-1-screening-determinations-and*, Table 60).

In two 90-day studies in Beagle dogs, mortalities occurred at the high dose. In the subchronic feeding study with dogs (M-038758-01-1), one male and one female dog were sacrificed on nominal day 59 (male) and on day 58 (female) at the high dose (26.2 mg/kg bw/d in males and 30.2 mg/kg bw/d in females) due to clinical moribund. Also, in the subchronic oral toxicity study with dogs via capsules (M-513582-01-1), the dose level for the high dose group was reduced from 50 to 30 mg/kg bw/d from day 35 onwards, due to occurrence of severe signs of toxicity in dogs of either sex, one high dose male died on day 33 of treatment. In rats, no mortalities were reported up to doses of 150 mg/kg bw/d in subchronic oral studies.

Exposure to metribuzin caused adverse effects on red blood cells (mostly in dogs) and thyroid (mostly in rats).

Effects on red blood cell parameters became apparent in dogs from 15 mg/kg bw/d in females (decreased RBC, Hb and Hct in the 90-day study (M-513582-01-1)) and from 26.2 mg/kg bw/d in males (decreased RBC, Hb and Hct in the 90 feeding study (M-038758-01-1)). Two dogs who where sacrificed prior to study termination in the 90-day feeding study, showed severe anemia. In rats generally effects on red blood cells were not observed, only slight decrease in males (-5%) in Hct and Hb were observed at the high dose of 150 mg/kg bw/d in the 28-day oral toxicity study in rats (M-018443-01-1). In summary, hematologically relevant effects were mainly observed in a 90-day study in dogs observed as lower red blood cell parameters (RBC, Hb, Hct) in males dogs at 26.2 mg/kg bw/d and signs of anaemia in the two dogs which were sacrificed early due to moribundity. Decreased red blood cell parameters were also noted in the other two dog studies as well as 28-day and 90-day rat oral studies.

The effect of metribuzin on the thyroid pathway was shown in rat studies, evidenced by changes in thyroid hormone levels, histopathological changes and increased thyroid weight. Changes in the levels of T4, T3 and TSH were noted in a rat oral 28-day study (M-018443-01-1) as well as 9-week mechanistic study (M-018468-01-1), rat developmental study (M-018676-01-1) and rat combined chronic toxicity/oncogenicity study (M-017948-02-1), rat pubertal development study (US EPA, 2011), rat repeated-dose inhalation study (M-018391-01-1) and in rabbit dermal repeat-dose study (M-018488-01-1). . Statistically significant changes to the levels of T4 and T3 were recorded already from the lowest doses tested in the rat 9-week study (from 2.41 mg/kg bw/d) and in the rat chronic toxicity study (from 1.3 (m)/1.6 (f) mg/kg bw/d). Increased iodine concentration accompanied the thyroid hormone change in the rat mechanistic study also from the low dose (2.41 mg/kg bw/d). Changes in the colloidal staining of thyroid or thyroid follicular cell hyperplasia or hypertrophy were observed in the rat 28-day oral study (from 30 mg/kg bw/d), 9-week mechanistic study (from 2.41 mg/kg bw/d) as well as rat chronic study (from 13.8 (m)/17.7 (f) mg/kg bw/d) and rat pubertal development study (at 120 mg/kg bw/d). Thyroid toxicity was often accompanied by increased liver weight, changes in liver enzyme activities and reductions in food consumption and bodyweight in rats.

In addition to the study reports provided by the applicant, the Tier 1 test battery of US EPA's Endocrine Disrupter Screening results are available, including also a review of the entire submitted toxicology data base on metribuzin to identify possible effects of the compound on endocrine organs, tissues or parameters *in vivo*. In pubertal assays with male and female rats convincing evidence was observed of potential interaction of metribuzin with the thyroid pathway. At the low dose (60 mg/kg bw/d), serum T4 levels were decreased and serum TSH levels were increased in males. At the high dose (120 mg/kg bw/d), serum T4 levels were decreased in both sexes, serum TSH levels were increased in males, thyroid weights were increased in both sexes. Males at this dose exhibited enlarged thyroids and minimal to slight follicular cell hypertrophy, colloid alteration, and an increased number of mitoses.

Furthermore, in an acceptable 2-year combined chronic toxicity/carcinogenicity feeding study in rats (M-017948-02-1) thyroid effects were evident. Changes in thyroid hormones were evident from the lowest dose tested (1.3 (in males) and 1.6 (in females) mg/kg bw/d). The serum T4 levels were increased (without dose-dependency) at the low- and mid-dose (13.8 (in males) and 17.7 (in females) mg/kg bw/d) at all timepoints (statistically significant and at times, marked increase) in males and females, whereas the T4 levels at the high-dose (42.2 (in males) and 53.6 (in females) kg/kg bw/d) were inconsistent in terms of magnitude, direction, and time of occurrence. General pattern (without dose-dependency) of decrease can be observed in the serum T3 levels. In male rats of the mid- and high-dose groups increased incidence of thyroid follicular cell hyperplasia was observed. Enlargement of thyroids was noted in a dose-related manner and at the high dose, thyroid and liver weights were increased, but no occurrence of thyroid tumours was observed. ALT, AST, AP were decreased at the mid- and high-dose and cholesterol was increased without clear dose-response-relationship and within normal physiological limits.
In the rat oral developmental toxicity study (M-018676-01-1), maternal toxicity as changes in T4 levels were observed from the mid-dose of 70 mg/kg bw/d (T4↓ on day 16, T4 ↑ on day 20), as well as increased thyroid weight.
Repeated dermal administration of metribuzin to rabbits caused changed in thyroid hormone levels from 200 mg/kg bw/d. Repeated inhalation exposure of rats to metribuzin for 3 weeks resulted in decreased bodyweight gains, changes in liver enzymes, increased T4 and increased thyroid weight from 93 mg/m3.
Effects on thyroid pathway were not observed in dogs up to the highest dose tested of 50/30 mg/kg bw/d, except increased thyroid weight in high dose females at 50/30 mg/kg bw/d, whereas marked toxicity was already evident at this dose level as anaemic effects.
Based on the available test data the main targets were the red blood cells in dogs and the thyroid gland in rat and rabbits irrespective of the exposure route. In rats and rabbits the thyroid gland and in dogs the peripheral blood (red blood cells) was affected at dose levels which are above the guidance values for STOT RE classification.
Effects on the liver were also reported in the rat and dog studies as well as in the rabbit dermal study and consisted of increased liver weights, increased activities of liver enzymes, and chronic inflammation of the liver and Kupffer cell aggregates. Increased liver weights were noted already at dose levels of < 10 mg/kg bw/d in rats. In the subchronic studies, at dose levels of 9 and 30 mg/kg bw/d in dogs and > 100 mg/kg bw/d in rats signs of damage to the liver parenchyma became apparent and higher activities of ALAT, ASAT, and ALP were recorded in rats from the 30 mg/kg bw/d dose levels. With increasing dose levels, i.e. 150 mg/kg bw/d oral in rats, around 90 mg/m^3 air in rats, and 200 mg/kg bw/d dermal in rabbits, microsomal hepatic enzyme induction was apparent from elevated levels of the mixed-function oxidases (N-demethylase, O-demethylase, cytochrome P-450). In addition, these doses led to an effect on the hepatic function with dysregulation of the lipid, carbohydrate, glycoprotein and protein metabolism, as was apparent from the changes which occurred in the cholesterol, glucose, triglyceride and protein levels in the blood. Only at doses of >100 mg/kg bw/d in rats and at 30 mg/kg bw/d in dogs histopathological changes in the liver were reported (mild hepatocellular hypertrophy, slight fatty change of hepatocytes). In addition, vacuolar degeneration of the hepatocytes, nuclear damage and focal liver cell necroses were noted at these dose levels.
In general in the rat studies, at higher doses, adverse effects on thyroid were accompanied by increased liver weights, increased activities of liver enzymes and histopathological changes of the liver. Therefore, a liver enzyme-related thyroid mode of action in rats could not be excluded.
Overall, the results indicate that rats were more sensitive to metribuzin-induced thyroid effects. Rodents are known in general to be more sensitive to liver enzyme-related thyroid mode of action via thyroid hormone clearance by phase II catabolism enzymes and subsequent increased TSH levels and proliferation of the follicular cells in the thyroid gland. However, this does not rule out the effect of metribuzin on thyroid hormone levels in humans via other mechanisms such as disruption of thyroid hormone synthesis, thyroperoxidase, deiodinase, leading to potential developmental effects.

10.12.2 Comparison with the CLP criteria

According to the CLP Regulation, substances that have produced significant toxicity in humans or that, on the basis of evidence from studies in experimental animals, can be presumed to have the potential to produce significant toxicity in humans or have the potential to be harmful to human health following repeated exposure are classified as specific target organ toxicants.

Substances are classified in Category 1 for target organ toxicity (repeat exposure) on the basis of: reliable and good quality evidence from human cases or epidemiological studies; or observations from appropriate studies in experimental animals in which significant and/or severe toxic effects, of relevance to human health, were produced at generally low exposure concentrations.

Substances are classified in Category 2 for target organ toxicity (repeat exposure) on the basis of observations from appropriate studies in experimental animals in which significant toxic effects, of relevance to human health, were produced at generally moderate exposure concentrations.

Guidance values are ≤ 10 mg/kg bw for Category 1 and ≤ 100 mg/kg bw for Category 2 for oral exposure to rats in 90-day study; ≤ 20 mg/kg bw for Category 1 and ≤ 200 mg/kg bw for Category 2 for dermal exposure to rats or rabbits in 90-day study; and ≤ 0.02 mg/L/6h/d for Category 1 and ≤ 0.2 mg/L/6h/d for Category 2 for inhalation exposure to dust/mist/fume to rats in 90-day study.

Toxic effects in humans and/or animals taken into consideration in the classification process include:

"All available evidence, and relevance to human health, shall be taken into consideration in the classification process, including but not limited to the following toxic effects in humans and/or animals:

(a) morbidity or death resulting from repeated or long-term exposure. Morbidity or death may result from repeated exposure, even to relatively low doses/concentrations, due to bioaccumulation of the substance or its metabolites, and/or due to the overwhelming of the de-toxification process by repeated exposure to the substance or its metabolites;

(b) significant functional changes in the central or peripheral nervous systems or other organ systems, including signs of central nervous system depression and effects on special senses (e.g. sight, hearing and sense of smell);

(c) any consistent and significant adverse change in clinical biochemistry, haematology, or urinalysis parameters;

(d) significant organ damage noted at necropsy and/or subsequently seen or confirmed at microscopic examination;

(e) multi-focal or diffuse necrosis, fibrosis or granuloma formation in vital organs with regenerative capacity;

(f) morphological changes that are potentially reversible but provide clear evidence of marked organ dysfunction (e.g., severe fatty change in the liver);

(g) evidence of appreciable cell death (including cell degeneration and reduced cell number) in vital organs incapable of regeneration."

Effects in repeated-dose studies with metribuzin considered potentially relevant for classification of STOT-RE include mortality (in dogs), haematological effects (mainly in dogs) and effects on thyroid (mainly in rats, supported by data in rabbits).

In rats, no mortalities occurred up to the highest dose tested of 1355 mg/kg bw/d in females and 2384 mg/kg bw/d in males in a 5-day oral toxicity study and 150 mg/kg bw/d in 90-day oral toxicity studies.

Adverse findings were reported in subchronic toxicity studies with metribuzin in dogs (M-038758-01-1). In a 90-day study with metribuzin in dogs, mortality was noted on day 33 at 50 mg/kg bw/d. In another 90-day study in dogs, 2 dogs were prematurely sacrificed in a moribund condition on days 59 (1 male dog) and 58 (1 female dog) at the highest dose levels of 26.2 and 30.2 mg/kg bw/d, respectively. The mortalities and moribundity occurred below the guidance value for Category 2 of 100 mg/kg bw/d and therefore justify classification to STOT-RE Category 2.

Effects on the thyroid pathway below the STOT-RE Category 2 guidance value were observed in a rat 28-day study, 9-week rat mechanistic study, 2-year rat combined chronic toxicity/carcinogenicity and rat developmental study and rat pubertal development study.

In the Table 61 below relevant effects on morbidity or death, hematology and thyroid parameters are highlighted with reference to the extrapolated guidance values for STOT RE classification categories.

Table 61: Summary of relevant key *in vivo* data referring to findings related to morbidity and death, haematological and thyroid parameters compared with STOT-RE guidance values for 90-day exposure and extrapolated values for studies of greater or lesser duration according to the Guidance on the Application of the CLP Criteria.

Study	Dose (mg/kg bw/d)	Haematological effects	Thyroid effects	Morbidity or death
Guidance value STOT-RE 1 Oral 28-day: ≤ 30 mg/kg bw/d; Guidance value STOT-RE 2 Oral 28-day: ≤ 300 mg/kg bw/d				
28-day Rat oral (M-018443-01-1) Acceptable GLP	150	↓ Hct (males -5%), ↓ Hb (males -5%)	↑ rel. thyroid wt. (females +33%); ↑ incidence and severity of colloidal vacuoles in the thyroid gland (average grades of tissues in dose groups 0/5/30/150 mg/kg bw/d were 1.0/1.0/2.0/2.8 for males and 0/0/0/2.0 for females) and severely altered colloidal staining (average grades of tissues in dose groups 0/5/30/150 mg/kg bw/d were 1.6/2.2/2.4/4.8 for males and 1.3/1.0/2.3/4.2 for females) in both sexes. ↓ T4 (males -55% and females -67%), ↑ TBC (males +15% and females +11%) and ↑ TSH conc. (males ~8-fold and females ~5-fold)	
28-day Rat oral (M-513577-01-1) Acceptable GLP	89.5	↑ Hct (+12%, combined sex), ↓ MCH (-11%, combined sex), ↓ MCHC (-12%, combined sex);		
Pubertal development 21-31 days Rat (US EPA, 2011) Acceptable GLP	60		↓ serum T4 (males 43%); ↑ TSH (changes were marked and statistically significant in males 109%; in females non-significant decrease by 29%);	
	120		↓ T4 (males 72%; females 44%), ↑ TSH (changes were marked and statistically significant in males 236%; in females non-significant decrease by 33%); ↑ thyroid wt. (45-46% in males; 15% in females); enlarged thyroids (6/15 males; 1/15 females); minimal to slight follicular cell hyprtrophy, colloid alteration, and an increased number of mitoses in thyroid (2/15	

			males);	
Guidance value STOT-RE 1 Oral 90-day: ≤ 10 mg/kg bw/d; Guidance value STOT-RE 2 Oral 90-day: ≤ 100 mg/kg bw/d				
90-day Dog dietary (M-038758-01-1) Acceptable GLP	26.2/30.2 (m/f)	↓ RBC (males -22%); ↓ Hb (males -25%); ↓ Hct (males -24%). There was substantial evidence of anemia in both animals sacrificed on days 58 and 59.		Two animals were sacrificed prior to study termination (one female at day 58 and one male at day 59) due to marked body weight decrease, attributed to treatment, animals showed severe anemia and platelet counts were very low in the female dog.
90-day Dog via capsules (M-513582-01-1) Acceptable with reservations GLP	15	↑ MCV (males +13% on day 90); ↑ MCH (males +6% on day 90); ↓ RBC (females -19% on day 45); ↓ Hb (females -17% on day 45); ↓ Hct (females -19% on day 45);		
	50/30 (30 from day 35 due to severe toxicity)	↑ RBC (males 16% on day 90); ↓ RBC (males -24% on day 45; females -17% on day 45); ↑ Hb (males +21% on day 90); ↓ Hb (males -26% on day 45; females -17% on day 45, -14% on day 90); ↓ Hct (males -28% on day 45; females -16% on day 45, -10% on day 90); ↑ MCHC (males +3% on day 45); ↑ reticulocytes (females ~5-fold on day 90);	↑ rel. thyroid gland wt. (females +43%);	50 mg/kg bw was reduced to 30 mg/kg bw from day 35 onwards since one male dog died on day 33 and the other males and one female showed toxic signs (dullness, weakness, blood tinged faeces, eye discharge)
Guidance value STOT-RE 1 Oral 9-week: ≤ 14 mg/kg bw/d; Guidance value STOT-RE 2 Oral 9-week: ≤ 140 mg/kg bw/d				
9-week Rat (male) dietary (M-018468-01-1) Supplementary Non-GLP	2.41		Day 7: ↑ T4 (+22%); ↓ T3 (-19%); Day 21: ↑ T4 (+45%); ↑ iodine concentration (+33%) Day 63: ↑ T4 (+50%); ↑ iodine quantity (+64%); ↑ iodine concentration (+46%); ↓ rel. thyroid wt. (-11%, day 7); Change in stainability of thyroid colloid in 10/10 animals (on day 63) (0/10 in	

			controls).	
	7.43		↑ T4 (+30%); ↓ T3 (-15%); ↑ iodine quantity (+38%), ↑ iodine concentration (+60%) Day 21: ↑ T4 (+58%); ↑ iodine concentration (+19%) Day 63: ↑ T4 (+60%); ↑ iodine quantity (+37%), ↑ iodine concentration (+24%) Change in stainability of thyroid colloid in 10/10 animals (on day 63) (0/10 in controls).	
	21.69		↑ T4 (+34%); ↓ T3 (-19%); ↑ iodine quantity (+40%), ↑ iodine concentration (+93%) Day 21: ↑ T4 (+51%); Day 63: ↑ T4 (+55%); ↑ iodine quantity (+51%); ↓ rel. thyroid wt. (-22%, day 7); Change in stainability of thyroid colloid in 10/10 animals (on day 63) (0/10 in controls).	
	65.06		↓ T3 (-20%); ↑ iodine quantity (+31%), ↑ iodine concentration (+83%); Day 21: ↑ T3 (+39%); Day 63: ↑ T4 (+24%); ↓ T3 (-13%); ↑ iodine quantity (+37%); ↓ rel. thyroid wt. (-11%, day 7); Change in stainability of thyroid colloid in 10/10 animals (on day 63) (0/10 in controls).	
Guidance value STOT-RE 1 Oral 2-year: ≤ 1.25 mg/kg bw/d; Guidance value STOT-RE 2 Oral 2-year: ≤ 12.5 mg/kg bw/d				
2-year Rat combined chronic toxicity / carcinogenicity (M-017948-02-1) Acceptable GLP	1.3/1.6 (m/f)		↓ T3 (males -17% at day 91; -19% at day 182; -15% at day 364; females -16% at day 91; -23% at day 182; -26% at day 546); ↑ T4 (males +57% at day 91; +48% at day 182; +41% at day 364; +21% at day 546; females +28% at day 91; +27% at day 182; +93% at day 364; +22% at day 546; +42% at day 728);	
	13.8/17.7 (m/f)		↓ T3 (males -37% at day 182; -24% at day 364; -16% at day 546; -22% at day 728; females -16% at day 182; -13% at day 546; -16% at day 728); ↑ T4 (males +25% at day 91; +41% at day 182; +43% at day 364; females +30% at day 91; +31% at day 182; +84% at	

			day 364; +32% at day 546; +57% at day 728); Enlarged thyroid (males 7/50, females 1/50 animals); ↑ absolute thyroid wt. (females +19%);	
	42.2/53.6 (m/f)		↓ T3 (males -17% at day 182; -27% at day 728; females -11% at day 182); ↑ T4 (males +7% at day 182, +27% at day 364; +29% at day 546; females +47% at day 364; +36% at day 728); Enlarged thyroid (males 8/50, females 3/50 animals); ↑ absolute thyroid wt. (males +24% and females +14%); thyroid follicular cell hyperplasia (males 11/20 animals, 1-yr; 38/50 animals, 2-yr).	
Guidance value STOT-RE 1 Oral 15 days: ≤ 60 mg/kg bw/d; Guidance value STOT-RE 2 Oral 15 days: ≤ 600 mg/kg bw/d				
Developmental Rat oral (M-018676-01-1) Supplementary GLP	70		Maternal toxicity: ↓ T4 (-52% on day 16)	
	200		Maternal toxicity: ↓ T4 (-85% on day 16); ↑ absolute thyroid wt. (+57% on day 16; +33% on day 20)	
Guidance value STOT-RE 1 Dermal 15 days: ≤ 120 mg/kg bw/d; Guidance value STOT-RE 2 Dermal 15 days: ≤ 1200 mg/kg bw/d				
Dermal repeated-dose 15 days Rabbit (M-018488-01-1) Acceptable GLP	200		↑ T4 (females +45%)	
	1000		↑ T4 (males +33%, females +53%); ↓ T3 (males -23%);	
Guidance value STOT-RE 1 Inhalation 15 days: ≤ 0.12 mg/L/6h/day=120 mg/m^3/6h/day; Guidance value STOT-RE 2 Inhalation 15 days: ≤ 1.2 mg/L/6h/day=1200 mg/m^3/6h/day				
Inhalation repeated-dose 15 days Rat (M-018391-01-1) Supplementary Non-GLP	Fist study: 93 mg/m^3		↑ T4 (males 63%, females 39%); ↑ re. thyroid wt. (females 25%);	
	Fist study: 219 mg/m^3		↑ T4 (males 24%, females 24%); ↑ rel. thyroid wt. (males and females 25%);	
	Fist study: 720 mg/m^3		↓ T4 (females 9%); ↑ rel. thyroid wt. (males 25% and females 50%);	
	Second study: 31 mg/m³		↑ T4 (males 50%, females 23%);	
	Second study: 93 mg/m³		↑ T4 (males 56%, females 37%) ↑ rel. thyroid wt. (males 25%, females 20%);	

10.12.3 Conclusion on classification and labelling for STOT RE

Metribuzin should be classified as STOT RE 2 based on the findings on red blood cell parameters (evidence of anemia and effects on RBC, Hct, and Hb) in dogs at ≥ 15 mg/kg bw/d after 90-days oral exposure and thyroid effects (changes in THs levels, hypertrophy and increased organ weight) in rats at ≥ 2.41 mg/kg bw/d after subchronic oral exposure and ≥ 1.3 mg/kg bw/d after chronic oral exposure as well as mortality in dogs at ≥ 26.2 mg/kg bw/d after 90-day oral exposure. Thyroid effects were also observed in rabbits after 15-day dermal exposure at ≥ 200 mg/kg bw/d and in rats after 15-day inhalation exposure at ≥ 31 mg/m^3. The following hazard statement should be added: H373 – May cause damage to organs (blood, thyroid) through prolonged or repeated exposure.

10.13 Aspiration hazard

This hazard class is not assessed in this dossier.

11 EVALUATION OF ENVIRONMENTAL HAZARDS

11.1 Rapid degradability of organic substances

A study on the ready biodegradability of metribuzin was not performed. However, water, water-sediment and soil degradation studies were performed which can be used in order to address biodegradability of metribuzin. The degradation studies in aquatic systems showed that metribuzin is not rapidly biodegradable. Please refer to section 11.1.4.3 for details on the simulation studies.

11.1.1 Ready biodegradability

No studies available for readily biodegradation.
Based on the data from degradation simulation studies in water and aquatic sediment as described under section 11.1.4.3, metribuzin is considered to be not rapidly biodegradable for the classification purpose according to CLP criteria as the substance is demonstrated not to be ultimately degraded in a surface water nor in an aquatic sediment with a half-life of < 16 days.

11.1.2 BOD_5/COD

No data available.

11.1.3 Hydrolysis

Study M-019238-01-1

The hydrolysis of metribuzin was investigated in 0.5 M buffer solutions, which were adjusted to pH 5, 6, 7 and 9. The test solutions were prepared with radiolabelled [5-^{14}C]metribuzin at a concentration of approximately 0.02 g/L. The solutions were incubated at 25 °C for a maximum period of 34 days under sterile conditions in the dark. Replicate samples were taken at 3, 8, 14, 20, 27 and 34 days post sample preparation. Analysis of the samples was performed using HPLC analyses followed by liquid scintillation measurement and in comparison with reference standards. Metribuzin was stable at all pH-values investigated over the course of the study of a period of 34 days. No degradation products were observed in amounts > 10 % AR.

Study M-513257-01-1

Metribuzin was investigated in test solutions buffered at pH 4, 7 and 9. Test concentration was 200 mg metribuzin/L; two replicates per temperature and pH were investigated. Test temperature was 50 °C for pH 4 and pH 7, but for pH 9 a temperature of 50, 60 and 70 °C was tested. Analytical

verification was done via HPLC-DAD. The test substance was stable at pH 4 and 7 (50 °C). At pH 9, hydrolysis at 50 °C amounted to ca. 50 %, which is why experiments at higher temperatures were conducted at pH 9. Since metribuzin was found to be stable at room temperature and conditions relevant to the environment (i.e. neutral to acidic pH range), no further investigations on the nature of hydrolysis products at elevated temperature and pH 9 were conducted. Metribuzin was found to be stable at neutral or acidic pH values, and was only slowly degraded under basic conditions (calculated DT_{50} for pH 9 = 1317 days at 20 °C). Thus, any metabolite, degradation or reaction product accounting for more than 10 % of the active substance can be excluded under environmentally relevant conditions.
Therefore metribuzin is considered stable to hydrolysis in buffer solutions (pH 5, 6, 7 and 9) at 25 ºC.

11.1.4 Other convincing scientific evidence

No data available.

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

No data available.

11.1.4.2 Inherent and enhanced ready biodegradability tests

No data available.

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

Route and rate of degradation of [5-^{14}C]metribuzin in surface water was investigated under defined laboratory conditions according to OECD TG 309 (M-513283-01-1). 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 was performed over a period of 56 days; sampling intervals were 0, 7, 14, 21, 28, 45 and 56 days. Duplicate samples were taken and the water phases were analysed with RP-radio-HPLC, HPLC-MS/MS and RP-radio-TLC. 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. From the analytical methods used, no degradation of test item could be observed throughout the study, and the mineralization rate was negligible under tested conditions. On the other hand, the fast degradation of the reference item (> 90 % AR) within 14 days proofed that the test system was viable. The dissipation times (DT_{50} and DT_{90}) of the test item were calculated. For each concentration single first order (SFO) was the kinetic model with the best fit kinetic. Thereby, 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 optimized degradation parameters of metribuzin in total water and sediment system, 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. The resulting modelling endpoint, i.e. the geometric mean SFO-DT_{50} value of metribuzin in total system was 42.2 days, in water phase 31.2 days and in sediment phase 55.2 days, indicating slow primary degradation. Ultimate degradation of metribuzin, i.e. 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 is formed (M-016862-01-2, M-513735-01-1, M-537471-01-1).

The rate of degradation of metribuzin in soil under aerobic conditions was investigated in four studies (M-493037-01-1, M-030613-02-1, M-035363-01-1, M-537473-01-1, M-517209-01-1) using 13 soils. The normalised single first order laboratory DT_{50} values ranged from 4.73 to 12.5 days. A

geometric mean value of 7.1 days was established. The maximum not normalised laboratory DT_{50} value was 12.8 days. However, only one study and four soils were considered to comply with OECD Test Guideline 307 (M-517209-01-1). In this study the normalised laboratory DT_{50} values ranged from 5.98 to 7.72 days. A geometric mean value of 6.99 days was established. The maximum not normalised laboratory DT_{50} / DT_{90} value was 7.76/ 25.8 days. The results of anaerobic studies in soil (M-016937-02-1, M-016965-01-1) demonstrated that the degradation of metribuzin was even slower (first-order DT_{50} values of 109 and 439 days were determined), but not different under anaerobic conditions as compared to the aerobic environment.

Based on the results of degradation simulation studies in surface water and in water-sediment system metribuzin is considered to be not rapidly biodegradable for the classification purpose according to CLP criteria as the substance is demonstrated not to be ultimately degraded in a surface water nor in an aquatic sedimentwith a half-life of < 16 days.

11.1.4.4 Photochemical degradation

The photo-transformation of [5-^{13}C/^{14}C]metribuzin in soil was studied on thin layers of the sandy loam soil. Dose rate was 110 µg/g soil (dry substance). Irradiation of the soil samples was carried out in a soil photolysis module exposed to natural sunlight. Under irradiated conditions metribuzin was degraded with a half-life of 14.25 days. No degradation was observed in the dark control samples. The only metabolite observed was Desamino-metribuzin (DA-metribuzin) amounting to a maximum of 21.0 % of AR at the end of irradiation period (M-016898-01-1).

The environmental half-life of metribuzin in water has been estimated by simulation models and studied in sterile water. Model calculations resulted in environmental direct photolysis half-lives shorter than one day. Under the study conditions the experimental half-life of metribuzin in steril water was determined by liner regression analysis to be 4.34 hours indicating the rapid degradation of the parent compound under the influence of light. Thus the direct phototransformation in water contributes to the overall elimination of metribuzin from the environment to a great extent. In natural water, 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 in phototransformation studies. It can be concluded that solar radiation will significantly contribute to the degradation of metribuzin in aquatic environmental systems (M-024959-01-1, M-019848-01-1, M-019301-01-1).

Theoretical Atkinson calculation of the potential for photo-oxidation of metribuzin led to quite short DT_{50} values in the lower atmosphere of 7.03 hours under consideration of a 12-hours day at 1.5 106 OH radicals/cm^3, and 21 hours under consideration of a 24-hours day at 0.5 106 OH radicals /cm^3 (M-040702-01-1, M-513737-01-1).

11.2 Environmental transformation of metals or inorganic metals compounds

Not applicable.

11.2.1 Summary of data/information on environmental transformation

Not applicable.

11.3 Environmental fate and other relevant information

Vapour pressure values extrapolated (vapour pressure balance method, M-462079-01-1):
1.7×10^{-4} Pa for 20 °C,
3.4×10^{-4} Pa for 25 °C,
7.0×10^{-3} Pa for 50 °C.

For characterization of the adsorption of metribuzin the results obtained by different authors and 6 soil types were considered. The geometric mean Koc value of 48.3 mL/g and the arithmetic mean 1/n of 0.922 are proposed to be used for metribuzin.

Table 62: Summary of adsorption constants to be considered for exposure assessments of metribuzin:

Report/Article Ref.	1st Author (year)	Soil type	OC%	pH (measured in $CaCl_2$	K_f	K_{OC}	1/n
M-023902-01-1	Hein (2000)	Sandy loam	1.35	6.4	0.500	37.0	0.91
M-014973-01-1	Lenz (1979)	Sandy loam	1.62	6.6	1.320	81.0	0.92
		Silt loam	2.90	7.9	1.900	65.7	0.89
M-085111-01-1	Sneikus (2003)	Silt loam	0.83	6.5	0.303	36.5	0.92
		Silt	2.11	6.7	0.565	26.8	0.94
		Silty clay	1.00	6.0	0.657	65.7	0.95
Overall geometric mean						**48.3**	
Overall arithmetic mean							**0.922**

11.4 Bioaccumulation

Low potential for bioaccumulation as log Pow < 4.

11.4.1 Estimated bioaccumulation

No data available.

11.4.2 Measured partition coefficient and bioaccumulation test data

Partition coefficients n-octanol/water were determined according to OECD 117 (M-464868-01-1). The log Pow of 1.8 at all three pH values have been reported.

11.5 Acute aquatic hazard

Metribuzin is already classified as Aquatic Acute 1 with M-factor 10 based on growth inhibition studies of freshwater algae (*Pseudokirchneriella subcapitata, Desmodesmus subspicatus*) (M-042548-01-1, M-468327-01-1, M-456768-01-1) and freshwater aquatic plants (*Lemna* sp, *Myriophyllum spicatum*) (M-455636-01-1, M-663178-01-1).

A summary of available information on the acute aquatic toxicity of metribuzin is presented in Table 63. Studies were conducted according to internationally agreed standard test guidelines and conformed to GLP certification. In the following sections, executive summaries of the available studies on metribuzin are provided that give more detailed information on acute aquatic toxicity.

Table 63: Summary of acute aquatic studies

Method	Species	Test material (purity)	Exposure	Results[1]	Reference
Fish					
OECD 203 GLP	*Oncorhynchus mykiss*	Metribuzin (94.3 %)	96 hours, static	LC_{50} = 74.6 mg a.s./L (mm)	M-046241-01-1
OECD 203 GLP	*Oncorhynchus mykiss*	Metribuzin (95.3 %)	96 hours, static	LC_{50} = 80.3 mg a.s./L (nom)	M-513878-01-1
OECD 203 GLP	*Leuciscus idus melanotus*	Metribuzin (93.5 %)	96 hours, static	LC_{50} = 141.6 mg a.s./L (nom)	M-013913-01-2
OECD 203 GLP	*Leuciscus idus melanotus*	Metribuzin (95.3 %)	96 hours, static	LC_{50} = 169.4 mg a.s./L (nom)	M-513882-01-1

Method	Species	Test material (purity)	Exposure	Results[1]	Reference
FIFRA / ASTM GLP	*Cyprinodon variegatus*	Metribuzin (92.6 %)	96 hours, static	LC_{50} = 85 mg a.s./L (mm)	M-013993-01-1
OECD 203 GLP	*Cyprinus carpio*	Metribuzin (93.7 %)	96 hours, static	LC_{50} > 100 mg a.s./L (nom)	M-104023-01-1
Aquatic invertebrates					
OECD 202 GLP	*Daphnia magna*	Metribuzin (94.3 %)	48 hours, static	EC_{50} = 49.6 mg a.s./L (nom)	M-021792-04-1
OECD 202 GLP	*Daphnia magna*	Metribuzin (95.3 %)	48 hours, static	EC_{50} = 49.0 mg a.s./L (nom)	M-513889-01-1
Algae					
OECD 201 GLP	***Pseudokirchneriella subcapitata***	**Metribuzin (99.0 %)**	**72 hours, static**	**E_rC_{50} = 0.0265 mg a.s./L (nom)**	**M-042548-01-1 (recalculation of M-013856-01-1)**
OECD 201 GLP	***Desmodesmus subspicatus***	**Metribuzin (95.3 %)**	**72 hours, static**	**E_rC_{50} = 0.02657 mg a.s./L(1) (nom)**	**M-468327-01-1 (recalculation of M-493049-01-1)**
OECD 201 GLP	***Desmodesmus subspicatus***	**Metribuzin (93.9 %)**	**72 hours, static**	**E_rC_{50} = 0.0664 mg a.s./L (mm)**	**M-456768-01-1**
FIFRA §123-2 GLP	*Skeletonema costatum*	Metribuzin (94.2 %)	72 hours, static	E_rC_{50} = 0.0306 mg a.s./L(2) (nom)	M-043409-01-1 (recalculation of M-013735-01-1)
FIFRA §123-2 GLP	*Navicula pelliculosa*	Metribuzin (99.0 %)	72 hours, static	E_rC_{50} = 0.0429 mg a.s./L(3) (mm)	M-431222-01-1 (recalculation of M-013747-02-1)
OECD 201 GLP	*Anabaena flos-aquae*	Metribuzin (91.0 %)	96 hours, static	E_rC_{50} = 0.061 mg a.s./L (nom)	M-493055-01-1
Aquatic plants					
OECD 221 GLP	***Lemna gibba G3***	**Metribuzin (94.4 %)**	**7-days, semi static**	**E_rC_{50} = 0.0385 mg a.s./L (nom)**	**M-455636-01-1**
OECD 239 GLP	***Myriophyllum spicatum***	**Metribuzin tech. (91.9 % w/w)**	**14 days, semi static**	**E_rC_{50} (total shoot lenght) = 0.154 mg a.s./L E_rC_{50} (fresh weight) = 0.0457 mg a.s./L E_rC_{50} (dry weight) = 0.0313 mg a.s./L (nom)**	**M-663178-01-1**
ASTM E 1415-91 GLP	*Lemna minor*	Metribuzin (91.0 %)	7-days, semi static	EC_{50} = 0.0178 mg a.s./L (nom)	M-426036-01-1 (recalculation of M-513929-01-1)
OECD 221 GLP	*Lemna minor*	Metribuzin (93.9 %)	24 hours exposure on day 0, followed by 6 days in untreated growth	24h peak E_rC_{50} > 0.519 mg a.s./L (mm)	M-456786-01-1

Method	Species	Test material (purity)	Exposure	Results[1]	Reference
			media		
OECD 221 GLP	*Lemna minor*	Metribuzin (93.9 %)	Two 24 hours exposures on day 0 and day 1, followed by 5 days in untreated growth media	48h Peak E_rC_{50} > 0.1047 mg a.s./L (mm)	M-456787-01-1
Other aquatic organisms					
No guideline GLP	*Xenopus laevis*	Metribuzin (94.4 % w/w)	48 hours, static	48 h LC_{50} > 97.4 mg a.s./L (mm)	M-397568-01-1

Notes:
Studies, species and endpoints used in the aquatic hazard classification have been highlighted **in bold**.
mm refers to mean measured concentrations
nom refers to nominal concentration
(1) The original study (M-493049-01-1) reported an E_rC_{50} of 0.020 mg a.s./L and an E_bC_{50} of 0.030 mg a.s./L. Since growth rate endpoints are usually higher than biomass endpoints, it has been considered advisable to recalculate endpoints for this study using state of the art statistics (M-468327-01-1)
(2) This statement presents the recalculated E_rC_x values based on the original study data of a 120h static marine diatom growth inhibition test, where results are based on cell density. According to the current guideline on algae testing (OECD 201, 2011) the recommended response variable in the evaluation of algae study results is average specific growth rate (E_rC_x). Moreover, for EU requirements the standard test period is set to 72h.
(3) This statement presents the recalculated E_rC_x values based on the original study data of a 120h static freshwater diatom growth inhibition test, where results are based on cell density. According to the current guideline on algae testing (OECD 201, 2011) the recommended response variable in the evaluation of algae study results is average specific growth rate (E_rC_x). Moreover, for EU requirements the standard test period is set to 72h.

11.5.1 Acute (short-term) toxicity to fish

Six acute fish studies were submitted. The studies were conducted according to OECD Guideline 203 principles and were run under GLP conditions. All six studies were considered valid.

Study 1, M-046241-01-1

In a 96-hour static acute toxicity laboratory study, rainbow trout (*Oncorhynchus mykiss*, mean body length 3.7 cm, mean body weight 0.6 g) were exposed to a dilution water control and to nominal (mean measured) concentrations of 26.0 (24.8), 43.3 (39.7), 72.0 (68.2), 120 (113), and 200 (187) mg as/L in groups of 20 fish per test concentration. Fish were observed for mortalities and signs of intoxication four hours after the start of the exposure and then at 24, 48, 72 and 96 hours. Mean measured concentrations were between 97 % and 101 % of nominal values and were stable throughout the test. The results of this study are based on mean measured concentrations. The lowest lethal concentration (LLC) was 68.2 mg a.s./L and the lowest-observed-effect-concentration (LOEC) was 39.7 mg a.s./L. The minimum concentration causing 100% mortality (96h) was 113 mg a.s./L. The no-observed-effect-concentration (NOEC) was 24.8 mg a.s./L. In the LOEC fish showed the following symptoms: lying on the bottom of aquarium, vertical oriented, darkened coloration, swollen belly. The 96-hour LC_{50} based on mean measured concentrations was 74.6 mg a.s./L.

Study 2, M-513878-01-1

In a 96-hour static acute toxicity laboratory study, rainbow trout (*Oncorhynchus mykiss*, mean body length 4.9 cm, mean body weight 1.1 g) were exposed to a dilution water control and to nominal concentrations: 32, 58, 100, 180 and 320 mg as/L in groups of 7 fish per test concentration. Fish were observed for mortalities and signs of intoxication at 24, 48, 72 and 96 hours. Mean measured concentrations were between 88 and 96 % of nominal. The results are given based on nominal concentrations of the test substance. The test concentration of 32 mg/L caused no mortality or non

lethal effects. Therefore the NOEC was laid down as 32 mg/L. The LC_{50} after 96 h is 80.3 mg/L (nominal concentration).

Study 3, M-013913-01-2

In a 96-hour static acute toxicity laboratory study, golden orfe (*Leuciscus idus melanotus,* mean body weight. 1.86 g, mean body length: 6.01 cm) were exposed to a dilution water control and to nominal concentration of 18.9, 33.6, 59.7, 106.2 and 188.9 mg as/L in groups of 10 fish per test concentration. The fish were examined daily for symptoms of intoxication and mortality. All measured concentrations were between 80 % and 120 % of nominal and the test substance was stable under test conditions. Therefore, all reported results are related to nominal concentrations. The 96-hour LC_{50} of the test substance was determined to be 141.6 mg a.s./L with a range from 106.2 to 188.9 mg a.s./L. This range is derived from the two adjacent concentrations with a spacing factor of 1.778, in which 0 and 100 % mortality were observed. The LC_{50} (96 h) reported was reached after 48 hours of exposure. This study is considered as valid but with restrictions due to some shortcomings in reporting and the test species is not part of the species list of “recommended fish species” in the updated and adopted version of OECD 203 (update 2019). The values of LC_{50} of 141.6 mg a.s./L and NOEC of 18.9 mg a.s./L should be used with care.

Study 4, M-513882-01-1

In a 96-hour static acute toxicity laboratory study, golden orfe (*Leuciscus idus melanotus*, mean length: 6.0 cm, mean body weight: 1.4 g;) were exposed to a dilution water control and to nominal concentration of 32, 58, 100, 180, and 320 mg as/L in groups of 7 fish per test concentration. Mean measured concentrations were between 81 and 96 % of nominal. Therefore results are given based on nominal concentrations of the test substance. The test concentration of 58 mg/L (nominal concentration) caused no mortality or non-lethal effects. Therefore the NOEC was determined as 58 mg/L (nominal concentration). The LC_{50} after 96 h is 169.4 mg/L (nominal concentration). The study is regarded as valid, although the species golden orfe (*Leuciscus idus melanotus*) is not part of the species list of “recommended fish species” in the updated and adopted version of OECD 203 (update 2019).

Study 5, M-013993-01-1

In a 96-hour static acute toxicity laboratory study, sheepshead minnow (*Cyprinodon variegatus*, mean body length 22 mm, mean body weight 0.2 g) were exposed to a dilution water control and to nominal (mean measured) concentrations of 7.8 (8.3), 13 (13), 22 (23), 36 (36), 60 (60), and 100 (102) mg as/L in groups of 10 fish per test concentration. Fish were examined after 0, 24, 48, 72 and 96 hours of exposure. All measured concentrations were within ± 20 % of the nominal concentrations. The LC_{50} of metribuzin to sheepshead minnow has been calculated as 85 mg as/L based on measured concentrations.

Study 6, M-104023-01-1

In a 96-hour static acute toxicity laboratory study, common carp (*Cyprinus carpio*, mean body length 4.4 cm, mean body weight 1.04 g) were exposed to a dilution water control and to nominal concentrations of 3.13, 6.25, 12.5, 25, 50 and 100 mg as/L in groups of 10 fish per test concentration. Observations regarding mortality and any adverse sublethal effects resulting from the exposure to metribuzin were made at test initiation, hour 4 and daily thereafter. The measured concentrations ranged from 98.4 to 109 % of the nominal concentrations. Since all concentrations were within ± 20 % of the nominal concentrations biological results were based on nominal concentrations. Sublethal effects (i.e. dark fish and fish with partial loss of equilibrium) were observed in the test solutions with 50 and 100 mg a.s./L. The highest concentration with neither sublethal nor lethal test item effects, i.e. the NOEC was 25 mg a.s./L (LOEC = 50 mg a.s./L). The 96 hour LC_{50} was determined to be >100 mg a.s./L based on nominal concentrations.

11.5.2 Acute (short-term) toxicity to aquatic invertebrates

Two valid acute aquatic studies were submitted, both according to GLP.

Study 1, M-021792-04-1

In a 48-hour static acute toxicity laboratory study according to OECD 202, water flea (*Daphnia magna*) neonates were exposed to a dilution water control and to nominal concentrations of 0, 0.4. 1, 2.5, 6.25, 15.6, 39.1, 97.7 and 200 mg as/L in three replicates per treatment containing 10 daphnids each. The daphnids were observed for mobility and symptoms of toxicity after 24 and 48 hours of exposure. Analytical determination of metribuzin on days 0 and 2 revealed that all measured concentrations were between 80 % and 120 % of nominal (avarage 100.3 % on day 0 and 103 % on day 2) and the test substance was stable under test conditions. Therefore, all reported results are related to nominal concentrations. The study meets the validity criteria and the endpoints based on nominal concentrations are: EC_{50} 48 hours 49.6 mg a.s./ L, LOEC 39.1 mg a.s./L and NOEC 15.6 mg a.s./L.

Study 2, M-513889-01-1

In a 48-hour static acute toxicity laboratory study according to OECD 202, water flea (*Daphnia magna*) neonates were exposed to a dilution water control and to nominal concentrations of 10, 18, 32, 58 and 100 mg as/L in four replicates per treatment containing 5 daphnids each. The daphnids were observed for mobility and symptoms of toxicity after 24 and 48 hours of exposure. The measured concentrations of the active substance in all treatment solutions were between 88-94 % of nominal on day 0 and between 93-99 % of nominal on day 2 of the experiment, and all effect concentrations were therefore based on nominal concentrations of the test item. The study meets the validity criteria and the endpoints with respect to the immobilisation of *Daphnia magna* based on nominal concentrations are: EC_{50} 48 hours 49 mg a.s./ L and NOEC 48 hours 32 mg a.s /L.

11.5.3 Toxicity to algae or other aquatic plants

Effects of the toxicity of metribuzin were tested on five species of algae (all conducted according to OECD Guideline 201 or similar guideline FIFRA §123-2) and aquatic plants *Lemna gibba*, *Lemna minor* (OECD guideline 221 and similar guideline ASTM E 1415-91) and *Myriophyllum spicatum* (OECD guideline 239). All studies were run under GLP. All acceptable and supplementary studies are presented below.

Study 1, M-013856-01-1 and M-042548-01-1

In a 120-hour static study conducted according to OECD 201, the effect of metribuzin on the growth of the green alga *Pseudokirchneriella subcapitata* was investigated. The green algae were exposed to a dilution water control, a solvent control and to nominal concentrations of 2.5, 5.0, 10, 20 and 40 µg a.s./L, which corresponded to mean measured concentrations of 2.33, 4.69, 9.43, 18.2 and 36.5 µg 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. The mean measured concentrations of the test substance were 2.33, 4.69, 9.43, 18.2 and 36.5 µg a.s./L which represents 91 to 94 % of the nominal test concentrations.

The original study ran for a total exposure period of 5 days. To fulfill reporting requirements of this OECD guideline, 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 are: E_rC_{50} 72h 26.5 µg a.s./L, LOE_rC 72 h 5.0 µg a.s./L and NOE_rC 72 h 2.5 µg a.s./L. The recalculated data comply with the validity criteria of the OECD Guideline 201. No E_rC_{10} and E_rC_{20} was derived from this study.

Study 2, M-493049-01-1 and M-468327-01-1

In a 72-hour static study conducted according to OECD 201, the effect of metribuzin on the growth of the green alga *Desmodesmus subspicatus* was investigated. 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 treatment groups and the controls consisted of 3 replicates each containing 1.0 – 1.6 × 10^4 cells/mL at test initiation. The cell density was measured at the beginning of the test and every 24 h. The measured soncentrations 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. All effect concentrations were based on nominal concentrations. The study met the validity criteria and the endpoints based on nominal concentrations are: biomass growth E_bC_{10} (72 h) 10.0 µg/L, E_bC_{50} (72 h) 30.0 µg/L, NOEC (72 h) 3.2 µg/L; and growth rate E_rC_{10} (72 h) 4.0 µg/L, E_rC_{50} (72 h) 20.0 µg/L, NOEC (72 h) 3.2 µg/L.

However, here the E_rC_{50} values were lower than E_bC_{50} values and therefore the E_rC_{50} and NOE_rC values were derived from recalculations of the same data by using state of the art statistics. According to the recalculation results, the study is still valid if the criteria as given in the most recent version of OECD guideline 201 are applied. The recalculated endpoints are summarised below, results are based on nominal concentrations of the test item: growth rate E_rC_{50} 26.57 µg a.s./L, E_rC_{20} 9.06 µg a.s./L, E_rC_{10} 5.16 µg a.s./L, LOE_rC 3.20 µg a.s./L, NOE_rC 1.00 µg a.s./L and biomass growth E_yC_{50} 12.79, E_yC_{20} 6.12 µg a.s./L, E_yC_{10} 4.16 µg a.s./L, LOE_yC 10.00 µg a.s./L, NOE_yC 3.20 µg a.s./L. In conclusion, the endpoint recalculation which was based on the original study data delivered a 0-72 h E_rC_{50} of 26.57 µg a.s./L, E_rC_{10} 5.16 µg a.s./L and the corresponding NOE_rC was calculated to be 1.00 µg a.s./L.

Study 3, M-456768-01-1

In a 72-hour static study conducted according to OECD 201, the effect of metribuzin on the growth of the green alga *Desmodesmus subspicatus* was investigated. The green algae were exposed to a dilution water control and to nominal concentrations of 7.27, 14.5, 29.1, 58.2 and 131 µg a.s./L, which corresponded to initial measured concentrations of 5.58, 11.6, 24.4, 49.9 and 142 µg a.s./L. The treatment groups consisted of 3 replicates per each concentration and the controls consisted of 6 replicates each containing 5,000 cells/mL at test initiation. The cell density was measured at the beginning of the test and every 24 h. Measured concentrations ranged between 76.8 and 109 % of nominal values at test start and between 78.6 and 95.9 % of the nominal values at the end of the exposure period. Concentrations at test end ranged between 88.3 and 102 % of the measured initial concentrations, indicating that the metribuzin concentrations were maintained over the test period. The test was evaluated using the measured initial concentrations and the endpoints are: EC_{50} (yield) 25.6 µg a.s./L and EC_{50} (growth) 66.4 µg a.s./L; EC_{20} (yield) 16.2 µg a.s./L and EC_{20} (growth) 24.7 µg a.s./L; EC_{10} (yield) 12.7 µg a.s./L and EC_{10} (growth) 14.7 µg a.s./L; NOEC 11.6 µg a.s./L. The study met all validity criteria of the OECD guideline.

Study 4, M-013735-01-1 and M-043409-01-1 (supplementary)

In a 120-hour static study conducted according to guideline FIFRA §123-2 (similar to OECD 201), the effect of metribuzin on the growth of the marine diatom *Skeletonema costatum* was investigated. The diatoms were exposed to a dilution water control, a solvent control and to nominal concentrations of 6.25, 12.5, 25, 50 and 100 µg a.s./L, which corresponded to mean measured concentrations of 5.25, 10.6, 25.9, 46.0 and 90.0 µg a.s./L. The treatment groups and the controls consisted of 3 replicates each containing 1.0 × 10^4 cells/mL at test initiation. The cell density was determined at every 24 h. Measured concentrations at test initiation (day 0) ranged from 84-104% of nominal and at test termination (day 5) from 75-111% of the nominal concentrations. Based on initial measured concentrations 5-day exposure results are: EC_{25} = 22.2 µg a.i./L, EC_{50} = 31.0 µg a.i./L and NOEC = 10.6 µg a.i./L. The E_rC_x values based on the original study data of the 120 h static marine diatom growth inhibition test were recalculated to 72-h exposure based on nominal concentrations. The following results were obtained: E_rC_{50} (72-h) 30.6 µg a.s./L, LOE_rC (72-h) 25.0 µg a.s./L and NOE_rC 12.5 µg a.s./L. The study is considered to be supplementary because of

deviations: 14.1 fold biomass increase in the control over 72 h and the mean coefficient of variation for section-by-section specific growth rates in the control cultures = 65.8%.

Study 5, M-013747-02-1 and M-431222-01-1 (supplementary)

In a 120-hour static study conducted according to guideline FIFRA §123-2 (similar to OECD 201), the effect of metribuzin on the growth of the freshwater diatom *Navicula pelliculosa* was investigated. The diatoms were exposed to a dilution water control, a solvent control and to nominal concentrations of 2.5, 5.0, 10, 20 and 40 µg a.s./L, which corresponded to initial measured concentrations of 2.17, 4.38, 8.90, 18.7 and 36.05 µg a.s./L. The treatment groups and the controls consisted of 3 replicates each containing 3000 cells/mL at test initiation. The cell density was determined at every 24 h. Measured concentrations of the active substance at the beginning of the test were 87 – 93 %, and at the end of the test 89 – 94 % of the nominal test concentrations. The 5-day exposure results were based on initial measured concentrations of metribuzin: E_rC_{50} 11.9 µg a.s./L, E_rC_{25} 8.0 µg a.s./L and NOE_rC 8.90 µg a.s./L.

These E_rC_x values based on cell density were recalculated to the standard test period of 72 hours based on initially measured concentrations. The following results were obtained for the growth E_rC_{50} (72-h) 42.90 µg a.s./L, E_rC_{10} (72-h) 25.49 µg a.s./L , LOE_rC (72-h) 36.10 µg a.s./L and NOE_rC 18.60 µg a.s./L; and for the biomass E_yC_{50} (72-h) 16.20 µg a.s./L, E_yC_{10} (72-h) 12.88 µg a.s./L , LOE_yC (72-h) 18.60 µg a.s./L and NOE_yC 8.90 µg a.s./L. It can be stated that the recalculation clearly met the first two validity criteria (increase of biomass (≥ 16-fold) and sectional growth rates of controls (CV ≤ 35%)) while it slightly failed to meet the criterion on control variation (CV = 12% instead of ≤ 10%). The calculated 72 h E_rC_{50} value 42.90 µg a.s./L exceeded the highest tested concentration 36.1 µg a.i./L because extrapolated from experimental results. Due to these limitations, the study along with the recalculations is considered supplementary.

Study 6, M-493055-01-1 (supplementary)

In a 96-hour static study conducted according to OECD 201, the effect of metribuzin on the growth of the blue-green alga *Anabena flos-aquae* was investigated. The algae were exposed to a dilution water control and to nominal concentrations of 1.0, 3.2, 10, 32, 100 and 320 µg/L. The treatment groups consisted of 3 replicates per each concentration and the controls consisted of 6 replicates each containing 1.0×10^4 cells/mL at test initiation. The cell density was measured at the beginning of the test and every 24 h. The concentration of the active substance in the test solutions was analysed. Recovery rates of the active substance at the beginning of the test were 92 – 107 %, and at the end of the test 92 – 110 %. Since all deviations were below 20 % of nominal, all effect concentrations were based on nominal concentrations. The effects of metribuzin on algal growth after 96 hours of exposure, based on nominal concentrations, are as follows: E_bC_{50} (biomass) 52.0 µg/L, E_rC_{50} (growth) 61.0 µg/L, NOEC (growth/biomass) 3.2 µg/L. The study is supplementary because compliance with the validity criteria of the OECD TG 201 did not either reached or were not reported.

Study 7, M-455636-01-1

In a 7-day semi static toxicity laboratory study conducted according to OECD 221, the effect of metribuzin on the growth of the duckweed *Lemna gibba G3* was investigated. The plants were exposed to a dilution water control and to nominal concentrations of 0.205, 0.512, 1.28, 3.20, 8.00, 20.0 and 50.0 µg a.s./L. Treatment groups and the controls consisted of 3 replicates each containing 12 fronds per vessel at test initiation. Visual observations were made on study day 0, 1, 2, 3, 4, and 7, but day 0, 2, 4 and 7 were reported. 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. Thus all reported results were based on nominal concentrations of the test item metribuzin. The following results were obtained:

Endpoint (0-7 day)	Effect on mean growth rate of frond no. [µg a.s./L]	Effect on mean growth rate of total frond area of plants [µg a.s./L]	Effect on mean growth rate of biomass [µg a.s./L]
E_rC_{10} (CI 95 %)	5.9 (0.85-10.99)	6.78 (3.28-9.97)	5.06 (0.02-9.8)
E_rC_{20} (CI 95 %)	11.25 (3.43-17.7)	11.16 (6.79-14.87)	7.54 (0.17-13.03)
E_rC_{50} (CI 95 %)	38.5 (25.4 – 86.5)	29.0 (23.0 – 37.9)	16.1 (6.49 – 41.2)
LOE_rC	0.512	0.512	0.512
NOE_rC	0.205	0.205	0.205

In this study the experimental conditions of OECD TG 221 were adhered to and the validity criteria were met, as the frond numbers had a doubling time of 1.6 days in toxicant-free controls.

Study 8, M-663178-01-1

In a 14-day semi static toxicity laboratory study conducted according to OECD 239, the effect of metribuzin to the aquatic plant *Myriophyllum spicatum* was investigated. The plants were exposed to a dilution water control and to nominal concentrations of 1.03, 2.95, 8.46, 24.3, 69.7, 200 µg a.s./L. Treatment groups consisted of 4 replicates and the controls consisted of 6 replicates each containing 3 shoots per vessel. Visual assessments of plant health were made on day 0, 7 and 14 and root health on day 14. Determinations of total shoot length were made on days 0, 7 and 14. Only on day 7, red shoot tips were observed in treatment groups 1.03, 2.95 and 8.46 µg a.s./L, but not in the three highest test concentrations and the control. On day 14, the roots in all treatment groups were healthy and comparable to those in the control. Chemical analysis showed that in overlaying water the measured concentrations of fresh media (day 0/day 7) ranged between 90.5 and 123% of nominal. Measured concentrations of aged media (day 7/day 14) ranged between 88.7 and 110% of nominal. In pore water the measured concentrations ranged between 22.7 and 47.5% of the nominal test concentrations. In sediment metribuzin was found at concentrations > LOQ in all treatment groups except in the lowest concentration of 1.03 µg a.s./L and the control. Endpoints were calculated based on nominal concentrations as follows:

Endpoint (0-14 days)	Growth rate total shoot length [µg a.s./L]	Growth rate fresh weight [µg a.s./L]	Growth rate dry weight [µg a.s./L]
E_rC_{50} (95 % C.I.)	154 (120 – 212)	45.7 (38.4 – 54.5)	31.3 (24.8 – 39.5)
E_rC_{20} (95 % C.I.)	21.4 (15.0 – 28.1)	13.5 (9.83 – 17.1)	9.46 (6.12 – 12.8)
E_rC_{10} (95 % C.I.)	7.64 (4.32 – 11.4)	7.13 (4.61 – 9.78)	5.07 (2.78 – 7.52)
LOE_rC	8.46	24.3	8.46
NOE_rC	2.95	8.46	2.95

The validity criteria of OECD TG 239 were fulfilled, as control plants showed no signs of contamination or chlorosis, the coefficient of variation of control shoot fresh weight was 6.9% (<35% necessary), and control plants exhibited more than a doubling of both total shoot length and total shoot fresh weight.

Study 9, M-513929-01-1 and M-426036-01-1 (supplementary)

In a 14-day semi static toxicity laboratory study conducted according to ASTM E 1415-91, the effect of metribuzin on the growth of the duckweed *Lemna minor* was investigated. The plants were exposed to a dilution water control and to nominal concentrations of 0.58, 1.0, 1.8, 3.2, 5.8, 10, 18, 32, 58 and 100 µg/L. Treatment groups and the controls consisted of 3 replicates each containing 12

fronds per vessel at test initiation. Frond numbers were determined and observations of change in colour, break-up of plants and destructions of roots were made on days 7 and 14. Analytical results showed that metribuzin concentrations remained within ± 20 % of nominal over 3 days which represents the longest renewal interval. All effect values are based on the nominal test concentrations. The following results were obtained based on the growth rate: EC_{50} (7d) 17.8 µg/L, EC_{50} (14 d) 13.3 µg/L and EC_{50} (14 d, dry weight) 7.9 µg/L; NOEC (7d) 1.0 µ/L, NOEC (14 d) 0.58 µ/L and NOEC (14 d, dry weight) 0.58 µ/L. The experimental conditions deviated slightly from the requirements of OECD TG 221 (the control pH increased more than 1.5 units and the temperature range was 25 ± 2 °C instead of 24 ± 2 °C, test substance concentrations lack analytical verification, because the used concentrations remain below the limit of detection) and is considered to be supplementary.

Study 10, M-456786-01-1 (supplementary)

In a toxicity study conducted according to OECD 221, the effect of metribuzin on the growth of the duckweed *Lemna minor* was investigated. The plants were exposed to a dilution water control and to nominal concentrations of 13.1, 28.1, 60.4, 130, 279 and 600 µg a.s./L (which correspond to geometric mean measured concentrations of 2.38, 3.43, 13.4, 72.6, 192 and 519 µg a.s./L) for 24 hours on day 0, followed by 6 days in untreated growth media. Treatment groups consisted of 3 replicates and the controls consisted of 6 replicates each containing 12 fronds per vessel at test initiation. Visual observations of plants were made on study day 0, 1, 2, 5 and 7. No physical abnormalities were observed in any test concentrations during the study. Chemical analysis was performed at test start and at the end of the 24 hour exposure period. Since the measured concentrations deviated by more than 20% from nominal values, geometric mean measured concentrations were used for the evaluation. Seven day effects on *Lemna minor* after 24 h exposure to metribuzin (mean measured concentrations) based on frond number growth rate: E_rC_{50} >519 µg a.s./L and the lowest NOEC value for both growth rate and yield was determined as 3.43 µg a.s./L. Because the exposure regime did not adhere to OECD TG 221 requirements and the reasons for such deviations were not explained thoroughly, data from this study are considered supplementary.

Study 11, M-456787-01-1 (supplementary)

In a toxicity study conducted according to OECD 221, the effect of metribuzin on the growth of the duckweed *Lemna minor* was investigated. The plants were exposed to a dilution water control and to nominal concentrations of 5.0, 10, 20, 40, 80 and 160 µg a.s./L for the first 24 hours (first expoure) and to nominal concentrations of 2.3, 4.6, 9.2, 18.5, 36.9 and 73.9 µg a.s./L for next 24 hours (second exposure) followed by 5 days in untreated growth media. Corresponding geometric mean measured concentrations were for the first exposure peak (0-24 h): 1.0, 1.7, 7.82, 13.1, 44.6 and 104.7 µg a.s./L and for the second exposure peak (24- 48 h): 0.5, 1.01, 1.97, 4.72, 16.0 and 44.2 µg a.s./L. Treatment groups consisted of 3 replicates and the controls consisted of 6 replicates each containing 12 fronds per vessel at test initiation. Visual observations of plants were made on study day 0, 1, 2, 5 and 7. No physical abnormalities were observed for the 4 lowest treatments during the study. On day 2, all fronds were separated in the replicates of the 104.7 µg a.s./L test concentration, and on day 5 all fronds were partly light green in all replicates of the highest treatment level 160 µg a.s./L. Chemical analysis was performed at test start and at the end of the 24 hour exposure period. Since the measured concentrations deviated by more than 20% from nominal values, geometric mean measured concentrations were used for the evaluation. Seven-day effects on *Lemna minor* after 2x24 h exposure to metribuzin (mean measured concentrations): E_rC_{50} >104.7 µg a.s./L and the NOEC value for both growth rate and yield was determined as 13.1 µg a.s./L. The validity criteria of the guideline are fulfilled, but effect concentration values were not provided and the experimental setup did not adhere to OECD TG 221 conditions, nor was an explanation provided for such deviations. Therefore, this study is considered supplementary.

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

One non-standard acute toxicity study with African clawed frog (*Xenopus laevis*) is available for metribuzin which is considered supplementary.

Study M-397568-01-1 (supplementary)

The acute toxicity of metribuzin to African clawed frog (*Xenopus laevis*) was investigated in a 48 hour static study without a specific guideline followed. The frogs were exposed to a dilution water control and to nominal concentrations of 6.25, 12.5, 25, 50 and 100 mg a.s./L, which corresponded to mean measured concentrations of 5.77, 11.9, 24.1, 48.2 and 97.4 mg a.s./L. Recoveries on day 0 and day 2 were between 93 and 100% of nominal concentrations, results were based on mean measured concentrations. Treatment groups and the controls consisted of 3 replicates each containing 10 organisms per vessel. Observations for death and sub-lethal behavioural effects were made after 4, 24 and 48 hours. Sublethal effects were limited to organisms at the surface of the water and were noted during the 6 hour observations in the highest test concentration (97.4 mg a.s./L), and after 24 hours in the two highest test concentrations (48.2 and 97.4 mg a.s./L). No effects were seen after 48 hours; all organisms appeared normal. There were no mortalities in the control or in any test concentration during the study. The 48h-LC_{50} is determined to be >97.4 mg a.s./L based on the mean measured concentrations. Only fish-specific protocols were cited as guidelines for this study, e.g. OECD TG 203 and the study did not adhere to the guidelines to amphibian assays such as OECD TG 231. It remained unclear, which sublethal and behavioral effects were studied in addition to mortality. Due to the non-standard experimental setup and unspecified sublethal endpoints, this study is considered supplementary.

11.6 Long-term aquatic hazard

The substance is already classified as Aquatic Chronic 1, but no M-factor assigned for chronic effects. In this section relevant chronic studies are presented.

A summary of available valid information on the long-term aquatic toxicity of metribuzin is presented in Table 64. All studies below were conducted according to internationally agreed standard test guidelines and corresponding validity criteria were met for key studies.

Table 64: Summary of information on chronic aquatic toxicity

Method	Species	Test material (purity)	Exposure	Results	Reference
Fish					
OECD 210 US EPA OCSPP 850.1400 Non-GLP	*Oncorhynchus mykiss*	Metribuzin (94 %)	95 days, flow-through	EC_{10} = 4.432 mg a.s./L[(1)] (mm)	M-042516-01-1
OECD 210 GLP	*Pimephales promelas*	Metribuzin (93.9 %)	36 days, flow through	NOEC = 13.1 mg a.s./L (mm)	M-073884-01-1
Aquatic invertebrates					
OECD 211 GLP	*Daphnia magna*	Metribuzin (93.0 %)	21 days, flow-through	NOEC = 1.29 mg a.s./L EC_{10} = 1.44 mg a.s./L (mm)	M-654189-01 (recalculation of M-013774-01-1)
OECD 211 GLP	*Daphnia magna*	Metribuzin (95.3 %)	21 days, semi static	NOEC = 0.32 mg a.s./L (nom)	M-604351-01-1 (recalculation of M-513894-01-1)
Algae					
OECD 201 GLP	***Pseudokirchneriella subcapitata***	**Metribuzin (99.0 %)**	**72 hours, static**	**NOE_rC = 0.0025 mg a.s./L (nom)**	**M-042548-01-1 (recalculation of M-013856-01-1)**

Method	Species	Test material (purity)	Exposure	Results	Reference
OECD 201 GLP	***Desmodesmus subspicatus***	**Metribuzin (95.3 %)**	**72 hours, static**	**E_rC_{10} = 0.00516 mg a.s./L NOE$_r$C = 0.001 mg a.s./L[(2)] (nom)**	**M-468327-01-1 (recalculation of M-493049-01-1)**
OECD 201 GLP	*Desmodesmus subspicatus*	Metribuzin (93.9 %)	72 hours, static	E_rC_{10} = 0.0147 mg a.s./L NOE$_r$C = 0.0116 mg a.s./L (mm)	M-456768-01-1
FIFRA §123-2 GLP	*Skeletonema costatum*	Metribuzin (94.2 %)	72 hours, static	NOE$_r$C = 0.0125 mg a.s./L[(3)] (nom)	M-043409-01-1 (recalculation of M-013735-01-1)
FIFRA §123-2 GLP	*Navicula pelliculosa*	Metribuzin (99.0 %)	72 hours, static	E_rC_{10} = 0.02549 mg a.s./L NOE$_r$C = 0.0186 mg a.s./L[(4)] (mm)	M-431222-01-1 (recalculation of M-013747-02-1)
OECD 201 GLP	*Anabaena flos-aquae*	Metribuzin (91.0 %)	96 hours, static	NOE$_r$C = 0.0032 mg a.s./L (nom)	M-493055-01-1
Aquatic plants					
OECD 221 GLP	***Lemna gibba G3***	**Metribuzin (94.4 %)**	**7-days, semi static**	**E_rC_{10} = 0.0059 mg a.s./L NOE$_r$C = 0.000205 mg a.s./L (nom)**	**M-455636-01-1**
OECD 239 GLP	***Myriophyllum spicatum***	**Metribuzin tech. (91.9 % w/w)**	**14 days, semi static**	**E_rC_{10} (total shoot lenght) = 0.00764 mg a.s./L E_rC_{10} (fresh weight) = 0.00713 mg a.s./L E_rC_{10} (dry weight) = 0.00507 mg a.s./L NOE$_r$C = 0.00295 mg a.s./L (nom)**	**M-663178-01-1**
ASTM E 1415-91 GLP	*Lemna minor*	Metribuzin (91.0 %)	7/14-days, semi static	NOE$_r$C (7d) = 0.001 mg a.s./L NOE$_r$C (14d) = 0.00058 mg a.s./L (nom)	M-426036-01-1 (recalculation of M-513929-01-1)

Notes:
Studies, species and endpoints used in the aquatic hazard classification have been highlighted **in bold**.
mm refers to mean measured concentrations
nom refers to nominal concentration
(1)The endpoint of 4.4 mg a.s./L which has been recalculated (M-042516-01-1) is an EC_{10} value; 'NOEC' in the EU list of endpoints of the active substance metribuzin is a typo
(2)The original study (M-493049-01-1) reported an ErC50 of 0.020 mg a.s./L and an EbC50 of 0.030 mg a.s./L. Since growth rate endpoints are usually higher than biomass endpoints, it has been considered advisable to recalculate endpoints for this study using state of the art statistics (M-468327-01-1)
(3) This statement presents the recalculated ErCx values based on the original study data of a 120h static marine diatom growth inhibition test, where results are based on cell density. According to the current guideline on algae testing (OECD 201, 2011) the recommended response variable in the evaluation of algae study results is average specific growth rate (ErCx). Moreover, for EU requirements the standard test period is set to 72h.
(4) This statement presents the recalculated ErCx values based on the original study data of a 120h static freshwater diatom growth inhibition test, where results are based on cell density. According to the current guideline on algae testing (OECD 201, 2011) the recommended response variable in the evaluation of algae study results is average specific growth rate (ErCx). Moreover, for EU requirements the standard test period is set to 72h.

11.6.1 Chronic toxicity to fish

Two chronic fish studies were submitted and considered acceptable. Both were early life stage studies in accordance with OECD Guideline 210.

Study 1, M-042516-01-1

The early life stage toxicity of metribuzin to the rainbow trout (*Oncorhynchus mykiss*) was investigated under flow-through conditions for 95 days. The study was conducted at the nominal (mean measured) test concentrations of 3.2 (3.0), 6.3 (5.7), 13 (11.7), 25 (23.5) and 50 (48.0) mg as/L. The mean measured values ranged from 90 to 96 percent of nominal during the test period for all test levels. The reported results are related to the mean measured concentrations. Hatchability, survival rate and behaviour of embryos and fry were assessed throughout the study. Individual fish lengths and weights were measured on day 67 and at test termination.

Egg hatching began on day 27 and continued until day 32. There was no statistical difference in time to hatch in any test treatment compared to the pooled controls. Percent hatch was significantly different for the 3.0 mg/L level compared to the pooled control data. There was no dose-response relationship observed since no other test level showed any significant difference in hatchability. The difference observed in the 3.0 mg/L test level is probably due to biological variability and is not considered compound related.

Fry survival was analyzed on day 67 (36 days post-hatch) and on day 95 (64 days post hatch; study termination). On day 67 fry survival was 100 percent in all controls, 5.7 mg/L and 11.7 mg/L test levels. Day 67 survival was 72 and 80 percent in the 23.5 and 48.0 mg/L test levels, respectively. Survival on day 95 was 100 percent in all controls and the 5.7 mg/L test level. Day 95 survival was 53 and 2 percent in the 23.5 and 48.0 mg/L test levels, respectively.

Newly hatched fry began swimming up from the bottom of the test chambers on day 42 (10 days post-hatch). Swim-up was observed for a 14 day period between study day 42 and 56. 100 percent swim-up was achieved on day 45 in the controls, solvent controls, 3.0 mg/L, 5.7 mg/L and 23.5 mg/L test levels. During the post-hatch period morphological and behavioral effects observed included scoliosis, erratic movement, lethargy, darkened coloration, vertical orientation and surfacing. The majority of these anomalies were observed in the highest test concentration. The results of the statistical analysis showed that there was a significant difference in length between the pooled controls and the 3.0, 5.7 and 11.7 mg/L test levels. Statistical analysis of weight data for day 95 showed that there was a significant difference between the control and solvent control #2 data. Therefore, test treatment data were compared to solvent control #2 data. For weight, the results of the ANOVA and Dunnett's test showed no significant difference between the solvent control and the 3.0, 5.7 and 11.7 mg/L test levels.

The study meets the validity criteria and EC_{10} (based on body length) was determined as 4.432 mg a.s./L and NOEC 5.7 mg a.s./L based on mean measured concentrations.

Study 2, M-073884-01-1

The chronic toxicity of metribuzin to fathead minnow (*Pimephales promelas*) was determined in a flow-through early-life stage toxicity test with a study duration of 36 d (32 d post-hatch). The study was conducted at the nominal test concentrations of 1.4, 3.1, 6.8, 15.0 and 33.0 mg as/L. The mean measured concentrations of metribuzin were 1.3, 2.7, 6.0, 13.0 and 29.0 mg as/L, which ranged from 87 % to 90 % of nominal during the test period for all test levels. All reported results are related to the mean measured concentrations. Hatchability, survival rate and behaviour of embryos and fry were assessed throughout the study. Individual fish lengths and weights were measured at test termination.

At the end of the exposure, two deformed fish were observed, one in a control and one in the 6.8 mg/L treatment level. On day 19 after start of the exposure, one deformed fish (exophthalmus) was observed in the nominal 33 mg/L treatment level. Since there was no dose dependency and the

number of deformed larvae was low, it is concluded that the test item has no effect on the deformity of the exposed early life-stages of fathead minnow.

The test item had no statistically significant negative effect on egg survival, time of hatch, and hatchability. However, post-hatch success (day 32 post-hatch), individual wet and dry weight, and length of the fish were statistically significantly reduced at the highest treatment level. Thus, based on mean measured concentrations, the NOEC (based on survival and growth) was determined as 13.1 mg a.s./L and the LOEC 29.0 mg a.s. /L. The LC_{50} (survival = 32d post-hatch success) was calculated to be 22 mg a.s./L. The study meets the validity criteria.

11.6.2 Chronic toxicity to aquatic invertebrates

Two chronic aquatic invertebrate studies were performed according OECD Guideline 211, both conformed to GLP and were considered acceptable.

Study 1, M-013774-01-1 and M-654189-01

A 21-day reproduction study was performed on metribuzin according to OECD guideline 211 using *Daphnia magna* (first instar <24 hours) under flow-through conditions. The study was conducted at the nominal test concentrations of 0.32, 0.63, 1.25, 2.5 and 5.0 mg a.s./L. The corresponding measured concentrations were 0.30, 0.65, 1.29, 2.62 and 5.74 mg a.s./L. The mean measured concentrations of test substance ranged from 94 to 115 % of nominal during the test period for all test levels. Treatement groups and diluted water and solvent controls consisted of 4 replicates each containing 10 daphnides. Survival of parent daphnids was monitored daily until release of first broods, after which observations for mortality and number of offspring produced were made three times in week. Observations for sublethal and behavioral effects were also made. The body length of parent daphnids from all test chambers was measured at test termination.

Number of offspring per adult per reproduction day decreased significantly at 2.62 and 5.74 mg a.s/L compared to controls. Time to first brood was significantly longer at the highest tested concentration (5.74 mg a.s/L) of metribuzin compared to controls. No adverse effects were identified for parental survival up to concentration 5.74 mg a.s/L. The summary of endpoints for *Daphnia magna* based on mean measured concentrations are:

Parameters	Survival and Time to First Brood	Number of offspring / parent / reproduction-day	Body length (parents)	Dry weight (parents)
NOEC [mg as/L]	2.62	1.29	> 5.74	> 5.74
LC_{50} [mg as/L]	> 5.74			

In addition, LOEC 2.62 mg a.s./L and MATC 1.84 mg a.s./L was determined. The validity criteria of the study were fulfilled as mortality of control parent animals was 3 % (<20 % required) and the mean number of living offspring was 110 and 94 in solvent control and control, respectively (>60 required). The study adhered to experimental condition requirements specified in OECD TG 211.

No EC_{10} and EC_{20} was derived from the initial study report. This deficiency was amended by the recalculation and the 21-day EC_{10} and EC_{20} (offspring per adult per repro day) were determined as 1.44 mg a.s./L and 1.885 mg a.s./L.

Study 2, M-513894-01-1 and M-604351-01-1

A 21-day reproduction study was performed on metribuzin according to OECD guideline 211 using *Daphnia magna* (first instar <24 hours) under semi static conditions. The study was conducted at the nominal test concentrations of 0.32, 1.0, 3.2, 10 and 32 mg a.s./L. The recovery rates of the test

substance were in the range of 90 - 111 % in fresh, and 83 - 98 % in aged test media. Thus, the measured concentrations were within ± 20 % of nominal, and all effect concentrations were based on nominal concentrations of the test item. Treatement groups and diluted water control consisted of 10 replicates each containing 1 daphnid. First appearance of juveniles was checked daily during the first nine days. Number of total juveniles and immobilisation of females was determined in all replicates every day. At every renewal of water winter eggs, eggs laid but not hatched, motility and appearance was recorded. Growth and appearance of the parental animals was visually compared to the control group. At the end of the test the total length of each *Daphnia* and the dry weight of the ten daphnids at each concentration and control were determined.

Significant effects on immobilisation, growth of parental animals and number of juveniles were found at nominal test concentrations of 1.0 to 32 mg/L. The summary of endpoints based on nominal concentrations are:

Endpoint	**Parameter**	**Concentration [mg/L]**
Reproduction	NOEC (21 d)	0.32
	LOEC (21 d)	1.0
	EC_{50} (21 d)	2.2
Mortality	EC_0 (21 d)	0.32
	EC_{50} (21 d)	3.2
	EC_{100} (21 d)	32

This study adheres to OECD TG 211 test condition requirements and fulfils all the validity criteria as mortality of the parent animals in control at the end of the test was 0 and mean number of living offspring produced per parent animal surviving in control at the end of the test was 165. No EC_{10} and EC_{20} was derived from this study. This deficiency was amended by the recalculation and the 21-day EC_{10} and EC_{20} as follows: number of juveniles EC_{10} 1.95[5] mg a.s./L and EC_{20} 2.57[6] mg a.s./L; immobilisation EC_{10} 0.75 and EC_{20} 1.29 mg a.s./L; lenght EC_{10} 3.52 mg a.s./L and EC_{20} 73.44 mg a.s./L; weight EC_{10} 0.29 mg a.s./L and EC_{20} 0.42 mg a.s./L.

11.6.3 Chronic toxicity to algae or other aquatic plants

Please see Table 63 and 64 and section 11.5.3.

11.6.4 Chronic toxicity to other aquatic organisms

Not available.

11.7 Comparison with the CLP criteria

11.7.1 Acute aquatic hazard

The substance has harmonised classification as Aquatic Acute 1 with M factor 10. The lowest ErC_{50} value of 0.0265 mg a.s./L was obtained in *Pseudokirchneriella subcapitata* Growth Inhibition Test. Metribuzin is not considered to be rapidly degradable and shows low potential to bioaccumulate because of log Kow value of 1.8. Therefore, the CLP classification for acute aquatic hazard remains unchanged as Aquatic Acute 1 with M factor 10 ($0.01 < ErC_{50} < 0.1$).

[5] EC_x estimates not robust because concentration/response relationship not stat. sig.

[6] ECx estimates not robust because concentration/response relationship not stat. sig.

11.7.2 Long-term aquatic hazard (including bioaccumulation potential and degradation)

The substance has harmonised classification as Aquatic Chronic 1. The lowest E_rC_{10} was 0.00516 mg a.s./L obtained in *Desmodesmus subspicatus* Growth Inhibition Test and the lowest NOE_rC 0.000205 mg a.s./L obtained in *Lemna* sp. Growth Inhibition Test. Available E_rC_{10} and NOEC values for fish and daphnia are not in the range of the classification criteria for Aquatic Chronic 1.
Metribuzin is regarded as not readily biodegradable based on available degradation studies. It shows low potential for bioaccumulation because of log Kow value of 1.8.
Considering the NOE_rC value of 0.000205 mg a.s./L that is in the range 0.0001 mg/l < NOEC ≤ 0.001 mg/l, the M-factor of 100 for chronic aquatic toxicity should be assigned.
The lowest E_rC_{10} value of 0.00516 mg a.s./L would lead to a classification of Aquatic Chronic 1 with an M-factor of 10. In this case a worst case approach has been considered and a more sensitive endpoint has been selected that leads to a more severe chronic classification for metribuzin.

11.8 CONCLUSION ON CLASSIFICATION AND LABELLING FOR ENVIRONMENTAL HAZARDS

The reliable and the most sensitive 7-day NOE_rC for *Lemna gibba G3* of 0.000205 mg/L should be used for chronic aquatic hazard classification. This is in the range 0.0001 mg/l < NOEC ≤ 0.001 mg/l, and since metribuzin is 'non-rapidly degradable' according to CLP criteria, the substance should be classified as:

Aquatic Acute Category 1 with an acute M-factor of 10

Aquatic Chronic Category 1 with a chronic M-factor of 100.

12 EVALUATION OF ADDITIONAL HAZARDS

12.1 Hazardous to the ozone layer

Not evaluated.

12.1.1 Short summary and overall relevance of the provided information on ozone layer hazard

Not evaluated.

12.1.2 Comparison with the CLP criteria

Not evaluated.

12.1.3 Conclusion on classification and labelling for hazardous to the ozone layer

Not evaluated.

13 ADDITIONAL LABELLING

Not applicable.

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15 ANNEXES

Confidential Annex I – List of References