MCL Report

Proposal for mandatory classification and labelling based on the retained CLP Regulation (EU) No. 1272/2008 as amended for Great Britain, Annex VI Part 2

Chemical name: Cinmethylin (ISO); rac-(1R,2S,4S)-1-methyl-4-(1-methylethyl)-2-[(2-methylphenyl)methoxy]-7-oxabicyclo[2.2.1]heptane

EC Number: 402-410-9

CAS Number: 87818-31-3

MCL Index Number: 603-093-00-1

Prepared by:

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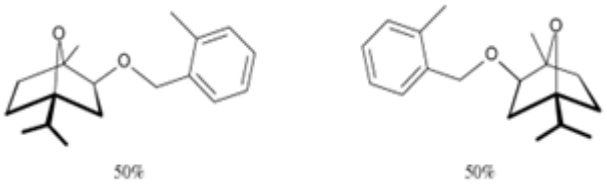
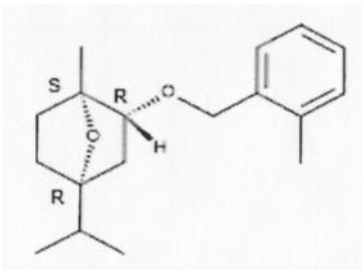
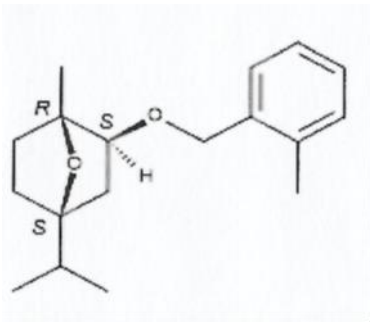
CONTENTS

1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

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

Name(s) in the IUPAC nomenclature or other international chemical name(s)	rac-(1R,2S,4S)-1-methyl-4-(1-methylethyl)-2-[(2-methylphenyl)methoxy]-7-oxabicyclo[2.2.1]heptane Also; exo-(±)-1-methyl-2-(2-methylbenzyloxy)-4-isopropyl-7-oxabicyclo(2.2.1)heptane
Other names (usual name, trade name, abbreviation)	BAS 684 H Luximo, Teqimo, Consuris
ISO common name (if available and appropriate)	Cinmethylin
EC number (if available and appropriate)	402-410-9
EC name (if available and appropriate)	exo-(±)-1-methyl-2-(2-methylbenzyloxy)-4-isopropyl-7-oxabicyclo(2.2.1)heptane
CAS number (if available)	87818-31-3
Other identity code (if available)	603-093-00-1
Molecular formula	$C_{18}H_{26}O_2$
Structural formula	
SMILES notation (if available)	Not available
Molecular weight or molecular weight range	274.40 g/mol

Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	Cinmethylin is a racemic mixture containing two enantiomers in approximately 50:50 ratio: (1S,2R,4R)-1-methyl-2-[(2-methylbenzyl)oxy]-4-(propan-2-yl)-7-oxabicyclo[2.2.1]heptane:  (1R,2S,4S)-1-methyl-2-[(2-methylbenzyl)oxy]-4-(propan-2-yl)-7-oxabicyclo[2.2.1]heptane: 
Description of the manufacturing process and identity of the source (for UVCB substances only)	Cinmethylin is not a UVCB substance.
Degree of purity (%) (if relevant for the entry in Annex VI)	*Min. 910 g/kg (pilot)* ***Min. 940 g/kg (full scale)***

1.2 Composition of the substance

Cinmethylin consists of two enantiomers, in approximately 50:50 ratio (Tables 1 and 2). Both enantiomers are biologically active, although in general the R (-) enantiomer is considered more biologically active than the S (+). All batches of cinmethylin used for toxicological studies had an enantiomeric ratio of approximately 50:50 except for two, which had an enantiomeric ratio of 70:30 R:S– the 28 day range findings study in rats and the developmental toxicity range finding study in rabbits.

There are two non-confidential toxicologically relevant impurities present in cinmethylin, (1SR,2RS,4RS)-1-methyl-4-(propan-2-yl)-7-oxabicyclo[2.2.1]heptan-2-ol and toluene. Both substances have mandatory classification and labelling (refer to Table 2).

However, noting the concentration at which they are present and the data available on cinmethylin, neither impurity is considered to contribute to the classification and labelling of the substance.

Table 2: Constituents

Constituent (Name and numerical identifier)	Concentration range (% w/w minimum and maximum in multi-constituent substances)	Current MCL in GB MCL List (GB CLP)
Enantiomer: (1S,2R,4R)-1-methyl-2-[(2-methylbenzyl)oxy]-4-(propan-2-yl)-7-oxabicyclo[2.2.1]heptane (no numerical identifier)	50 – 70 %	No mandatory classification and labelling
Enantiomer: (1R,2S,4S)-1-methyl-2-[(2-methylbenzyl)oxy]-4-(propan-2-yl)-7-oxabicyclo[2.2.1]heptane (no numerical identifier)	30 – 50 %	No mandatory classification and labelling

Table 3: Relevant impurities (non-confidential information)

Impurity (Name and numerical identifier)	Concentration range (% w/w minimum and maximum in multi-constituent substances)	Current MCL in GB MCL List (GB CLP)	
Impurity: (1SR,2RS,4RS)-1-methyl-4-(propan-2-yl)-7-oxabicyclo[2.2.1]heptan-2-ol EC: 402-470-6 CAS: 87172-89-2	4 g/kg (max)	Acute Tox. 4; H302 Eye Dam. 1, H318	
Impurity: Toluene EC: 601-021-00-3 CAS: 203-625-9	0.5 g/kg (max)	Flam. Liq. 2, H225 Skin Irrit. 2, H315 Asp. Tox. 1, H304 STOT SE 3, H336 STOT RE 2, H373 Repr. 2, H361d	

2 PROPOSED MANDATORY CLASSIFICATION AND LABELLING

2.1 Proposed mandatory classification and labelling under GB CLP

Table 4: Proposed mandatory classification and labelling

	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 entry in MCL List	603-093-00-1	cinmethylin (ISO); exo-(±)-1-methyl-2-(2-methylbenzyloxy)-4-isopropyl-7-oxabicyclo(2.2.1)heptane	402-410-9	87818-31-3	Acute Tox. 4* Aquatic Chronic 2	H332 H411	GHS07 GHS09 Dgr	H332 H411			
Proposed Classification	603-093-00-1	Cinmethylin (ISO); rac-(1R,2S,4S)-1-methyl-4-(1-methylethyl)-2-[(2-methylphenyl)methoxy]-7-oxabicyclo[2.2.1]heptane	402-410-9	87818-31-3	**Remove** Acute Tox 4* Aquatic Chronic 2 **Add** Skin Sens 1 STOT-SE 2 Aquatic Acute 1 Aquatic Chronic 1	**Remove** H332 H411 **Add** H317 H371 (Nervous system) H400 H410	GHS07 GHS08 GHS09 Dgr	**Remove** H332 H411 **Add** H317 H371 H410		Add M-factor(s): Aquatic acute, M-factor = 10 Aquatic chronic, M-factor = 1	
Proposed Entry in MCL List	603-093-00-1	Cinmethylin (ISO); rac-(1R,2S,4S)-1-methyl-4-(1-methylethyl)-2-[(2-methylphenyl)methoxy]-7-oxabicyclo[2.2.1]heptane	402-410-9	87818-31-3	Skin Sens 1 STOT-SE 2 Aquatic Acute 1 Aquatic Chronic 1	H317 H371 (Nervous system) H400 H410	GHS07 GHS08 GHS09 Dgr	H317 H371 H410		M-factor(s): Aquatic acute, M-factor = 10 Aquatic chronic, M-factor = 1	

Table 5: Reason for not proposing mandatory classification and status under public consultation

Hazard class	Reason for no classification	Within the scope of public consultation
Explosives	No classification, data conclusive but not sufficient for classification	Yes
Flammable gases (including chemically unstable gases)	Hazard class not applicable	No
Oxidising gases	Hazard class not applicable	No
Gases under pressure	Hazard class not applicable	No
Flammable liquids	No classification, data conclusive but not sufficient for classification	Yes
Flammable solids	Hazard class not applicable	No
Self-reactive substances	No classification, data conclusive but not sufficient for classification	Yes
Pyrophoric liquids	No classification, data conclusive but not sufficient for classification	Yes
Pyrophoric solids	Hazard class not applicable	No
Self-heating substances	No classification, data conclusive but not sufficient for classification	Yes
Substances which in contact with water emit flammable gases	No classification, data conclusive but not sufficient for classification	Yes
Oxidising liquids	No classification, data conclusive but not sufficient for classification	Yes
Oxidising solids	Hazard class not applicable	No
Organic peroxides	No classification, data conclusive but not sufficient for classification	Yes
Corrosive to metals	No classification, data lacking	Yes
Acute toxicity via oral route	No classification, data conclusive but not sufficient for classification	Yes
Acute toxicity via dermal route	No classification, data conclusive but not sufficient for classification	Yes
Acute toxicity via inhalation route	No classification, data conclusive but not sufficient for classification	Yes
Skin corrosion/irritation	No classification, data conclusive but not sufficient for classification	Yes
Serious eye damage/eye irritation	No classification, data conclusive but not sufficient for classification	Yes
Respiratory sensitisation	No classification, data lacking	Yes
Skin sensitisation	**Skin Sens. 1; H317**	Yes
Germ cell mutagenicity	No classification, data conclusive but not sufficient for classification	Yes
Carcinogenicity	No classification, data conclusive but not sufficient for classification	Yes
Reproductive toxicity	No classification, data conclusive but not sufficient for classification	Yes
Specific target organ toxicity-single exposure	**STOT-SE 2; H371 (Nervous system)**	Yes
Specific target organ toxicity-repeated exposure	No classification, data conclusive but not sufficient for classification	Yes
Aspiration hazard	No classification, data lacking	Yes

Hazard class	Reason for no classification	Within the scope of public consultation
Hazardous to the aquatic environment	**Aquatic Acute 1; H400, M-factor 10** **Aquatic Chronic 1; H410, M-factor 1**	Yes
Hazardous to the ozone layer	No classification, data lacking	Yes

3 HISTORY OF THE CLASSIFICATION AND LABELLING

Cinmethylin is a new pesticidal active substance in Great Britain (GB) under Regulation (EC) 1107/2009 as it has effect in Great Britain (hereinafter referred to as Regulation 1107/2009).

Cinmethylin is a new herbicidal active substance developed by the applicant (BASF). BASF provided a dossier in support of their application for the first approval of this pesticide in Great Britain in accordance with Regulation 1107/2009. No registrations or authorisations of cinmethylin-containing plant protection products currently exist in the UK or EU Member States, however, there is an authorisation for a product in Australia.

There is also an ongoing application for the approval of cinmethylin as a new active substance in the EU, with the evaluation being performed by the Netherlands as Rapporteur Member State (RMS) and France as co- Rapporteur Member State (co-RMS). The applicant has not provided details of any other evaluations by non-EU countries or international organisations, nor of any information exchange within the OECD. Furthermore, no other relevant EU-evaluations of the active substance have been carried out under other EU-legislation.

The substance is listed in the GB Mandatory Classification and Labelling List and in Annex VI of EU CLP with the following classification and labelling; Acute Tox 4* H332 (Harmful if inhaled) and Aquatic Chronic 2; H411 (Toxic to aquatic life with long lasting effects). The classification and labelling of this substance has not been considered by ECHA's Risk Assessment Committee (RAC) to date.

4 JUSTIFICATION THAT ACTION IS NEEDED

Cinmethylin is a new pesticidal active substance in the scope of Regulation 1107/2009. As such, it is subject to the mandatory classification and labelling process in accordance with Article 36 (2) and Article 37A of the retained CLP Regulation (EC) No. 1272/2008 as amended for Great Britain (hereinafter referred to as GB CLP). This report has been prepared by technical experts at HSE, acting in its capacity as the GB CLP Agency (hereinafter referred to as 'the Agency').

5 IDENTIFIED USES

Cinmethylin is a herbicide intended for use against annual weeds including black grass and Italian rye grass in cereals and oilseed rape.

6 DATA SOURCES

This MCL report relies exclusively on the data submitted in the context of the application for approval as an active substance under Regulation 1107/2009.

Draft assessment report (DAR): volume 3 (B1) Identity of the active substance (2020)

Draft assessment report (DAR): Volume 3 (B2) Physical Chemistry (2020)

Draft assessment report (DAR): Volume 3 (B6) Toxicology (2020)

Draft assessment report (DAR): Volume 3 (B8) Environmental fate (2020)

Draft assessment report (DAR): Volume 3 (B9) Ecotoxicology (2020)

Draft assessment report (DAR): Volume 4 Confidential information (2020)

At the time of preparation of this report, there is no REACH registration dossier for cinmethylin (October 2020).

7 PHYSICOCHEMICAL PROPERTIES

The physico-chemical properties of cinmethylin are summarised below. Reference should be made to the Draft Assessment Report – DAR – Volume 3, Annex B.2; Physical and Chemical properties – November 2020.

All studies were conducted to appropriate quality standards and are considered adequate and reliable.

Table 6: Summary of physicochemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
Physical state at 20°C and 101,3 kPa	Clear colourless liquid, faint fruity smell	Daum, A; 2015 DAR: B.2.3.1	Visual and olfactory inspection GLP Purity: 99.0 %
Melting/freezing point	No melting point observed. Product is liquid at room temperature. The solidification point was determined to -56 °C (pure) and -58 °C (technical material).	Kroehl, T; 2019 DAR: B.2.1.1	Measured OECD 102 GLP Purity: 98.6 % (pure), 96.2 % (technical)
Boiling point	Boiling Point = 330 °C (pure) Boiling Point = 323 °C (technical material	Kroehl, T; 2019 DAR: B.2.1.2	Measured OECD 102 GLP Purity: 98.6 % (pure), 96.2 % (technical)
Relative density	Density at 20 °C = 1.016 g/cm^3	Daum, A; 2015 DAR: B.2.14.1	Measured OECD 109 EC A3 GLP Purity: 99.0 %

<table>
<tr><th>Property</th><th>Value</th><th>Reference</th><th>Comment (e.g. measured or estimated)</th></tr>
<tr><td>Vapour pressure</td><td><table><tr><td>Temp (°C)</td><td>Vapour Pressure (Pa)</td></tr><tr><td>20</td><td>8.1×10^{-3}</td></tr><tr><td>25</td><td>1.5×10^{-2}</td></tr></table></td><td>Daum, A; 2015
DAR: B.2.2.1</td><td>Measured

OECD 104
EC A4
GLP

Purity: 99.0 %</td></tr>
<tr><td>Surface tension</td><td>At 90 % of the saturation solubility in pure water: 50.3 mN/m.</td><td>Daum, A; 2015
DAR: 2.12.1</td><td>Measured

OECD 115
EC A5
GLP

Purity: 99.0 %</td></tr>
<tr><td>Water solubility</td><td><table><tr><th>Solvent</th><th>Solubility (g/L)</th><th>pH (measured)</th></tr><tr><td>deionised water</td><td>0.069</td><td>8.9</td></tr><tr><td>buffer pH 4</td><td>0.063</td><td>4.1</td></tr><tr><td>buffer pH 7</td><td>0.058</td><td>7.0</td></tr><tr><td>buffer pH 9</td><td>0.062</td><td>9.0</td></tr></table>Results were obtained at 20 °C.
Low water solubility, not significantly affected by pH.</td><td>Daum, A; 2015
DAR: 2.5.1</td><td>Measured

OECD 105
EPA 830.7840
GLP

Purity: 98.9 %</td></tr>
<tr><td>Partition coefficient n-octanol/water</td><td>Cinmethylin log P_{ow} = 4.5 at 20°C and pH=5.8</td><td>Daum, A; 2015
DAR: B.2.7.1</td><td>Measured

OECD 117
EC A8
GLP

Purity: 99.0 %</td></tr>
<tr><td>Flash point</td><td>156.5 °C</td><td>Smeykal H; 2017
DAR: B.2.10.1</td><td>Measured

EC A9 (Pensky-Martens)
GLP

Purity: 93.0 %</td></tr>
<tr><td>Flammability</td><td>Refer to flash point</td><td>Smeykal H; 2017
DAR: B.2.9.1</td><td>Refer to flash point</td></tr>
</table>

Property	Value	Reference	Comment (e.g. measured or estimated)
Explosive properties	Determination of thermal stability via DSC measurement indicated that the maximum energy of the exothermic decomposition was below the threshold of 500 J/g. Therefore, no further tests for explosive properties were performed.	Smeykal H; 2017 DAR: B.2.11.1	Measured OECD 113 (DSC) (EC A14) GLP Purity: 93.0 %
Self-ignition temperature	The auto-ignition temperature was determined to be 375 °C atmospheric pressure.	Smeykal H; 2017 DAR: B.2.10.1	Measured EC A15 GLP Purity: 93.0 %
Oxidising properties	Not oxidising. The mean pressure rise time for cinmethylin is greater than the mean pressure rise time for the reference item nitric acid 65 %.	Smeykal H; 2017 DAR: B.2.13.1	Measured EC A21 GLP Purity: 93.0 %
Granulometry	No data	-	-
Stability in organic solvents and identity of relevant degradation products	No data	-	-
Dissociation constant	No dissociation was observed for cinmethylin in the range between pH 3.2 and pH 10.9 (no pKa can be determined).	Daum, A; 2017 DAR: B.2.8.1	Measured OECD 112 GLP Purity: 98.9 %

8 EVALUATION OF PHYSICAL HAZARDS

8.1 Explosives

Table 7: Summary table of studies on explosive properties

Method	Results	Remarks	Reference
OECD 113 (DSC) (EC A14 – explosive properties)	Not explosive In the DSC measurement, the maximum energy of exothermic decomposition was 50 J/g in the temperature range of 100 – 210 °C.	GLP	Smeykal, H; 2017 DAR: B.2.11.1

8.1.1 Short summary and overall relevance of the information provided on explosive properties

The maximum exothermic decomposition energy in a preliminary thermal stability study (DSC) was below the threshold of 500 J/g. As such, the criteria for classification as explosive are not met.

8.1.2 Comparison with the GB CLP criteria

Cinmethylin did not meet the criteria for classification as an explosive substance.

8.1.3 Conclusion on classification and labelling for explosive properties

Not classified – conclusive but not sufficient for classification

8.2 Flammable liquids

Table 8: Summary table of studies on flammable liquids

Method	Results	Remarks	Reference
EC A9	Flash point of 156.5 °C.	GLP	Smeykal, H; 2017 DAR: B.2.9.1

8.2.1 Short summary and overall relevance of the provided information on flammable liquids

The flash point of cinmethylin was determined to be 156.5 °C, according to EC A9 (Pensky-Martens closed cup) test method. According to the guidance on the application of the CLP criteria, a flammable liquid means a liquid having a flash point of not more than 60 °C. As such, cinmethylin does not meet the criteria for classification as a flammable liquid.

8.2.2 Comparison with the GB CLP criteria

Cinmethylin does not meet the criteria for classification as a flammable liquid.

8.2.3 Conclusion on classification and labelling for flammable liquids

Not classified – conclusive but not sufficient for classification

8.3 Self-reactive substances

Table 9: Summary table of studies on self-reactivity

Method	Results	Remarks	Reference
OECD 113 (DSC) (EC A14) -	In the DSC measurement, the maximum energy of exothermic decomposition was 50 J/g.	GLP	Smeykal, H; 2017 DAR: B.2.11.1

8.3.1 Short summary and overall relevance of the provided information on self-reactive substances

The maximum exothermic decomposition energy was 50 J/g (using DSC) in the temperature range of 100 – 210 °C. As the heat of decomposition is less than 300 J/g the substance does not need to be considered for this hazard class.

8.3.2 Comparison with the GB CLP criteria

Cinmethylin does not meet the criteria for classification as a self-reactive substance.

8.3.3 Conclusion on classification and labelling for self-reactive substances

Not classified – conclusive but not sufficient for classification

8.4 Pyrophoric liquids

8.4.1 Short summary and overall relevance of the provided information on pyrophoric liquids

Experience in manufacture and handling of cinmethylin shows that the liquid does not ignite spontaneously on coming into contact with air at room temperature.

8.4.2 Comparison with the GB CLP criteria

Cinmethylin does not meet the criteria for a pyrophoric liquid.

8.4.3 Conclusion on classification and labelling for pyrophoric liquids

Not classified – conclusive but not sufficient for classification

8.5 Self-heating substances

Table 10: Summary table of studies on self-heating substances

Method	Results	Remarks	Reference
Melting/freezing point	The substance is liquid at room temperature. The solidification point was determined to -56 °C (pure) and -58 °C (technical material).	Measured OECD 102 GLP Purity: 98.6 % (pure), 96.2 % (technical)	Kroehl, T; 2019 DAR: B.2.1.1
Self-ignition temperature EC A15	The auto-ignition temperature was determined to be 375°C.	A15 GLP	Smeykal, H; 2017

8.5.1 Short summary and overall relevance of the provided information on self-heating substances

The substance is a liquid at room temperature with a solidification point of -56 °C. As such, it is not classified as a self-heating substance.

8.5.2 Comparison with the GB CLP criteria

Cinmethylin does not meet the criteria for classification as a self-heating substance.

8.5.3 Conclusion on classification and labelling for self-heating substances

Not classified – conclusive but not sufficient for classification

8.6 Substances which in contact with water emit flammable gases

8.6.1 Short summary and overall relevance of the provided information on substances which in contact with water emit flammable gases

The chemical structure of cinmethylin does not contain metals or metalloids. In addition, experience in handling and use shows that cinmethylin forms a stable mixture with water.

8.6.2 Comparison with the GB CLP criteria

Cinmethylin does not meet the criteria for classification as a substance which in contact with water emits flammable gases.

8.6.3 Conclusion on classification and labelling for substances which in contact with water emit flammable gases

Not classified – conclusive but not sufficient for classification

8.7 Oxidising liquids

Table 11: Summary table of studies on oxidising liquids

Method	Results	Remarks	Reference
EC A21	No oxidising properties. The mean pressure rise time for cinmethylin is greater than the mean pressure rise time for the reference item, nitric acid 65 %.	GLP	Smeykal, H; 2017 DAR: B.2.13.1

8.7.1 Short summary and overall relevance of the provided information on oxidising liquids

The results of test method EC A 21 showed that the mean pressure rise time for cinmethylin was greater than that of the reference substance (65% nitric acid). Therefore, cinmethylin does not meet the criteria for classification as an oxidising liquid.

8.7.2 Comparison with the GB CLP criteria

Cinmethylin does not meet the criteria for an oxidising liquid.

8.7.3 Conclusion on classification and labelling for oxidising liquids

Not classified – conclusive but not sufficient for classification

8.8 Organic peroxides

8.8.1 Short summary and overall relevance of the provided information on organic peroxides

Cinmethylin does not contain the bivalent -O-O- structure and can therefore is not considered for classification in this hazard class.

8.8.2 Comparison with the GB CLP criteria

Cinmethylin does not meet the criteria for classification as an organic peroxide.

8.8.3 Conclusion on classification and labelling for organic peroxides

Not classified – conclusive but not sufficient for classification

8.9 Corrosive to metals

8.9.1 Short summary and overall relevance of the provided information on the hazard class corrosive to metals

There is no test data on cinmethylin with regards to its corrosivity to metals. However, the pH of cinmethylin is 8.9 at 20 °C and is therefore not considered extreme. Further, experience from handling and use indicates it is not corrosive to metals.

8.9.2 Comparison with the GB CLP criteria

There is no evidence to suggest that cinmethylin is corrosive to metals. As such, the criteria for classification are not met. However, a corrosivity test has not been carried out.

8.9.3 Conclusion on classification and labelling for corrosive to metals

Not classified – data lacking

9 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

The ADME of cinmethylin has been investigated in rats, via oral and iv dosing. Two data sets are available, a modern data set consisting of 3 studies (^{14}C-phenyl labelled cinmethylin and ^{14}C-cyclohexyl labelled cinmethylin) and an older dataset (^{14}C-phenyl labelled cinmethylin only). Also available is an in vitro comparative metabolism study using hepatocytes from humans, rats, dogs and rabbits exposed to ^{14}C-phenyl labelled cinmethylin and ^{14}C-cyclohexyl labelled cinmethylin. Together, these studies provide a thorough understanding of the ADME of cinmethylin in experimental animals following oral dosing.

Reference should be made to the Draft Assessment Report – DAR – Volume 3, Annex B.6; Toxicology and metabolism data – November 2020.

Table 12: Summary table of toxicokinetic studies

Method	Results	Remarks	Reference
In vivo toxicokinetic study in rats OECD 417 GLP Wistar rats 6/sex/dose ^{14}C-cinmethylin (labelled at cyclohexyl and phenyl rings) Chemical purity: 95.3 – 99 % Radiochemical purity: 97.9 – 99.4 % Dose: 1 mg/kg bw (i.v.), 15 or 350 (oral gavage) mg/kg bw	**Absorption (72 h)** **15 mg/kg bw/day**: Oral absorption: 98.5/87.5 % in males/females Tmax: 1 h **350 mg/kg bw/day:** Oral absorption: 91.24/91.73 % in males/females Tmax: 4 h **Distribution** Widely distributed – mainly liver, kidney, thyroid, adrenals and adipose tissue. **Excretion (168 h)** **15 mg/kg bw/day (single dose)**: 58/59 % in urine in males/females 38/33 % in faeces in males/females **350 mg/kg bw/day (single dose):** 58/52 % in urine in males/females 35/41 % in faeces in males/females **350 mg/kg bw/day (15 days, repeated dosing):** 57/60 % in urine in males/females 41/37 % in faeces in males/females **Toxicokinetics** Dose corrected AUC: 66/73 % in males/females	Rapid and complete oral absorption within 24 h – no sex or dose-related differences. High oral bioavailability. Rapid excretion (largely within 48 h), mainly by the urinary route – no sex or dose-related differences.	Anon, 2018a DAR: B6.1.1

<table>
<tr><th>Method</th><th>Results</th><th>Remarks</th><th>Reference</th></tr>
<tr><td>In vivo tissue distribution

OECD 417
GLP

Wistar rats
3-4/sex/dose

^{14}C-cinmethylin (labelled at cyclohexyl and phenyl rings)

Chemical purity: 95.9 and 97.9 %
Radiochemical purity: 97.9 and 99.6 %

Dose: 15 or 350 mg/kg bw

Route: oral gavage</td><td><u>Distribution of radioactivity in plasma and selected tissues at Tmax:</u>

<table><tr><th rowspan="3">Tissue</th><th colspan="2">15 mg/kg bw (Tmax = 1h)</th><th colspan="2">350 mg/kg bw (Tmax = 4h)</th></tr><tr><th>Males</th><th>Females</th><th>Males</th><th>Females</th></tr><tr><th colspan="4">% Dose#</th></tr><tr><td>Liver</td><td>8.4/7.1</td><td>7.7/9.6</td><td>2.2/2.4</td><td>2.3/1.7</td></tr><tr><td>Kidney</td><td>0.9/0.4</td><td>0.6/0.6</td><td>0.4/0.3</td><td>0.3/0.2</td></tr><tr><td>Plasma</td><td>0.5/0.5</td><td>0.7/0.6</td><td>0.5/0.4</td><td>0.3/0.2</td></tr><tr><td>Blood</td><td>0.15/0.13</td><td>0.17/0.16</td><td>0.24/0.15</td><td>0.16/0.08</td></tr><tr><td>Testes/ovaries</td><td>0.09/0.08</td><td>0.02/0.02</td><td>0.1/0.08</td><td>0.02/0.01</td></tr><tr><td>Abdominal fat</td><td>0.1/0.05</td><td>0.12/0.09</td><td>0.18/0.09</td><td>0.56/0.18</td></tr><tr><td>Muscle</td><td>0.04/0.04</td><td>0.04/0.05</td><td>0.06/0.05</td><td>0.08/0.04</td></tr><tr><td>Thyroid</td><td><0.01</td><td><0.01</td><td><0.01</td><td><0.01</td></tr></table>#% dose represents the two values obtained for each radiolabel – ^{14}C- phenyl/^{14}C-cyclohexyl label.</td><td>No significant sex, dose or label-specific differences in radioactive levels in investigated tissues, plasma and blood observed.</td><td>Anon, 2017a

DAR: B.6.1.1</td></tr>
<tr><td>In vivo Metabolism

OECD 417
GLP

Wistar rats
3-4/sex/dose

^{14}C-cinmethylin (labelled at cyclohexyl and phenyl rings)</td><td><u>Metabolism:</u>

Rapid and extensive metabolism in vivo in rats.

The main biotransformation steps of the metabolic pathway for cinmethylin are:

Hydroxylation at the cyclohexane and / or benzyl ring
Hydroxylation of the alkyl groups at the benzyl and / or cyclohexane ring
Oxidation of the hydroxylated methyl group at the benzyl ring to a carboxy group
Cleavage of the ether bridge
Conjugation with glucuronic acid
Conjugation with glycine

Conjugation with sulphate and glutathione and subsequent degradation of the glutathione conjugate were noted as minor pathways</td><td>No significant sex, dose or label specific differences were noted.</td><td>Anon, 2018b

DAR: B.6.1.1</td></tr>
</table>

Method	Results	Remarks	Reference
Chemical purity: 95.4 and 94.3-95.9 % Radiochemical purity: 98.9-99.6 and 97.9-99.4 % Dose: 15 or 350 mg/kg bw Route: oral gavage Metabolites identified from tissues harvested in previous study (Anon, 2017a)			
In vitro metabolism of cinmethylin in hepatocytes Non-guideline GLP Primary hepatocytes from: Humans (male and female donors) Wistar rats Beagle dogs New Zealand White rabbits ^{14}C-cinmethylin (labelled at cyclohexyl and phenyl rings) (10µM) Incubation: 10, 30, 60 and 180	Cell viability: 86 – 104 % Humans: unchanged cinmethylin was detected up to 60 min but none remained at 180 min. Faster metabolism was noted in rat, rabbit and dog hepatocytes compared to humans (cinmethylin was completely metabolised in these species within 60 min) No unique human metabolites were identified.	Positive controls behaved accordingly.	Funk-Weyer 2017 DAR: B.6.1.2

Method	Results	Remarks	Reference
min, 37°C, 5 % CO_2 Positive controls: 7-ethoxycoumarin and testosterone (10 µM)			

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

The toxicokinetics of cinmethylin have largely been investigated in rats via oral (p.o.) and intravenous (i.v.) dosing only. Three modern in vivo studies in rats and one in vitro study using human, rat, dog and rabbit hepatocytes are presented. Each study was conducted using ^{14}C-phenyl labelled and ^{14}C-cyclohexyl labelled cinmethylin. An older set of studies is available, conducted with ^{14}C-phenyl labelled cinmethylin only. Detailed summaries of these studies were not provided in the DAR but are taken into account in the overall summary below.

In vivo studies were carried out in male and female Wistar rats (n=3-6) using doses of 1 mg/kg bw (i.v.) (single dose), 15 mg/kg bw (p.o.) (single dose) or 350 mg/kg bw (p.o.) (single dose or 15 days repeated dosing).

Absorption

Cinmethylin was well absorbed from the gastrointestinal tract (75.4 % to 98.5 % of the administered dose) following doses of 15 and 350 mg/kg bw. The maximum plasma concentrations for oral administration, were achieved 1 hour after low dose (15 mg/kg bw) administration (both radiolabels) and 4 - 8 hours after high dose (350 mg/kg bw) administration (for the ^{14}C-phenyl and ^{14}C-cyclohexane radiolabels, respectively). Absorption appeared to be independent of dose, sex and position of the radiolabel.

Although uptake from the gastrointestinal tract was extensive, it appeared that post-hepatic systemic exposure to unchanged cinmethylin and/or its metabolites accounted for around 50 % of the administered dose, with a significant amount excreted in bile within 6 hours of gavage dosing (33.77 % to 56.72 % of the administered dose noted at the low dose and 12.56 % to 35.36 % of the administered dose at the high dose). It is possible that cinmethylin and/or its metabolites excreted into the bile within 6 hours of gavage dosing might not be systemically available.

However, there are relevant data available from a toxicokinetic experiment employing i.v. and orally dosed rats (DAR B6.1.1) which enable derivation of a more scientifically robust value for post-hepatic systemic bioavailability of cinmethylin based on the plasma AUCoral:AUCiv ratios. From this toxicokinetic experiment, dose corrected post-hepatic systemic bioavailability values of 66 % and 73 % for males and females respectively were obtained and an overall average value of 70 % can be calculated. This value is considered to be more robust and less uncertain than that derived from the bile-duct cannulation experiment. Overall, the Agency proposes a post-hepatic systemic bioavailability value of 70 %.

Distribution

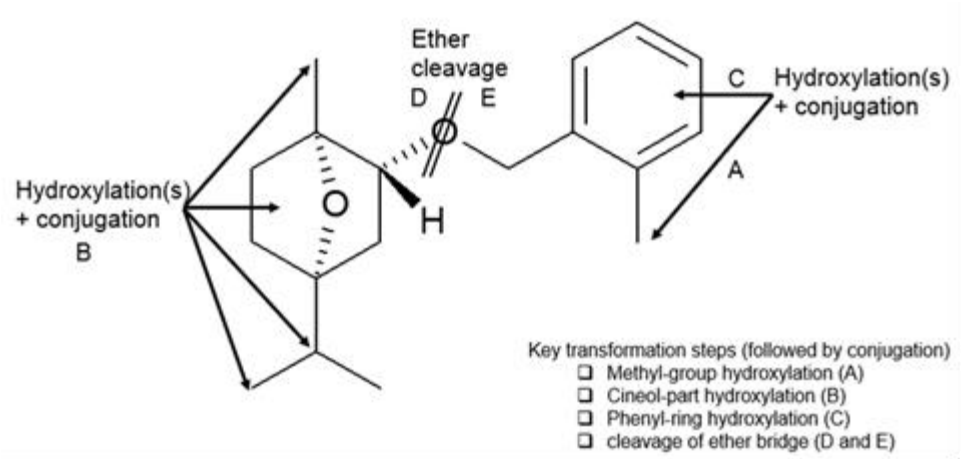
Distribution of radiolabelled cinmethylin and/or its metabolites was predominately to the organs of metabolism and excretion, the liver and kidneys and also the adrenals and adipose tissues. There was no evidence of retention in any organs/tissues at any dose level. Limited information on plasma concentrations of cinmethylin and four of its metabolites from lifetime toxicodynamic studies conducted in rats and mice confirmed that there is little or no detectable unchanged cinmethylin in blood plasma following repeated exposure (Anon 2018c and Anon 2018d). The levels of the metabolites in plasma were significantly higher than those of the parent in these studies.

Metabolism

In vivo toxicokinetic investigations of metabolism found that cinmethylin was rapidly and extensively metabolised such that there was no significant, post-hepatic exposure to unchanged cinmethylin. The main biotransformation reactions identified are:

- Hydroxylation at the cyclohexane and/or benzyl ring
- Hydroxylation of the alkyl groups at the benzyl and/or cyclohexane ring
- Oxidation of the hydroxylated methyl group at the benzyl ring to a carboxy group
- Cleavage of the ether bridge
- Conjugation with glucuronic acid
- Conjugation with glycine

Cinmethylin showing sites of key metabolic transformation reactions:



The ratio of the (-)/(+) enantiomers of unchanged cinmethylin in representative methanol extracts of liver and faeces shifted from 50:50 (-)/(+) in the starting material towards higher relative amounts of the (-) enantiomer and ranged from approximately 70:30 to 76:24 (-)/(+) in faeces extracts and from approximately 63:37 to 69:31 (-)/(+) in liver extracts. These data indicate some preferential metabolism for the (+) enantiomer.

Limited information from an in vitro comparative metabolism study employing primary hepatocytes from humans, rats, dogs and rabbits exposed to ^{14}C-phenyl labelled cinmethylin and ^{14}C-cyclohexyl labelled cinmethylin found no unique metabolites were formed by human primary hepatocytes.

Excretion

Excretion via both the urine and faeces was rapid, and essentially complete within 48 hours of oral dosing. There was no evidence for a preferential route, although excretion via the urine was slightly higher (52 – 60 % of the administered dose) than faecal excretion. Faecal excretion was mainly due to biliary elimination. The expired air did not appear to be a significant route of excretion for cinmethylin. There was no evidence to suggest dose or sex-dependent differences in urinary or faecal excretion. Comparing urinary excretion in non-bile duct cannulated animals with bile duct cannulated animals suggests that there is some enterohepatic recirculation but it was of relatively minor importance. There do not appear to be any clear differences between single high dose and repeated high dose animals for both ^{14}C cyclohexyl and ^{14}C-phenyl labelled cinmethylin, in males or females.

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

The earlier toxicokinetic studies, conducted with ^{14}C-phenyl labelled cinmethylin only do not contradict the conclusions on the toxicokinetics of cinmethylin from the more recently performed data set.

Overall, there were no findings from the toxicokinetic studies that might influence the proposed classification of cinmethylin. In general, cinmethylin was well-absorbed and fully eliminated. There was no evidence of tissue accumulation or the presence of any unidentified or toxic metabolites.

10 EVALUATION OF HEALTH HAZARDS

The applicant provided the following statement regarding the available data:

"The acute toxicity of BAS 684 H [cinmethylin] was already investigated between 1981-1988, partly according GLP. However, the current production process is different to that used in the 80s and registration in Asian countries requires GLP-compliant acute toxicity studies with the material produced under the current production process. Therefore, all acute studies had to be repeated for global registration. Those new/modern studies are now used for classification. For skin and eye irritation, the chosen approach was based on sequential *in vitro* followed by *in-vivo* testing to comply with global data requirements, including Regulation (EU) No 283/2013."

"The acute toxicity of BAS 684 H [cinmethylin] was already investigated between 1981-1988, partly according GLP. However, the current production process is different to that used in the 80s and especially registration in Asian countries requires acute GLP-conform toxicity studies with the material produced under the current production process. Therefore, all acute studies had to be repeated for global registration. Those new/modern studies are now used for classification, and at least for skin sensitisation resulted in the need for classification although two former studies did not indicate this property. For skin and eye irritation, the chosen approach was based on sequential *in vitro* followed by *in-vivo* testing to comply with global data requirements, including Regulation (EU) No 283/2013."

Acute toxicity

10.1 Acute toxicity - oral route

There are three well-conducted studies available to inform on the acute oral toxicity of cinmethylin, two in rats and one in mice. The first study in rats was conducted according to test guidelines and GLP, the second study in rats and the study in mice are older and pre-date OECD guidelines. Further information is available from a recently performed acute neurotoxicity study in rats. Reference should be made to the Draft Assessment Report – DAR – Volume 3, Annex B.6; Toxicology and metabolism data – November 2020.

Table 13: Summary table of animal studies on acute oral toxicity

Method, guideline, deviations if any	**Species, strain, sex, no/group**	**Test substance,**	**Dose levels, duration of exposure**	**Value** LD_{50}	**Reference**
Acute oral toxicity in rats OECD 423 (2001) GLP *Oral gavage* No deviations	Rat, Wistar 6 females (two groups of n=3 tested sequentially)	Cinmethylin Purity = 93.5 % Enantiomeric ratio: 48: 52 (-/+) No vehicle (undiluted material administered)	Limit dose of 2000 mg/kg bw	> 2000 mg/kg bw (females only)	Anon, 2016a DAR: Volume 3 – B.6.2.1
Acute oral toxicity in rats	Rat, Fischer 344 5/sex/dose	Cinmethylin Purity = 93.3 %	0, 1.0, 1.8, 3.2 and 5.6 mL/kg	4550 mg/kg bw (males and	Anon, 1982a DAR: Volume 3 – B.6.2.1

Method, guideline, deviations if any	**Species, strain, sex, no/group**	**Test substance,**	**Dose levels, duration of exposure**	**Value** **LD_{50}**	**Reference**
No guideline – similar to OECD 401 (1981) GLP *Oral gavage* Deviations to guideline included body weights of animals that died were not measured at the time of death and clinical signs of toxicity were not observed on day 10.		Enantiomeric ratio: not specified	(Equivalent to 1016, 1829, 3251 and 5690 mg/kg bw)	females combined)	
Acute oral toxicity in mice No guideline – similar to OECD 401 (1981) with no deviations. GLP *Oral gavage*	Mouse, B6C3F1 5/sex/dose	Cinmethylin Purity = not specified Enantiomeric ratio: not specified	Limit dose of 5 mL/kg (equivalent to 5072 mg/kg bw)	> 5072 mg/kg bw (males and females combined)	Anon, 1982b DAR: Volume 3 – B.6.2.1

Table 14: Summary table of other studies relevant for acute oral toxicity

Type of study/data	**Test substance,**	**Relevant information about the study (as applicable)**	**Observations**	**Reference**
Acute oral neurotoxicity study in rats OECD 424 (1997) with no deviations that impacted the validity of the study. GLP *Oral gavage*	Cinmethylin Purity = 93.5 % Enantiomeric ratio: 48:52 (-/+)	Rats, Wistar 10/sex/dose Doses: 0, 300, 1000 and 2000 mg/kg bw In CMC (0.5 %) + Tween®80 (3 drops/ 1000 mL) Observation period: 14 days	No treatment-related mortality up to 2000mg/kg bw **<u>Clinical signs, FOB and motor activity:</u>** ↑ Salivation ↓ Righting response ↓ Number of rearings ↓ Decreased motor activity in females from 1000 mg/kg bw and in males at 2000 mg/kg bw on day 0 only.	Anon, 2018e DAR: Volume 3 – B.6.7.1

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
			Histopathology: **2000 mg/kg bw:** ↑ Axonal degeneration in tibial (distal and proximal) and sciatic (proximal) nerves in females (minimal) ↑ Axonal degeneration in sciatic (proximal) nerves in males (minimal)	

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

In a recently conducted study by Anon 2016a, performed according to OECD 423 and GLP, two groups of 3 female Wistar rats received a limit dose of 2000 mg/kg bw cinmethylin by oral gavage. The study was performed in a stepwise manner as per the guideline, with the first group of rats being monitored for mortality and clinical signs before treating the second group. Animals were observed until study termination on day 14 and then subjected to macroscopic examination.

No mortality occurred during this study. Clinical signs included poor general state and piloerection. This occurred in the first group of animals from 3-5 hours after administration of cinmethylin and from 0-5 hours in the second group of animals. All animals were observed to have diarrhoea 2-5 hours following administration of cinmethylin, followed by reduced defecation on day 1. There were no adverse treatment-related changes to body weight and no macroscopic pathological findings at the end of the observation period.

The LD_{50} derived from this study in female rats is > 2000 mg/kg bw.

A second, older study in rats is available (Anon 1982a). Whilst the study was GLP-compliant, it was not conducted strictly according to guidelines but was found to broadly follow OECD 401 (1981). Deviations to the guideline included measurements of bodyweights were not recorded at the time of death and clinical signs of toxicity were not observed on day 10. These deviations are considered to be minor and do not affect the reliability of the study.

A dose range-finding study was initially conducted in order to determine the selection of doses used in the main study. Groups of Fischer rats (5/sex/dose) were administered a single oral dose of 1.0, 1.8, 3.2 and 5.6 mL/kg bw undiluted cinmethylin, equivalent to 1016, 1829, 3251 and 5690 mg/kg bw (calculated using a density of 1.016 g/mL). Control rats received 5.0 mL/kg bw deionised water. Observations were carried out hourly for a 6 hour period, at 24 hours and then twice daily until study termination on day 14.

At the top dose of 5690 mg/kg bw, four males and four females died on day 2. One female of the 3251 mg/kg bw dose group also died on day 3. Clinical signs observed in all dose groups included ataxia, hypoactivity, piloerection and lacrimation, occurring more frequently with increasing dose. At higher doses only, hypothermia prostration, hypotonus, pale extremities, ptosis, hypopnea, loss of righting reflex, depression of myotactic placing reflex, red discharge from nose and/or eye and a lack of pain response were also noted. There were no treatment-related effects on bodyweight. Observations at necropsy of unscheduled deaths were limited by generalised autolysis. One female in each of the 3246 and 5680 mg/kg bw dose groups showed slight fatty change of the liver that was considered treatment-related.

The LD_{50} for this study was found to be 4.49 mL/kg for both males and female rats (equivalent to 4550 mg/kg bw).

A study in mice is also available (Anon 1982b). Again, this study was not conducted according to guidelines but was found to be consistent with OECD 401 (1981) and was compliant with GLP.

A dose range-finding study was carried out using dose levels of 1.0, 2.5 and 5.0 mL/kg (equivalent to 1014, 2536 and 5072 mg/kg bw) in which no mice died. For the main study, a limit dose of 5072 mg/kg bw undiluted cinmethylin was administered to groups of five males and female B6C3F1 mice via oral gavage. Control mice received a dose of saline (5.0 mL/kg). Observations for clinical signs of toxicity were made hourly for a 6 hour period, at 24 hours and then twice daily thereafter until termination of the study on day 14.

Two deaths occurred overnight on the day of dosing. One female in the control group died due to incorrect gavage and one treated male died of undetermined cause. Clinical signs of toxicity included mucoid diarrhoea, polyuria, hypoactivity and hunched posture. These signs were observed within 1 hour of administration of cinmethylin with recovery occurring over 1 – 4 days. There were no adverse treatment-related changes in body weight. No remarkable changes were observed in any treated animal following necropsy.

The LD_{50} was determined to be > 5.0 mL/kg for both males and female rats (equivalent to > 5072 mg/kg bw).

Further information is available from an acute neurotoxicity study performed in Wistar rats (Anon 2018e). Animals (10/sex/dose) received a single dose of cinmethylin via oral gavage of 0, 300, 1000 or 2000 mg/kg bw and were observed for a period of 14 days. Clinical signs, body weight, functional observation battery (FOB) and motor activity (MA) were examined 7 days prior to dosing, on the day of administration and then every 7 days post-dosing. At termination, animals (5/sex/dose) were subject to neuropathological examinations.

There was no treatment-related mortality in this study and no adverse effects on bodyweight. Clinical signs and the results of the FOB and MA tests indicated treatment-related and adverse alterations in females from doses of 1000 mg/kg bw and in males of the top dose group. These findings included slight, transient salivation, retarded righting response, reduced numbers of rearings and decreased motor activity. Histopathology revealed 1/5 females of the top dose group with minimal axonal degeneration to the distal and proximal tibial nerves and the proximal sciatic nerve and 1/5 males with minimal axonal degeneration of the proximal sciatic nerve (further discussion of this is available in Section 10.11 – specific target organ toxicity following a single dose).

The LD_{50} in this study is > 2000 mg/kg bw for both males and females.

10.1.2 Comparison with the GB CLP criteria

The acute oral toxicity of cinmethylin was assessed in three well-performed studies in rats and mice. The results of all three studies consistently show that cinmethylin is of low acute oral toxicity with an LD_{50} of > 2000 mg/kg bw in both sexes of both species. In support of this is the findings from an acute neurotoxicity study performed in rats, in which no deaths occurred up to a dose of 2000 mg/kg bw.

In order to meet the criteria for classification in category 4, the lowest category for this endpoint, the LD_{50} must fall between the range $300 < ATE \leq 2000$ mg/kg bw. As this is not the case, no classification is proposed for acute toxicity following oral exposure.

10.1.3 Conclusion on classification and labelling for acute oral toxicity

No classification – conclusive but not sufficient for classification

10.2 Acute toxicity - dermal route

There are two studies available to inform on the acute dermal toxicity of cinmethylin, one in rats, conducted according to test guidelines and GLP and one in rabbits following a method similar to test guidelines.

Table 15: Summary table of animal studies on acute dermal toxicity

Method, guideline, deviations if any	**Species, strain, sex, no/group**	**Test substance,**	**Dose levels duration of exposure**	**Value LD_{50}**	**Reference**
Acute dermal toxicity in rats OECD 402 (1987) GLP *Semi-occlusive conditions* No deviations	Rat, Wistar 5/sex/dose	Cinmethylin Purity = 93.5 % Enantiomeric ratio: 48: 52 (-/+) No vehicle (undiluted material administered)	Limit dose of 5000 mg/kg bw 24 h exposure	> 5000 mg/kg bw (males and females combined)	Anon, 2016b DAR: Volume 3 – B.6.2.2
Acute dermal toxicity in rabbits No guideline – similar to OECD 402 (1981) GLP *Occlusive* Deviations to guideline include the use of an occluded dressing rather than a semi-occlusive dressing and an additional three animals of each were tested with abraded skin (results of these animals are not included in this table).	Rabbit, New Zealand White 3/sex/dose 4/sex/control group	Cinmethylin Purity = 93.3 % Enantiomeric ratio: unknown No vehicle (undiluted material administered)	Limit dose of 2029 mg/kg bw 24 h exposure	> 2000 mg/kg bw (males and females combined)	Anon, 1981a DAR: Volume 3 – B.6.2.2

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

In a GLP and OECD test guideline-compliant acute dermal toxicity study, groups of 5 female and 5 male Wistar rats were exposed to a single dermal dose of 5000 mg/kg bw undiluted cinmethylin (Anon, 2016b). The substance was applied to clipped skin on the dorsal and dorso-lateral areas of the trunk and covered with a semi-occlusive dressing for 24 hours. A check for dead or moribund animals was made at least once each workday. Animals were observed for clinical signs several times on the day of administration, and then at least once a day for a total of 14 days. Skin findings were scored 30 – 60 min after removal of the dressing, according to the Draize method, weekly thereafter and on the last day of observation. Individual body weights were determined shortly before administration (day 0), weekly thereafter and on the last day of administration. The animals were sacrificed and subjected to macroscopic examination on the last day of the observation period.

No mortality occurred in this study and there were no skin effects or signs of systemic toxicity in any animal. There were no adverse treatment-related effects on body weight and no macroscopic pathological abnormalities in animals examined at the end of the study.

The LD_{50} was > 5000 mg/kg bw in male and female rats.

A second, older study conducted in New Zealand white rabbits is available (Anon, 1981a). This study was not carried out according to guidelines or GLP, however the procedure was broadly consistent with OECD 402 (1981). Deviations to the guideline included the use of an occlusive dressing, rather than semi-occlusive and an additional 3 animals per sex had cinmethylin applied to abraded skin. These deviations are not thought to diminish the reliability of the study.

Cinmethylin (2029 mg/kg bw) was applied to the clipped skin of male and female rabbits (n=3/sex) and an occlusive dressing was applied for 24 hours. An additional three animals per sex were intentionally abraded and were exposed to the same dose of cinmethylin. The results of these animals are not included in this report as they are not relevant under GB CLP. At the end of the 24 hour period, the remaining substance was removed with a damp towel. Clinical signs were recorded several times on the day of administration, and twice daily thereafter for 14 days. At study termination, necropsy with gross pathology examinations were performed.

No mortality occurred in this study and there were no treatment-related clinical observations. Dermal reactions were limited to barely perceptible erythema and oedema at 24 hours post-dosing. The erythema was described as pale red in colour with definable edges and the oedema was definable, raised no more than 1 mm. All skin observations were found to have ceased by day 14.

The LD_{50} was > 2029 mg/kg bw in male and female rabbits.

10.2.2 Comparison with the GB CLP criteria

The acute dermal toxicity of cinmethylin has been investigated in two studies, one in rats and one in rabbits. Both show that cinmethylin is of low toxicity following dermal exposure with an LD_{50} > 5000 mg/kg bw in rats and > 2029 mg/kg bw in rabbits. In order to meet the classification criteria for category 4 for acute dermal toxicity, the LD_{50} must fall between 1000 < ATE ≤ 2000 mg/kg bw. As the results from neither study fulfil this criteria, no classification is proposed.

10.2.3 Conclusion on classification and labelling for acute dermal toxicity

No classification – conclusive but not sufficient for classification

10.3 Acute toxicity - inhalation route

Two acute inhalation toxicity studies in rats are available for cinmethylin. One modern, study following guidelines and GLP, using nose-only exposure and one older, non-guideline and non-GLP study, using whole-body exposure.

Table 16: Summary table of animal studies on acute inhalation toxicity

Method, guideline, deviations if any	**Species, strain, sex, no/group**	**Test substance, , form and particle size (MMAD)**	**Dose levels, duration of exposure**	**Value** LC_{50}	**Reference**
Acute inhalation toxicity in rats OECD 403 (2009) GLP *Nose-only* A minor deviation was that whilst the MMADs were within the recommended range, the geometric standard deviations (SD) were outside of the recommended range (1.5-3.0).	Rat, Wistar 5/sex/dose	Cinmethylin Purity = 93.5 % Enantiomeric ratio: 48: 52 (-/+) Liquid aerosol (mist) MMAD = 1.4 – 1.5 µm	Limit concentration of 5.268 mg/L 4 hours	> 5.268 (for both males and females)	Anon, 2017b DAR: Volume 3 – B.6.2.3
Acute inhalation toxicity in rats No guideline – similar to OECD 403 (1981) without deviations GLP *Whole-body*	Rat, Fischer 344 6/sex/conc.	Cinmethylin Purity = 91.8 % Enantiomeric ratio: unknown Liquid aerosol (mist) MMAD = 2.2-3.4 µm	0, 0.9, 2.2 and 3.5 mg/L 4 hours	> 3.5 mg/L (for both males and females)	Anon, 1986a DAR: Volume 3 – B.6.2.3

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

In a recently performed acute inhalation toxicity study (Anon, 2017b), groups of Wistar rats (5/sex) were exposed to a single, limit concentration of 5.268 mg/L cinmethylin (liquid aerosol, nose-only exposure, MMAD 1.4-1.5 µm) for 4 hours. Animals were then observed for 14 days.

No mortality occurred during the study period. General signs of toxicity were noted in some animals, these included changes in respiration, closed or semi closed eyelids, red discharge around the nose and piloerection. Also noted during the study was incidences of substance-contaminated fur. It is unclear why

this finding would occur in a nose-only exposure study. However; as there were no deaths at all during the course of the study, the finding makes little impact. There were no adverse treatment-related changes to body weight and no gross pathological abnormalities detected during necropsy at study termination.

Following inhalation exposure to cinmethylin, the LC_{50} was found to be > 5.268 mg/L in both male and female rats.

In a second, older acute inhalation toxicity study, also carried out in rats, animals (6/sex/concentration) underwent whole-body exposure to a liquid aerosol of cinmethylin (0, 0.9, 2.2 or 3.5 mg/L, MMAD 2.2 – 3.4 µm) for 4 hours (Anon, 1986a). The top exposure level was reported to be the maximum attainable concentration. Animals were observed for 14 days.

Two females of the top exposure level were found dead on days 3 and 4 respectively. No other mortalities were noted during the study period. Clinical signs included visibly wet fur and chewing and rubbing the face against the cage bottom. The wet fur had disappeared in most rats by day 3-4 but persisted in a few rats for the entire 14-day period. Males and females exposed to 3.5 mg/L showed either transient weight-loss during the first week, with gain in the second or body weight stagnation during the entire study period. Stagnation of weight gain was also noted in low and mid-dose concentration females during the first week, with a gain of weight noted in the second. These changes to body weight are considered treatment-related.

No treatment-related gross morphological lesions were observed in any animal. Of the females that died, one was found to have moderate autolysis and slight red crusting of the eyes. The other was shown to have a moderate decrease in fat deposits in adipose tissue, slight red crusting around the nostrils, minimal red crusting around the eyes and dark luminal contents in the small intestine. These effects did not appear to be specific effects relating to treatment with cinmethylin.

Two out of six females and 0/6 males were found dead after inhalation exposure to cinmethylin (3.5 mg/L). There were no deaths at lower doses. Therefore, the LC_{50} for both males and females is > 3.5 mg/L.

10.3.2 Comparison with the GB CLP criteria

Two studies are available to inform on the acute inhalation toxicity of cinmethylin. In the most recent study, no mortality occurred up to the limit dose of 5.268 mg/L and the LC_{50} was > 5.268 mg/L in male and female rats (nose-only exposure). In the second study, 2/6 (33 %) female rats died following exposure to the top concentration of 3.5 mg/L (whole-body exposure). This was reported to be the highest achievable concentration, however it is clear by the time the 2017 study was performed, higher concentrations were feasible. The LC_{50} derived in the second study was > 3.5 mg/L. Animals were noted to have wet fur following exposure that persisted until at least days 3-4. As animals were exposed to whole-body aerosols of cinmethylin, it is entirely plausible that, through grooming, animals received concentrations greater than the reported 3.5 mg/L.

Although a study using nose-only exposure seeks to rule out the effect of oral exposure through grooming, the Anon, 2017b study did indeed show that the animals had substance-contaminated fur. The reasons for this are unclear. However, neither study gave rise to an LC_{50} that was within the guidance limits for classification dusts and mists ($1.0 < ATE \leq 5.0$ mg/L). Therefore, no classification is proposed for this endpoint.

10.3.3 Conclusion on classification and labelling for acute inhalation toxicity

No classification – conclusive but not sufficient for classification

10.4 Specific target organ toxicity-single exposure

A possible target for toxicity, following a single dose of cinmethylin in animals, is the nervous system. The most relevant study for consideration of STOT-SE is an acute neurotoxicity study in rats (oral route). Further information is provided by two acute oral toxicity studies in rats (2016 and 1982) and two acute inhalation toxicity studies, also in rats (2017 and 1986). Supporting evidence is also available from the repeated dose toxicity studies.

Table 17: Summary table of animal studies on STOT SE

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results**	**Reference**
Acute oral neurotoxicity study in rats OECD 424 (1997) with no deviations that impacted the validity of the study. GLP Rats, Wistar 10/sex/dose	Cinmethylin Purity = 93.5 % Enantiomeric ratio: 48:52 (-/+) *Oral gavage* Doses: 0, 300, 1000 and 2000 mg/kg bw In CMC (0.5 %) + Tween®80 (3 drops/ 1000 mL) Observation period: 14 days	No treatment-related mortality. **<u>Clinical signs, FOB and motor activity (day 0 only):</u>** **<u>2000 mg/kg bw:</u>** ↑ Salivation males (1/10) and females (2/10) ↑ Laboured respiration: 3/10 males and 2/10 females (0 in controls) ↑ Unsteady gait: 2/10 females (0 in controls) ↓ Righting response: 2/10 males and 5/10 females (0 in controls) ↓ Number of rearings: N= 7 in females (versus N= 14 in controls) ↓ Decreased motor activity (sum of intervals 1-12): 49 %** in females **<u>1000 mg/kg bw:</u>** ↑ Salivation females only (1/10) ↓ Number of rearings: N= 7 in females (versus N= 14 in controls) ↓ Decreased motor activity (sum of intervals 1-12): 48.6 %** in females **<u>300 mg/kg bw:</u>** No adverse treatment-related findings **<u>Histopathology:</u>** **<u>2000 mg/kg bw:</u>** ↑ Axonal degeneration – see Table 18 for details	Anon, 2018e DAR: Volume 3 – B.6.7.1

Acute oral toxicity in rats No guideline – similar to OECD 401 (1981) Deviations to guideline included body weighs of animals that died were not measured at the time of death and clinical signs of toxicity were not observed on day 10. GLP Rat, Fischer 344 5/sex/dose Doses relevant for classification: STOT-SE 1: ≤ 300 mg/kg bw STOT-SE 2: 2000 ≤ C > 300 mg/kg bw	Cinmethylin Purity = 93.3 % Enantiomeric ratio: not specified *Oral gavage* 0, 1.0, 1.8, 3.2 and 5.6 mL/kg (Equivalent to 1016, 1829, 3251 and 5690 mg/kg bw) Observation period: 14 days	LD_{50} = 4550 mg/kg bw (males and females combined) **≤ 1000 mg/kg bw/day:** No adverse treatment-related findings **Clinical signs (all observed within first 24 h after dosing):** **5.6 mL/kg (5690 mg/kg bw):** ↑Ataxia: 5/5 males and females (versus 0 in controls) ↑Hypoactivity: 5/5 males and females (versus 0 in controls) Depression of myotactic placing reflex: 2/5 males and 4/5 females (not observed in controls) Loss of righting reflex: 3/5 females (not observed in controls) **3.2 mL/kg (3251 mg/kg bw):** ↑Ataxia: 5/5 males and females (versus 0 in controls) ↑Hypoactivity: 4/5 males and females (versus 0 in controls) Depression of myotactic placing reflex: 2/5 males and 2/5 females (not observed in controls) Loss of righting reflex: 1/5 females (not observed in controls) **1.8 mL/kg (1829 mg/kg bw):** ↑Ataxia: 4/5 males and females (versus 0 in controls) ↑Hypoactivity: 5/5 males and 4/5 females (versus 0 in controls) Depression of myotactic placing reflex: 1/5 males and 1/5 females (not observed in controls) Loss of righting reflex: 1/5 females (not observed in controls) **1.0 mL/kg (1016 mg/kg bw):** ↑Ataxia: 3/5 males and 4/5 females (versus 0 in controls) ↑ Hypoactivity: 4/5 males and females (versus 0 in controls)	Anon, 1982a DAR: Volume 3 – B.6.2.1

Acute inhalation toxicity in rats OECD 403 (2009) GLP Rat, Wistar 5/sex/dose Doses relevant for classification: STOT-SE 1: ≤ 1.0 mg/L/4h STOT-SE 2: 5.0 ≤ C > 1.0 mg/L/4h	Cinmethylin Purity = 93.5 % Enantiomeric ratio: 52: 48 (-/+) *Inhalation – nose-only* Liquid aerosol (mist) MMAD = 1.4 – 1.5 µm Limit concentration of 5.268 mg/L 4 hours	LC_{50} > 5.268 mg/L (for both males and females) **5.268 mg/L:** ↑ Hunched posture: 2/5 males and 1/5 females (day 0 only) (versus 0 in controls) ↑ Plough nose-first into bedding: 1/5 males and 4/5 females (day 0 only) (versus 0 in controls) ↑ Substance contaminated fur: 5/5 males (day 0) and 5/5 females (day 0-4)	Anon, 2017b DAR: Volume 3 – B.6.2.3
Acute inhalation toxicity in rats No guideline – similar to OECD 403 (1981) without deviations GLP Rat, Fischer 344 6/sex/conc. STOT-SE 1: ≤ 1.0 mg/L/4h STOT-SE 2: 5.0 ≤ C > 1.0 mg/L/4h	Cinmethylin Purity = 91.8 % Enantiomeric ratio: unknown *Inhalation – whole-body* Liquid aerosol (mist) MMAD = 2.2-3.4 µm 0, 0.9, 2.2 and 3.5 mg/L 4 hours	LC_{50} > 3.5 mg/L (for both males and females) **3.5 mg/L:** ↑ Mortality: 2/6 females (day 3 and 4) ↑Wet fur: 6/6 males (day 0-9) and 6/6 females (day 0-3) ↑ Burrowing: 6/6 males (day 0) and 6/6 females (day 0) ↑ Hunched posture: 1/6 males (day 4-10) and 2/6 females (day 1-2) ↑ Tip toe gait: 1/6 males (day 9) and 2/6 females (day 1) ↑ Hypoactivity: 1/6 males (day 2-9) and 2/6 females (day 1-3) ↑ Prostration: 1/6 females (day 2-3) **2.2 mg/L:** ↑ Hunched posture: 1/6 females only (day 1) ↑ Tip toe gait: 1/6 females only (day 1)	Anon, 1986a DAR: Volume 3 – B.6.2.3

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

The acute toxicity studies carried out by the oral and inhalation routes are summarised in section 10.1 (refer to tables 13 and 16).

In an acute neurotoxicity study performed in Wistar rats (Anon 2018e), there were no treatment-related effects on mortality. Slight, transient salivation was observed in the 1,000 (in 2/10 males and 1/20 females) and 2,000 mg/kg bw (in 1/10 males and 2/10 females) dose groups immediately after dosing. The lack of dose response in males, the transient appearance and immediate response in males and females indicates

that this is a reaction to bad taste or local effect on the upper digestive tract, rather than an indication of neurotoxicity.

Signs relating to neurotoxicity were observed in animals from a dose of 1000 mg/kg bw on day 0 only. At this dose, signs were limited to a reduced number of rearings (N = 7 versus 14 in controls) and a statistically significant decrease in motor activity, particularly during intervals 1-4, leading to reduced motor activity in the sum of intervals 1-12 of 48.6% (females only). These findings were also observed to a similar degree in top dose females (2000 mg/kg bw). In addition, laboured respiration was observed (3/10 males and 2/10 females), 2/10 females were noted to have an unsteady gait and a retarded righting response was noted in 2/10 and 5/10 females of the top dose group.

Histopathological examination revealed an increase in incidence in axonal degeneration in single fibres of the tibial and sciatic nerves at the top dose of 2000 mg/kg bw only (Table 18). In females, degeneration of the distal tibial nerve was observed in a single animal (severity grade 1 - minimal). No control animals were affected and the relevant historical control data showed no incidences of female rats with this finding, although single incidences in males have occurred. In the absence of any findings like this in control females, the distal tibial nerve degeneration is considered related to treatment. The same female was also observed to have axonal degeneration of the proximal tibial nerve (severity grade 1 – minimal) and the proximal sciatic nerve (severity grade 2 - slight). The HCD showed no females with degeneration of the proximal tibial nerve; therefore, this finding is also considered related to treatment. A second female was observed to have axonal degeneration of the proximal sciatic nerve (severity grade 1). According to the HCD, degeneration of the proximal sciatic nerve has been seen in females (2/40 females from 8 studies, mean severity grade of 1).

The applicant notes that the pathologists did not consider these findings as treatment-related effects as the incidence and severity were only slightly higher than in control animals.

However, in the opinion of the Agency, the observation of more than one female in a single study with sciatic nerve degeneration, together with the increased severity (grade 2 – minimal) means the finding is likely related to treatment with cinmethylin.

Table 18: Incidence of axonal degeneration in the tibial and sciatic nerves of male and female Wistar rats following a single oral dose of cinmethylin.

Sex		**Males**				**Females**			
Dose [mg/kg]		**0**	**300**	**1000**	**2000**	**0**	**300**	**1000**	**2000**
Tibial nerve, distal	# examined	5	5	5	5	5	5	5	5
- Axonal degeneration	Grade 1	1	1	-	1	-	-	-	1[#]
	[mean][$]	*[1.0]*	*[1.0]*	*[0.0]*	*[1.0]*	*[0.0]*	*[0.0]*	*[0.0]*	*[1.0]*
	HCD[§]	1/40 (0 – 1); *[2.0]*				-			
Tibial nerve, proximal	# examined	5	5	5	5	5	5	5	5
- Axonal degeneration	Grade 1	-	-	-	-	-	-	-	1[#]
	[mean][$]	*[0.0]*	*[0.0]*	*[0.0]*	*[0.0]*	*[0.0]*	*[0.0]*	*[0.0]*	*[1.0]*
	HCD[§]	1/40 (0 – 1); *[1.0]*				-			
Sciatic nerve, proximal	# examined	5	5	5	5	5	5	5	5
- Axonal degeneration	Grade 1	-	-	-	1	-	-	-	1
	Grade 2	-	-	-	-	-	-	-	1[#]
	[mean][$]	*[0.0]*	*[0.0]*	*[0.0]*	*[1.0]*	*[0.0]*	*[0.0]*	*[0.0]*	*[1.5]*

Sex		Males				Females			
Dose [mg/kg]		0	300	1000	2000	0	300	1000	2000
	HCD§	1/40 (0 – 1); *[1.0]*				2/40 (0 – 1); *[1.0]*			

$ *[ ]* mean severity grading; histopathological findings were graded minimal (Grade 1), slight (Grade 2), moderate (Grade 3), marked (Grade 4) and massive/severe (Grade 5). The mean severity is the sum of the gradings divided by the incidence

finding of the same animal #48

§ historical control data (HCD) = total incidence, study range in brackets and the mean severity in square bracket. HCD based on 8 acute oral neurotoxicity studies in Wistar rats performed at the same laboratory in a period between 2011 – 2016 under GLP conditions.

Clinical signs that could potentially be related to neurotoxicity were observed in one acute oral toxicity study and two inhalation studies, all in rats.

In an acute oral toxicity study (Anon, 1982a) performed in Fischer 344 rats, clinical signs such as ataxia and hypoactivity were observed in the majority of males and females at doses of 1016 mg/kg bw and above. At doses of 1829 mg/kg bw and above, depression of the myotactic placing reflex was also seen in 1/5 males and females and loss of righting reflex was observed in 1/5 females only. These signs increased with increasing dose, indicating a treatment-related effect. Similar findings were not observed in the more recent acute oral toxicity study, performed according to GLP and OECD guidelines (Anon, 2018e), with cinmethylin produced according to the current production process.

In a recently performed acute inhalation toxicity study (Anon, 2017b), Wistar rats were exposed, nose-only to a single limit concentration of 5.268 mg/L. At this dose level, hunching was observed in 2/5 males and 1/5 females on day 0. One male and 4/5 females were also observed to plough nose-first into their bedding. All treated animals were found to have substance-contaminated fur. As rats lick themselves clean, it is possible that exposure to cinmethylin occurred by both the oral and inhalation routes. As the concentration administered was already outside of the guidance value ranges for classification with STOT-SE 2 (STOT SE 2: 5.0 ≥ C > 1.0 mg/L/4h) and there is the possibility that animals were additionally exposed by the oral route following grooming, less weight is given to the results of this study.

In an older acute inhalation toxicity study (Anon, 1986a), F344 rats were exposed whole-body to concentrations of cinmethylin of 0, 0.9, 2.2 and 3.5 mg/L. Clinical signs that might be considered related to neurotoxicity included hunching and tip toe gait from a dose of 2.2 mg/L. At the top dose of 3.5 mg/L, there was an increase in incidence of wet fur in all males and females (indicating additional oral intake of the test substance through grooming), burrowing (all males and females), hypoactivity (1/6 males and 2/6 females) and prostration (1/6 females only). However, at the top exposure level, there was also an increase in mortality (2/6 females on days 3 and 4). Therefore, clinical signs at this concentration occurred in the presence of lethality and cannot contribute towards the classification with STOT-SE.

To conclude, in an acute neurotoxicity study in rats, axonal nerve degeneration (minimal to slight severity) was observed in two females with incidence or severity above that of the HCD at 2000 mg/kg bw. Clinical signs relating to neurotoxicity, at doses relevant for classification, were also observed in the same neurotoxicity study and in an old, non-guideline acute oral study in rats (doses ≥ 1000 mg/kg bw) (these signs were not observed in the modern acute oral toxicity study in rats). Signs of neurotoxicity in the absence of lethality were also observed following exposure via the inhalation route in rats (2.2 mg/L). There were no signs of neurotoxicity in a recent acute oral toxicity study, performed using a limit dose of 2000 mg/kg bw (Anon, 2016a) or at doses relevant for classification in an acute oral toxicity study in mice (Anon, 1982b). In a developmental toxicity study (1984b), which dosed up to 2000 mg/kg bw/d, clinical signs were limited to excess salivation on the first day of application and urine stained abdominal fur, chromorrhinorrhea and alopecia at the second day. All other signs of toxicity at 2000 mg/kg bw occurred

later in the study, after repeated administration. There was no evidence of neurotoxicity following repeated exposure in studies using lower doses for longer periods of time, with the exception of one 90-day study in rats (Anon, 2018f) in which a statistically significant decrease in grip strength was noted in males only treated with 792 mg/kg bw/day cinmethylin (5.7 versus 7.1 in controls). As this finding was observed after 90 days of dosing and there were no other signs of neurotoxicity, the toxicological relevance is unclear.

10.4.2 Comparison with the GB CLP criteria

Specific target organ toxicity (single exposure) is defined as specific, non-lethal target organ toxicity arising from a single exposure to a substance or mixture. All significant health effects that can impair function, both reversible and irreversible, immediate and/or delayed and not specifically covered by the acute toxicity classifications should be included.

Classification in categories 1 and 2 is for substances causing non-lethal "significant and/or severe toxic effects", with the dose level at which the effect occurs covering the basis for the categorisation. Classification with STOT-SE 3 is reserved for substances/mixtures causing "transient effects" following a single exposure, specifically respiratory tract irritation (RTI) and narcotic effects. In the acute and repeated dose studies available there was no evidence of specific target organ toxicity relevant for classification in category 3.

In an acute neurotoxicity study in rats, there was evidence of minimal to slight axonal nerve degeneration in females dosed with 2000 mg/kg bw, which was outside of the HCD provided by the laboratory. The degeneration occurred in single nerve fibres only, mostly in one rat and were not associated with any loss of grip strength (forelimbs/hindlimbs). In addition, whilst the individual findings were outside of the HCD for females, they were occasionally observed in untreated males. Therefore, in isolation, they could be considered a spurious finding.

As part of the weight of evidence, findings to support a neurotoxic effect following oral exposure to cinmethylin include: a reduced number of rearings, and decreased motor activity in females and increased incidences of ataxia and hypoactivity in both sexes following dosing with ≥ 1000 mg/kg bw cinmethylin. An increase in incidence of females with unsteady gait, and a loss righting response and depression of myotactic placing reflex in males and females dosed with 1829-2000 mg/kg bw. Following inhalation exposure, hunching and tip toe gait were noted in a single female following exposure to 2.2 mg/L cinmethylin (STOT SE 2: 5.0 ≥ C > 1.0 mg/L/4h).

The finding of nerve degeneration is considered a significant toxic effect. Whilst it is acknowledged that the dose at which these effects occurred was at the limit of the guidance value ranges (STOT SE 2: 2000 ≥ C > 300 mg/kg bw), the guidance values and ranges are intended for guidance purposes only and are not intended to be strict demarcation values. The clinical signs observed in rats, in the absence of lethality, following both oral and inhalation exposure with cinmethylin support a treatment-related neurotoxic effect. There is no evidence to support a lack of relevance to humans and therefore classification with STOT SE 2 is supported.

10.4.3 Conclusion on classification and labelling for STOT SE

STOT-SE Category 2; H371: May cause damage to the nervous system.

10.5 Skin corrosion/irritation

The potential for cinmethylin to cause skin irritation/corrosion was investigated in a guideline in vivo skin irritation test in rabbits. Also available to inform on this endpoint is a guideline in vitro corrosion study and

a guideline in vitro irritation study, both using reconstructed human epidermis. Additional information is available from a non-guideline in vivo study, carried out in rabbits.

Table 19: 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,</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>Acute skin irritation/corrosion in rabbits
OECD 404 (2015) – no deviations
GLP
Semi-occlusive</td><td>Rabbit, New Zealand White
3 females</td><td>Cinmethylin
Purity = 93.5 %
Enantiomeric ratio: 48:52 (-/+)</td><td>0.5 mL undiluted, 4 hours</td><td>Mild irritation observed
<table><tr><th>Scores/animal</th><th>0 h</th><th>1h</th><th>Mean score (24-72 h)</th></tr><tr><td>Erythema</td><td>2, 2, 2</td><td>3, 2, 2</td><td>1.67, 0.67, 2</td></tr><tr><td>Oedema</td><td>0, 0, 0</td><td>0, 0, 0</td><td>0, 0, 0</td></tr></table>All findings were reversible by day 7.</td><td>Anon, 2016c
DAR: Volume 3 – B.6.2.4</td></tr>
<tr><td>Acute skin irritation/corrosion in rabbits
Non-guideline
Non-GLP
Occlusive</td><td>Rabbit, New Zealand White
3/sex</td><td>Cinmethylin
Purity = 93.3 %
Enantiomeric ratio: not specified</td><td>0.5 mL undiluted, 24 hours to both abraded and intact skin</td><td>Mild irritation observed
Very slight – slight oedema (grade 1-2)
All findings were reversible by day 7.</td><td>Anon, 1981a
DAR: Volume 3 – B.6.2.4</td></tr>
</table>

Table 20: Summary table of other studies relevant for skin corrosion/irritation

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
In vitro skin corrosion: reconstructed human epidermis model test OECD 431 (2015) GLP	Cinmethylin Purity = 93.5 % Enantiomeric ratio: 48:52 (-/+)	Reconstructed 3D human epidermis (Rhea) model (EpiDerm™) n=2 Dose: 50 µL Negative control: dH_20 Positive control: KOH (8N) MTT reduction control [killed control (KC)]: test substance or dH_20 on killed tissue	**Non-corrosive** <u>Exposure: 3 min</u> Viability of cinmethylin treated skin (as a % of negative control): 120 % Viability of positive control (as a % of negative control): 12 % <u>Exposure: 1 h</u> Viability of cinmethylin treated skin (as a % of negative control): 131 % Viability of positive control (as a % of negative control): 6 %	Remmele M. 2017a DAR: Volume 3 – B.6.2.4
In vitro skin irritation: reconstructed human epidermis	Cinmethylin Purity = 93.5 % Enantiomeric	Reconstructed 3D human epidermis (Rhe) model (EpiDerm™) n=3	**Inconclusive** <u>Exposure: 1 h, 42 h recovery</u> <u>1st experiment:</u> Viability of cinmethylin treated skin	Remmele M. 2017a DAR: Volume 3 – B.6.2.4

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
model test OECD 439 (2015) GLP	ratio: 52:48 (-/+)	Dose: 30 µL Negative control: PBS Positive control: 5% SDS in dH_20 MTT reduction control [killed control (KC)]: test substance or dH_20 on killed tissues.	(as a % of negative control): 42 % (individual values: 63, 32 and 33 %) Viability of positive control (as a % of negative control): 2.4 % 2nd experiment: Viability of cinmethylin treated skin (as a % of negative control): 56 % (individual values 45, 51 and 74 %) Viability of positive control (as a % of negative control): 3.3 %	

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

In a recently performed guideline and GLP in vivo study, 3 female New Zealand White rabbits were treated with cinmethylin (Anon, 2016c). The undiluted test substance (0.5 mL) was applied to the shaved flank of each animal for 4 hours under semi-occlusive conditions. The treated skin surface was examined 0, 1, 24, 48 and 72 hours after patch removal. Animals were observed for up to 14 days (for animals free of findings, the study was discontinued before this time).

All animals were found to have well-defined erythema (grade 2) immediately after patch removal. This persisted in one animal until 72 hours. No oedema was observed in any animal, at any time point. The observed skin reactions were reversible within 72 hours for one animal and within 7 days for two animals.

Under the conditions of this study, cinmethylin was found to be mildly irritating to the skin of rabbits.

Supporting information is available from a non-guideline study, also performed using New Zealand White rabbits (3/sex) (Anon, 1981a). Cinmethylin (0.5 mL, undiluted) was applied to the skin of rabbits on two application sites (intact and abraded) for a longer exposure time of 24 hours, under occlusive conditions. Signs of skin irritation were assessed at 15-20 minutes, 72 hours and 7 days after patch removal. The study was terminated on day 7 when all animals were observed to be free of any skin reactions.

Dermal observations in non-abraded skin included slight to well-defined erythema and very slight to slight oedema (both grade 1-2). As scores were not obtained at 48 hours, individual 24 – 72 hour scores are not available. All skin reactions were considered reversible within 7 days.

An in vitro study to investigate skin corrosion and skin irritation study using the reconstructed 3D human epidermis (Rhe) model (EpiDerm™) is available (Remmele M., 2017a). These tests were guideline and GLP compliant.

In the skin corrosion test, cinmethylin (50 µL, undiluted) was topically applied to two Epiderm™ skin models and incubated for 3 min and 1 hour respectively. Concurrent positive control (PC) tissues were treated with 8-N potassium hydroxide solution (8N KOH) and negative control (NC) tissues were treated with deionised water. Due to the ability of cinmethylin to directly reduce 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), freeze-killed control tissues were applied in parallel and the resulting mean value used for the correction of the negative control values. Tissue viability (a marker of skin corrosion) relative to the concurrent negative control (KC corrected) was calculated.

The results of the study showed that tissue viability after 3 min and 1 hour cinmethylin exposure was 120 % and 131 % respectively. All positive and negative controls behaved accordingly. Under the conditions of this in vitro study, cinmethylin was not corrosive.

In the skin irritation test, cinmethylin (30 µL, undiluted) to groups of three Epiderm™ skin models and incubated for 1 hour before washing out and further incubation for 42 hours (Remmele M., 2017a). The negative control was phosphate buffered saline (PBS) and 5 % SDS was used as a positive control. Again, due to the ability of cinmethylin to directly reduce MTT, freeze-killed control tissues were applied in parallel and the resulting mean value used for the correction of the negative control values. Tissue viability (a marker of skin irritation) relative to the concurrent negative control (KC corrected) was calculated.

The results of the first test run led to a corrected mean viability of 42 %. However, the individual values that this was based on were non-concordant (63, 32 and 33 %). Therefore, a second test run was performed with a corrected mean viability of 56 %. Individual values were again, non-concordant (44, 51 and 74 %). A third test run was not carried out. Positive and negative controls behaved accordingly. No conclusive prediction on the irritation potential of cinmethylin could be ascertained from this in vitro test.

10.5.2 Comparison with the GB CLP criteria

Cinmethylin was tested for its potential to cause skin corrosion/irritation both in vitro and in vivo.

The results of the skin corrosion test showed that treatment of reconstructed 3D human epidermis with cinmethylin led to a tissue viability of 120 % after 3 min, and 131 % after 1 hour exposure. According to the OECD test guideline, a tissue viability of ≥ 50 % after 3 min and ≥ 15 % after 1 hour exposure leads to the prediction that a substance is non-corrosive.

The in vitro Epiderm™ skin irritation study was seemingly inconclusive, with 2/6 tissues indicating non-irritancy (tissue viability > 50 %), 3/6 tissues indicating irritancy (tissue viability ≤ 50 %) and 1/6 tissue showing borderline results (tissue viability 50 ± 5 %).

The results of a modern, guideline in vivo study demonstrated that cinmethylin was mildly irritating to the skin of rabbits, but the gradings at 24, 48 and 72 h for erythema/oedema did not meet the criteria for classification (mean individual animal scores were < 2.3 and the effects resolved within 7 days). These results were supported by an older non-guideline study in which mild skin reactions in rabbits were noted that resolved within 7 days of exposure.

10.5.3 Conclusion on classification and labelling for skin corrosion/irritation

No classification – conclusive but not sufficient for classification

10.6 Serious eye damage/eye irritation

Two in vivo eye irritation studies in rabbits are available. One of these is modern, performed according to OECD and GLP and the other is older, broadly consistent with OECD guidelines but not carried out to GLP. Two in vitro eye irritation studies are also available. One utilised the Bovine Corneal Opacity and Permeability (BCOP) test method and the other was an EpiOcular™ eye irritation test.

Table 21: Summary table of animal studies on serious eye damage/eye irritation

<table>
<tr><th>Method, guideline, deviations if any</th><th>Species, strain, sex, no/group</th><th>Test substance,</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>Acute eye irritation in rabbits
OECD 405 (2012) – no deviations
GLP</td><td>Rabbit, New Zealand White, Females, n=3</td><td>Cinmethylin
Purity = 93.5 %
Enantiomeric ratio: 48:52 (-/+)</td><td>0.1 mL undiluted, 24 h
Observation period 4 days</td><td>Not irritating
<table><tr><th>Scores/animal</th><th>1 h</th><th>Mean score (24-72 h)</th></tr><tr><td>Corneal opacity</td><td>0, 0, 0</td><td>0, 0, 0</td></tr><tr><td>Iritis</td><td>0, 1, 0</td><td>0, 0, 0</td></tr><tr><td>Conjunctiva - redness</td><td>1, 1, 1</td><td>0.7, 0.3, 0</td></tr><tr><td>Conjunctiva - chemosis</td><td>2, 2, 1</td><td>0.3, 0, 0</td></tr></table>All findings were reversible by 48 h post exposure.</td><td>Anon, 2016d
DAR: Volume 3 – B.6.2.5</td></tr>
<tr><td>Acute eye irritation study in rabbits
No guideline but broadly consistent with OECD 405
Not GLP</td><td>Rabbit, New Zealand White, 3/sex</td><td>Cinmethylin
Purity = 93.3 %
Enantiomeric ratio not stated.</td><td>0.1 mL undiluted,
No wash-out
Observation period 14 days</td><td>Not irritating
<table><tr><th>Scores/animal</th><th colspan="2">1 h</th><th colspan="2">Mean score (24-72 h)</th></tr><tr><td></td><td>Males</td><td>Females</td><td>Males</td><td>Females</td></tr><tr><td>Corneal opacity</td><td>0, 0, 0</td><td>0, 0, 0</td><td>0, 0, 0</td><td>0, 0, 0</td></tr><tr><td>Iritis</td><td>0, 0, 0</td><td>0, 0, 0</td><td>0, 0, 0</td><td>0, 0, 0</td></tr><tr><td>Conjunctiva - redness</td><td>2, 1, 1</td><td>1, 1, 1</td><td>0.3, 1, 1</td><td>0.3, 1, 1</td></tr><tr><td>Conjunctiva - chemosis</td><td>2, 2, 2</td><td>2, 2, 2</td><td>0.3, 0.3, 0</td><td>0, 0, 0</td></tr></table>All findings were reversible by 14 d post exposure.</td><td>Anon, 1981a
DAR: Volume 3 – B.6.2.5</td></tr>
</table>

Table 22: Summary table of other studies relevant for serious eye damage/eye irritation

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
In vitro eye irritation: BCOP test OECD 437 (2013) GLP	Cinmethylin Purity = 93.5 % Enantiomeric ratio: 48:52 (-/+)	Isolated cornea from the eyes of freshly slaughtered cattle n=3 Dose: 750 µL undiluted cinmethylin	**Not classified** Corneal opacity: IVIS score = 0.0 Corneal permeability: IVIS score = 0.0	Remmele M., 2017b DAR: Volume 3 – B.6.2.5

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
		Negative control: dH_20 Positive control: EtOH (100 %) and DMF (100 %) MTT reduction control [killed control (KC)]: test substance or dH_20 on killed tissue		
In vitro eye irritation: EpiOcular™ OECD 492 (2015) GLP	Cinmethylin Purity = 93.5 % Enantiomeric ratio: 48:52 (-/+)	Reconstructed 3D human cornea model (EpiOcular™) n=2 Dose: 50 µL Negative control: dH_20 Positive control: >98% methyl acetate MTT reduction control [killed control (KC)]: test substance or dH_20 on killed tissues.	**Not irritating** Exposure: 30 min Viability of cinmethylin treated cornea (as a % of negative control): 104 % Viability of positive control (as a % of negative control): 30 %	Remmele M., 2017b DAR: Volume 3 – B.6.2.5

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

In a recently performed in vivo eye irritation study conducted according to OECD guidelines and GLP, cinmethylin (0.1 mL) was instilled into the conjunctival sac of the right eye of 3 New Zealand White rabbits using a stepwise procedure (Anon, 2016d). Twenty-four hours after instillation, the eye was rinsed with water. Ocular reactions were assessed 1, 24, 48 and 72 h after administration of cinmethylin.

No corneal opacity was observed in this study. Mild redness (grade 1) and mild-moderate chemosis (grade 1-2) of the conjunctiva were observed in all animals and mild iritis in one animal (grade 1) after 1 h. These findings resolved within 48 h and the individual mean scores for all observations at 24, 48 and 72 h were less than 1. The study was terminated on day 4 due to the absence of any ocular findings. Cinmethylin was not found to be irritating to the eye in this study.

In an older study (Anon, 1981a) pre-dating OECD guidelines and GLP, cinmethylin was tested for eye irritation using New Zealand White rabbits. Undiluted cinmethylin (0.1 mL) was instilled into the conjunctival sac of the right eye of male and female rabbits (3/sex). The test material was not washed out and ocular reactions were assessed 1, 24, 48 and 72 hours after instillation. Observations were also made on day 7 and day 14. The study was terminated on day 14 due to the absence of any ocular findings.

No corneal opacity or iritis was observed during the course of this study. Slight to moderate conjunctival redness and chemosis (grade 1 or 2) was observed in some males and females but mean scores did not indicate that cinmethylin was irritating to the eye. All scores were found to be 0 by day 14.

Two in vitro tests are available to assess the eye irritation potential of cinmethylin (Remmele M., 2017b).

In a BCOP test cinmethylin (750 µL) was applied to the surface of 3 isolated corneas from the eyes of freshly slaughtered cattle for 10 min, followed by a 2 h recovery period. Corneal opacity (light transmission through the cornea) and permeability (measured by sodium fluorescein dye leakage at λ = 490 nm) were assessed quantitatively and the in vitro irritation score (IVIS) was determined. Suitable positive and negative controls were used.

The results of the BCOP test showed no induction of corneal opacity or permeability through treatment with cinmethylin. The IVIS score for both was 0.0.

In an EpiOcularTM eye irritation test, undiluted cinmethylin (50 µL) was topically applied to groups of 2 EpiOcular™ corneal models and incubated for 30 min, followed by 2 hour recovery period. Suitable controls were used. Tissue viability relative to the concurrent negative control (KC corrected) was calculated.

Following cinmethylin exposure, the tissue viability was 104 % indicating cinmethylin does not cause irritation to the eye.

10.6.2 Comparison with the GB CLP criteria

Cinmethylin has been tested for its effects on the eye both in vitro and in vivo. In an in vitro BCOP test, the IVIS score was 0.0 for both corneal opacity and corneal permeability. The criteria for classification is:

IVIS	UN GHS
≤ 3	No category
> 3; ≤ 55	No prediction can be made
> 55	Category 1

As these values are < 3, no classification for eye irritation or serious eye damage is required.

The results of an EpiOcularTM eye irritation test showed that the mean tissue viability following treatment with cinmethylin was 104 %. The criteria for classification is:

Mean tissue viability	UN GHS
> 60 %	No category
≤ 60 %	No prediction can be made

As this value is > 60 %, cinmethylin is not considered classified for eye irritation.

The results of the in vitro studies are supported by the findings from two well-conducted in vivo studies carried out in rabbits. According to the CLP criteria, a substance shall be classified for reversible effects to the eyes (category 2) if, when applied to the eye of an animal, a substance produces:

At least in 2 of 3 tested animals, a positive response of:

Corneal opacity ≥ 1 and/or

Iritis ≥ 1, and/or

Conjunctival redness ≥ 2 and/or

Conjunctival chemosis ≥ 2

Calculated as the mean scores following grading at 24, 48 and 72 hours after instillation of the test material which fully reverses within an observation period of 21 days.

The results of two in vivo studies did not show any effects to the eye that were not fully reversed within an observation period of 14 days or less. The mean scores for corneal opacity and iritis in both studies were 0 (following gradings at 24, 48 and 72 hours). For conjunctival redness and chemosis, the mean scores were ≤ 1. Therefore, no classification for eye irritation is necessary.

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

No classification – conclusive but not sufficient for classification

10.7 Respiratory sensitisation

There are no data or appropriate studies conducted in animals to inform on the potential of cinmethylin to cause respiratory sensitisation.

Some medical surveillance of manufacturing plant personnel has been conducted. The medical monitoring program was designed as a general health check-up, with special interest in the primary target organs presumed to be relevant by analogy from animal experiments.

The surveillance program included a general physical examination including neurological status, red and white blood cell counts and liver enzymes. Adverse health effects suspected to be related to cinmethylin exposure were not observed. In particular, there are no reports of any hypersensitivity reactions such as asthma, rhinitis, conjunctivitis or alveolitis.

Therefore, whilst there is no evidence of a sensitising effect to the respiratory system in humans, the data available are somewhat lacking and not robust enough to make a conclusion on classification and labelling.

10.7.1 Conclusion on classification and labelling for respiratory sensitisation

No classification – data lacking

10.8 Skin sensitisation

Cinmethylin has been tested for its potential to cause skin sensitisation in guinea pigs in a recently performed Buehler test, an older Buehler test and a Guinea Pig Maximisation Test (GPMT). The latter two studies both contain deficiencies which impact on the reliability of the results (discussed below).

Table 23: Summary table of animal studies on skin sensitisation

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels duration of exposure	Results	Reference
Buehler test in guinea pigs OECD 406 (1992) No deviations GLP	Guinea pigs, Dunkin Hartley, females Preliminary test: 3/dose Main test: 20/dose Controls: n= 10	Cinmethylin Purity: 93.5 % Enantiomeric ratio: 48:52 (-/+) Vehicle: viscous paraffin Positive control: no concurrent positive control used.	Preliminary test: 25, 50, 75 and 100 % Main test: Induction: 100 % Challenge: 75 % Exposure: 6 h	**Sensitising** <u>Preliminary test</u>: 100 % - 3/3 animals with erythema (grade 1 in 2 animals and grade 2 in 1 animals) No skin reactions observed at lower concentrations. <u>Main test:</u> 24 h – 16/20 animals with discrete to moderate erythema (grade 1-2) 48 h – 5/20 animals with discrete to moderate erythema (grade 1-2)	Anon, 2016e DAR: Volume 3 – B.6.2.6
Buehler test in guinea pigs Similar to OECD 406 (1981) – deviations included no information on purity and the dose used for induction was too low. GLP	Guinea pigs, Dunkin Hartley Preliminary tests: 1/sex/dose Main test:5/sex	Cinmethylin Purity: not specified Enantiomeric ratio: not stated Vehicle: EtOH Positive control: 2,4-dinitrochlorobenzene (DNCB), 0.1% (w/v) in diethyl ether	Preliminary test: 1, 10, 50 and 100 % Main test: Induction: 1 % Challenge: 1 % Exposure: 6 h	**Not sensitising** <u>Preliminary test 1</u>: 100 % - slight erythema (grade 1) in males and females 50 % - moderate erythema with slight oedema (grade 3) in males and moderate erythema (grade 2) in females 10 % - slight erythema (grade 1) in males and females <u>Preliminary test 2:</u> 10 % - moderate erythema (grade 2) in males and slight erythema (grade 1) in females 1 % - slight erythema (grade 1) in males and minimal erythema (grade 0.5) in females. <u>Main test:</u> 24 h – 1/10 animals with slight erythema (grade 1) 48 h – 0/10 animals with erythema	Anon, 1982c DAR: Volume 3 – B.6.2.6

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels duration of exposure	Results	Reference
Guinea Pig Maximisation Test (GPMT) Similar to OECD 406 (1992) – deviations included no reliability check of the performing laboratory and no concurrent positive control was used. GLP	Guinea pigs, Dunkin Hartley Preliminary test: 2/sex/dose Main: 10/sex Controls: 5/sex	Cinmethylin Purity: 92 % Enantiomeric ratio: not stated Vehicle: Corn oil Positive control: none	Preliminary test: Intradermal injections: 0.05, 0.1, 0.5 and 1.0 % Topical: 10, 25, 50, 75 % Main test: Intradermal induction: 0.1 % Topical induction: 100 % Topical challenge: 75 %	**Not sensitising** <u>Preliminary test:</u> Grade 1-2 erythema was noted in all treated groups following intradermal injection. Grade 1 erythema was found in 1/4 animals following topical application of 100 % cinmethylin only. <u>Main test:</u> 0/20 animals with skin reactions.	Anon, 1988a DAR: Volume 3 – B.6.2.6

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

A modern, guideline and GLP compliant Buehler test in Dunkin Hartley guinea pigs is available (Anon, 2016e). The study comprised of a preliminary test to determine the highest dose causing mild skin irritation for use as an induction dose in the main study, and the main study itself.

In the preliminary test, cinmethylin was applied to the shaved skin of females (3/dose) at concentrations of 25, 50, 75 or 100 %. Skin reactions were determined 1, 24 and 48 h following patch removal. No skin reactions were observed at concentrations of 75 % or less. For those treated with 100 % cinmethylin, 2 animals showed discrete erythema (grade 1) and 1 animal showed moderate erythema (grade 2). As there were no skin reactions observed at concentrations below 100 %, this was selected for use for induction in the main study and 75 % cinmethylin was chosen for challenge.

In the main test, groups of 20 female guinea pigs were exposed to 100 % cinmethylin (0.5 mL) under occlusive conditions for 6 hours. Inductions were performed on days 0, 7 and 14. A challenge was performed 14 days after the last induction. Cinmethylin (75 %, 0.5 mL) was applied for 6 hours under occlusive conditions to the right posterior flank. The vehicle (viscous paraffin) was applied to the left posterior flank. Skin reactions were determined 24 and 48 hours after patch removal.

There were no local skin effects observed in the negative control group following induction. In the treated group, discrete to moderate erythema (grade 1 and 2) was observed in 17 animals after the first induction, 18 animals after the second induction and all 20 animals following the third induction. Following challenge with 75 % cinmethylin, no skin reactions were observed in negative control animals but 16/20 treated animals were observed to have discrete to moderate erythema (grade 1 or 2). The overall incidence rate was 80 % responding to a 100 % induction concentration. The results of this study indicate that cinmethylin is sensitising to skin.

A second, less reliable Buehler test is also available (Anon, 1982c). The study method broadly followed OECD guidelines, however, the test was not conducted using the highest induction dose to cause mild skin irritation.

A preliminary test was carried out to determine the appropriate induction dose to be used in the main study. Concentrations of cinmethylin (10, 50 or 100 %) in ethanol were applied topically to groups of male and female Dunkin Hartley guinea pigs (1/sex/dose). Skin reaction scores did not show a firm dose response, with skin reactions of up to grade 2 in animals treated with 100 % cinmethylin, up to grade 3 in animals treated with 50 % cinmethylin and up to grade 1 in animals treated with 10 % cinmethylin. As there were no doses at which skin reactions were not observed, a second preliminary test was carried out using concentrations of 1 and 10 % cinmethylin. Skin reactions up to grade 2 were noted in animals treated with 10 % cinmethylin and reactions up to grade 1 were noted with animals treated with 1 % cinmethylin. A concentration of 1 % cinmethylin was used for both induction and challenge in the main test.

Table 24: Preliminary test - skin reaction scores in male and female guinea pigs treated with cinmethylin

Concentration (%)	**Skin reaction score**			
	24 h		**48 h**	
	Males	**Females**	**Males**	**Females**
100	1	1	0.5	0.5
50	3	2	3	1
10	1[a]/2[b]	1[a]/1[b]	1	0.5
1	1	0.5	-	-

Grade 0.5 = Minimal erythema (barely perceptible with edges not defined)
Grade 1 = Sight erythema (pale red/pink in colour and edges of area defined)
Grade 2 = Moderate erythema (red in colour and edges well-defined)
Grade 3 = Moderate erythema with slight oedema (red in colour edges well-defined, raised less than 1 mm)
[a] first preliminary test
[b] second preliminary test

In the main test guinea pigs (5/sex) were exposed to a 1 % topical induction concentration of cinmethylin in ethanol. Inductions were performed on days 0, 7 and 14 for 6 hours under occlusive conditions. A challenge (1 % cinmethylin in ethanol) was performed 14 days after the last induction for 6 hours, under occlusive conditions. Ethanol (0.5 mL) and dinitrochlorobenzene (0.5 mL of a 0.1 % solution in diethyl ether) were used as negative and positive controls respectively.

During induction, only 1/10 animals showed a skin reaction. Grade 1 erythema was noted at the 1st induction at 24 h post application. Following challenge, 1/10 animals in the exposed group showed skin findings (grade 1 slight erythema). Positive and negative controls behaved accordingly. Under the conditions of this study, cinmethylin is not sensitising to the skin.

A guinea pig maximisation test is also available (Anon, 1988a). This test was carried out to a method similar to OECD 406, however the study did not include a positive control which inhibits the reliability of the results.

A preliminary test was performed in which Dunkin Hartley guinea pigs (2/sex/dose) received a single intradermal injection of 0.1 mL cinmethylin (0.05, 0.1, 0.5 or 1.0 %). A second group of animals (2/sex/dose) received dermal patches containing 0.3 mL cinmethylin (10, 25, 50, 75 or 100 %); exposure lasted for 24 hours. Following intradermal injection, all animals treated with ≥ 0.1 % cinmethylin had grade 1-2 skin reactions. Grade 1 skin reactions were observed in 2/4 animals treated with 0.05 % cinmethylin. Following

topical application, only 1/4 animals was observed to have a skin reaction following treatment with 100 % cinmethylin. No skin reactions were observed in any of the other treatment groups. The doses of cinmethylin selected for the main study were 0.1 % for intradermal induction, 100 % for topical induction and 75 % for topical challenge.

In the main study, guinea pigs (10/sex) received intradermal injections of 0.1 % cinmethylin to the shaved shoulder region [two injections of Fruend's complete adjuvant (FCA), two injections of the test material in corn oil and two injections of the test material in 50:50 FCA/corn oil]. Seven days after intradermal induction, 0.3 mL of cinmethylin (100 %) was applied to the same area of shaved skin. The test material was held in place with an adhesive bandage for 48 hours. Topical challenge using 75 % cinmethylin was carried out two weeks after the topical induction. There were no skin reactions observed in any animal within 24 or 48 hours of patch removal. No positive control was included in this study. Under the conditions of this study, cinmethylin was not sensitising to the skin.

10.8.2 Comparison with the GB CLP criteria

There are three skin sensitisation studies available in guinea pigs, two Buehler tests and one GPMT. Only the more recently performed Buehler test (2016) was performed strictly according to guidelines and a skin sensitising effect was observed.

Although the two older studies followed methods broadly similar to OECD guidelines, there were a number of deficiencies that rendered the results less reliable. In the 1982 Buehler test, the induction concentration chosen for the main study was not considered high enough to induce mild irritation in the majority of the animals tested. In the 1988 GPMT, a positive control was not included to assess the validity of the experiment. Cinmethylin was not considered sensitising in either of these two studies, however, due to the study deficiencies, more weight is given to the 2016 Buehler test.

The results of this recently performed Buehler test showed an 80 % response to a 100 % induction dose of cinmethylin. Cinmethylin appears to be a moderate sensitiser and meets the classification criteria for skin sensitisation category 1 B (≥ 15 % responding at > 20 % topical induction dose). However, as a lower topical induction dose was not tested, category 1A cannot be excluded. Therefore, the data are not sufficient for sub-categorisation and cinmethylin should be classified for skin sensitisation, category 1.

10.8.3 Conclusion on classification and labelling for skin sensitisation

Skin Sens. 1 (H317: May cause an allergic skin reaction)

10.9 Specific target organ toxicity-repeated exposure

The repeated dose oral toxicity of cinmethylin has been investigated in a number of studies in rats, mice and dogs. In rats, there are a 28-day study and two 90-day studies. Further information is also available from a two generation study, a combined chronic toxicity and carcinogenicity study and a 2-year carcinogenicity study. A second older reproduction study is also available, but was not included, as effects in parental animals were generally only observed at doses of 2000 mg/kg bw (twice that of the limit dose and 20 times above the guidance value for classification). In mice there are a 28-day study, two 90-day studies and two carcinogenicity studies (18 and 24 months respectively). In dogs, there is a 35-day, a 90-day and three 1-year studies. A 28-day study via the dermal route is also available in the rat.

Table 2518: Summary table of animal studies on STOT RE

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results	Reference
ORAL			
Repeated dose 28-day oral toxicity study in rats Wistar 5/sex/dose OECD 407 (2008) GLP Classification criteria: STOT-RE 1 ≤ 30 mg/kg bw/day STOT-RE 2 30 < C ≤ 300 mg/kg bw/day	Cinmethylin Purity: 97.5 % Enantiomeric ratio: 70:30 (-/+) Route: oral (diet) Dose: 0, 1500, 5000 and 15000 ppm (equivalent to 0, 137/141, 477, 1522/1331 mg/kg bw/day males/females) Administered daily in the diet for 28 days.	There were no treatment-related deaths or clinical signs of toxicity at any dose. **<u>1500 ppm (137/141 mg/kg bw/day):</u>** No adverse treatment-related findings. **<u>5000 ppm (477 mg/kg bw/day):</u>** *Clinical chemistry:* ↑ γ-Glutamyl transferase activity: 36 µkat/L** in males and 65 µkat/L** in females versus 0 in controls ↑ Total protein: 6.2 % in females ↑ Cholesterol: 34.6 % in males ↓ Glucose: 22.7 % in males *Organ weights:* ↑ Liver weight: abs. 23 %** and rel. 22 %** in males and rel. 18 %** in females ↑ Kidney weight: rel. 15 %* in males *Histopathology:* Thyroid follicular hypertrophy/hyperplasia 4/5 males and 2/5 females versus 0 in controls. **<u>15000 ppm (1522/1331 mg/kg bw/day):</u>** ↓ Body weight (day 0-28): 20.9 % in males and 11.4 % in females *Clinical chemistry:* ↑ γ-Glutamyl transferase activity: 412 µkat/L** in males and 337 µkat/L** in females versus 0 in controls ↑ Total protein: 4.6 %** in males and 7.7* % in females ↑ Cholesterol: 79.0 %** in males and 138.6 %** in females	Anon, 2015a DAR: B.6.3.1

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results**	**Reference**
		↓ Glucose: 25.1 %* in males and 21.2 %** in females ↑ Triglycerides: 91.7 %* in males *Organ weights:* ↑ Liver weight: abs. 39 %** and rel. 59 %** in males and abs. 55 %** and rel. 66 %** in females ↑ Kidney weight: rel. 19 %** in males ↑ Epididymides weight: rel. 16 %* ↑ Testes weight: rel. 18 %** *Histopathology:* *Liver:* Centrilobular hypertrophy: 5/5 females versus 0 in controls (slight – moderate) Diffuse hypertrophy: 5/5 males (minimal - slight) versus 0 in controls *Thyroid:* Follicular hypertrophy/hyperplasia: 5/5 males (slight – moderate) and 5/5 females (slight – moderate) versus 0 in controls Altered colloid:3/5 males (minimal – slight) versus 1/5 males in controls and 2/5 females (minimal) versus 0 in controls. **NOAEL:** 1500 ppm (equivalent to 137/141 mg/kg bw/day in males/females)	
Repeated dose 90-day oral toxicity study in rats Wistar 10/sex/dose OECD 408 (1998) – one minor deviation that clinical	Cinmethylin Purity: 96.2 % Enantiomeric ratio: 51:49 (-/+) Route: oral (diet) Dose: 0, 1000, 3000 and 10000 ppm (equivalent to 0, 67/79, 211/240, 792/814 mg/kg bw/day males/females)	There were no treatment-related deaths or clinical signs of toxicity at any dose. **1000 ppm (67/79 mg/kg bw/day):** No adverse treatment-related findings. **3000 ppm (211/240 mg/kg bw/day):** *Haematology and clinical chemistry:* ↓ Prothrombin time: 5.9 %** in females (% of controls)	Anon, 2018g DAR: B.6.3.2

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results**	**Reference**
examination was not performed for all animals on day 74 GLP Classification criteria: STOT-RE 1 ≤ 10 mg/kg bw/day STOT-RE 2 10 < C ≤ 100 mg/kg bw/day	Administered daily in the diet for 90 days.	↑ γ-Glutamyl transferase activity: 27 µkat/L* in males and 46 µkat/L** in females versus 0 in controls ↑ Total protein: 6.2 * % in females ↑ Cholesterol: 44.5 %** in females ↑ Globulin: 9.0 %** in females. *Organ weights:* ↑ Liver weight: rel. 18 %** in males and abs. 12 %* and rel. 11 %** in females. ↑ Kidney weight: rel. 13 %** in males *Histopathology:* *Liver:* Centrilobular hypertrophy: 3/10 females (minimal) versus 0 in controls Kidney: *Thyroid:* Hypertrophy/hyperplasia of follicular cells: 4/10 males (minimal – slight) and 1/10 females (minimal) versus 0 in controls *Nasal cavity:* Degeneration of the olfactory epithelium: 10/10 males and 4/10 females (minimal-slight) versus 0 in controls Exudate, proteinaceous: 9/10 males and 5/10 females (minimal-slight) versus 0 in controls *Ovaries:* Vacuolation of the interstitial glands: 7/10 females (slight-minimal) **<u>10000 ppm (792/814 mg/kg bw/day):</u>** ↓ Grip strength in males: 5.7** versus 7.1 in controls ↓ Body weight: 16.2 %** in males and 15.0 %** in females ↓ Body weight gain: 27.0 %** in males and 33.3 %** in females *Haematology and clinical chemistry:*	

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results	Reference
		↓ Prothrombin time: 6.9 %** in males and 13.8 %** in females (% of controls) ↑ γ-Glutamyl transferase activity: 292 µkat/L** in males and 268 µkat/L** in females versus 0 in controls ↑ Total protein: 4.3 %** in males and 7.3* % in females ↑ Cholesterol: 26.0 %** in males and 94.5 %** in females ↓ Glucose: 26.0 %** in males and 23.5 %** in females ↑ Creatinine: 17.7 %** in females ↑ Globulin: 10.1 %** in females ↑ Triglycerides: 44.8 %* in females *Organ weights:* ↑ Liver weight: abs. 35 %** and rel. 62 %** in males and abs. 22 %** and rel. 42 %** in females ↑ Kidney weight: abs. 11 % and rel. 33 %** in males ↑ Thyroid weight: rel. 25 %* in females *Histopathology:* *Liver:* Dark brown discolouration: 9/10 males and 10/10 females versus 0 in controls Centrilobular hypertrophy: 10/10 females (minimal – moderate) versus 0 in controls Diffuse hypertrophy: 9/10 males (minimal-slight) versus 0 in controls Fatty change, peripheral: 7/10 males (minimal – slight) versus 0 in controls Pigment storage, peripheral: 9/10 males and 10/10 females (slight) versus 0 in controls *Kidney:* Eosinophilic droplets: observed in all groups but ↑in severity (9/10 males and 1/10 grade 2 and 3 respectively, versus 10/10 grade 1 in controls) Chronic nephropathy: 9/10 males versus 2/10 in controls	

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results**	**Reference**
		Granular casts (tubular): observed in all treated groups but ↑ in severity (2/10 males and 1/10 grade 2 and 3 respectively, versus 0/10 in controls) *Thyroid:* Hypertrophy/hyperplasia of follicular cells: 9/10 males (minimal – moderate) and 1/10 females (slight) versus 0 in controls *Nasal cavity:* Degeneration of the olfactory epithelium: 10/10 males and 7/10 females (minimal-slight) versus 0 in controls Exudate, proteinaceous: 10/10 males and 9/10 females (minimal-slight) versus 0 in controls *Ovaries:* Vacuolation of the interstitial glands: 9/10 females (slight-marked) **NOAEL:** 1000 ppm (equivalent to 67 and 79 mg/kg bw/day) (males and females)	
Repeated dose 90-day oral toxicity study in rats F344 30/sex/dose (10/sex/dose were sacrificed at week 7 and 20/sex/dose were sacrificed at week 13) Non-guideline Non-GLP Classification criteria:	Cinmethylin Purity: Not stated Enantiomeric ratio: not stated Route: oral (diet) Dose: 0, 30, 100, 300 and 1000 ppm (equivalent to 0, 2.18/2.61, 7.51/8.73, 22.51/26.08 and 75.78/88.56 mg/kg bw/day males/females) Administered daily in the diet for 90 days.	There were no adverse treatment-related effects at any dose.	Anon, 1983a DAR: B.6.3.2

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results**	**Reference**
STOT-RE 1 ≤ 10 mg/kg bw/day STOT-RE 2 10 < C ≤ 100 mg/kg bw/day		**NOAEL:** 1000 ppm (equivalent to 75.8 and 88.6 mg/kg bw/day) (males and females)	
Two-generation reproduction toxicity study in rats Wistar 25/sex/dose OECD 416 (2001) GLP Classification criteria: STOT-RE 1 ≤ 10 mg/kg bw/day STOT-RE 2 10 < C ≤ 100 mg/kg bw/day	Cinmethylin Purity: 93.5 % Enantiomeric ratio: 48: 52 (-/+) Route: oral (diet) Dose: 0, 125/250, 500/1000 and 2500/5000 ppm (equivalent to 0, 19.7-21.8/20.6-23.8, 79.4-87.7/81.3-96.9 and 412-450/394-481 mg/kg bw/day males/females) (further details on dosing available in Section 10.12) Administered daily in the diet for 10 weeks prior to mating, throughout mating, gestation and lactation (equivalent to 90 days)	Findings relate only to parental toxicity. Full reproductive toxicity information can be found in Section 10.12. There were no treatment-related deaths or clinical signs of toxicity at any dose. **125/250 ppm (equivalent to 19.7-21.8 and 20.6-23.8 mg/kg bw/day in males and females respectively):** No adverse treatment-related findings. **500/1000 ppm (equivalent to 79.4-87.7 and 81.3-96.9 mg/kg bw/day in males and females respectively):** *Organ weights:* ↑ Liver weight: abs. 10.8 % and rel. 8 % in males only **2500/5000 ppm (equivalent to 412-450 and 394-481 mg/kg bw/day in males and females respectively):** *Organ weights:* ↑ Liver weight: abs. 23.5 % and rel. 26 % in males and abs. 20.2 % and rel. 24.5 % in females. ↑ Kidney weight: abs. 11.7 % and rel. 14.1 % in males only ↑ Thyroid weight: abs. 15.2 % and rel. 17 % in males and abs. 14.9 % and rel. 19 % in females. *Histopathology:* *Kidney:* Chronic nephropathy: 25/25 males versus 10/25 in controls Eosinophilic droplets: 22/25 males versus 0 in controls Granular casts: 17/25 males versus 0 in controls *Thyroid gland:* Hypertrophy/hyperplasia of follicular epithelial cells: 10/25 males and 16/25 females versus 0 in controls	Anon, 2018h DAR: B.6.6.1

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results**	**Reference**
		Nasal cavity: Degeneration/regeneration of the olfactory epithelium: 25/25 males and 25/25 females versus 0 in controls **<u>NOAEL:</u>** 500/1000 ppm (equivalent to approximately 80 mg/kg bw/day)	
Combined chronic toxicity/carcinogenicity study in rats – chronic phase Wistar 10/sex/dose OECD 453 (2009) GLP Classification criteria: STOT-RE 1 ≤ 2.5 mg/kg bw/day STOT-RE 2 2.5 < C ≤ 25 mg/kg bw/day	Cinmethylin Purity: 93.5 % Enantiomeric ratio: 48: 52 (-/+) Route: oral (diet) Dose: 0, 200, 1000 and 5000 ppm (equivalent to 0, 10/13, 51/69 and 265/351 mg/kg bw/day males/females) Administered daily in the diet for **12 months**.	There were no treatment-related deaths or clinical signs of toxicity at any dose. **<u>200 ppm (10/13 mg/kg bw/day):</u>** No adverse treatment-related findings. **<u>1000 ppm (51/69 mg/kg bw/day):</u>** No adverse treatment-related findings. ***<u>Histopathology:</u>*** *Thyroid gland:* Altered colloid: 4/10 males versus 1/10 controls **<u>5000 ppm (265/351 mg/kg bw/day):</u>** ↓ Body weight: 12.6 %* in females ↓ Body weight gain: 21.3 %* in females *Clinical chemistry:* ↑ γ-Glutamyl transferase: 77 nkat/L** in males and 47 nkat/L ** in females versus 0 in controls. *Organ weights:* ↑ Liver weight: abs. 16 %* and rel. 14 %** in males and rel. 17 %** in females. ↑ Kidney weight: abs. 15 %* and rel. 14 %** in males only *Histopathology:* *Liver:* Cytoplasmic alterations in 3/10 males (versus 0 in controls)	Anon, 2018c DAR: B.6.5.1

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results**	**Reference**
		Centrilobular hypertrophy in 4/10 males and 6/10 females (versus 0 in controls) *Kidney:* Eosinophilic droplets in males only *Nasal cavity:* Degeneration/regeneration of the olfactory epithelium (level III): 10/10 males and 10/10 females versus 0 in controls Proteinaceous exudate: 10/10 males and 10/10 females versus 0 in controls *Thyroid gland:* Hypertrophy/hyperplasia: 3/10 males versus 0 in controls Altered colloid: 8/10 males and 6/10 females versus 1/10 controls As this study was part of a combined chronic toxicity/carcinogenicity study of total duration 24 months, a NOAEL for 12 months was not determined.	
Combined chronic toxicity/carcinogenicity study in rats – carcinogenicity phase Wistar 50/sex/dose OECD 453 (2009) GLP Classification criteria: STOT-RE 1 ≤ 1.25 mg/kg bw/day	Cinmethylin Purity: 93.5 % Enantiomeric ratio: 48: 52 (-/+) Route: oral (diet) Dose: 0, 200, 1000 and 5000 ppm (equivalent to 0, 9/11, 45/59 and 242/317 mg/kg bw/day males/females) Administered daily in the diet for **24 months**.	Non-neoplastic findings presented only (for neoplastic findings, see Section 10.11). There was no treatment-related effect on mortality or clinical signs. **<u>200 ppm (9/11 mg/kg bw/day):</u>** No adverse treatment-related findings. **<u>1000 ppm (45/59 mg/kg bw/day):</u>** *Histopathology:* *Liver:* Centrilobular pigment storage: 22/50** females *Nasal cavity:* Degeneration/regeneration of the olfactory epithelium (level III): 2/50 males versus 0 in controls Inflammation (multifocal): 5/50 males versus 2/50 in controls	Anon, 2018c DAR: B.6.5.1

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results**	**Reference**
STOT-RE 2 1.25 < C ≤ 12.5 mg/kg bw/day		**<u>5000 ppm (242/317 mg/kg bw/day):</u>** ↓ Body weight: 13 %* in females ↓ Body weight gain: 12 %** in males and 18 %** in females *Organ weights:* ↑ Liver weight: rel. 13 %** in males and rel. 20 %** in females. ↑ Kidney weight: rel. 18 %** in males and rel. 11 %* in females *Histopathology:* *Liver:* Cytoplasmic alterations: 18/50* males (versus 0 in controls) Centrilobular hypertrophy: 1/50 male and 12/50* females (versus 0 in controls) Periportal hypertrophy: 7/50* females versus 1/50 females in controls Periportal pigment storage: 24/50* males and 15/50* females versus 0 in controls. Centrilobular pigment storage: 27/50** females Multinucleated hepatocytes: 17/50* males versus 8/50 males in control group *Kidney:* Mineralisation (tubular): 8/50* males versus 1/50 in controls Mineralisation (papilla): 7/50* males versus 0/50 in controls *Nasal cavity:* Degeneration/regeneration of the olfactory epithelium (level III): 50/50** males and 50/50** females versus 0 in controls Proteinaceous exudate: 20/50** males and 30/50** females versus 0 in controls Metaplasia of the respiratory epithelium: 14/50** males and 12/50** females versus 0 in controls Inflammation (multifocal): 12/50** males versus 2/50 in controls and 8/50* females versus 1/50 in controls. *Thyroid gland:* Hyperplasia (follicular cell): 10/50* males versus 2/50 in controls	

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results**	**Reference**
		Altered colloid: 24/50** males versus 7/50 in controls and 33/50** females versus 5/50 in controls **NOAEL:** 200 ppm (9 mg/kg bw/day in males and 11 mg/kg bw/day in females)	
A 2-year feeding study in rats F344 50/sex/dose (2 year carcinogenicity group) 15/sex/dose (18-month chronic group) 10/sex/dose (6 and 12 month chronic groups) Non-guideline Non-GLP Classification criteria (2 years): STOT-RE 1 ≤ 1.25 mg/kg bw/day STOT-RE 2 1.25 < C ≤ 12.5 mg/kg bw/day	Cinmethylin Purity: 92 % Enantiomeric ratio: not specified Route: oral (diet) Dose: 0, 30, 100 and 3000 ppm (equivalent to 0, 1.4/1.7, 4.7/5.8 and 144.2/177.4 mg/kg bw/day males/females) Administered daily in the diet for 6,12,18 or 24 months.	Non-neoplastic findings presented only (for neoplastic findings, see Section 10.11). **30 ppm (1.4/1.7 mg/kg bw/day):** No adverse treatment-related effects observed. **100 ppm (4.7/5.8 mg/kg bw/day):** *Thyroid:* Focal colloidal basophilia/mineralisation: 18/50** females versus 8/50 in controls **3000 ppm (144.2/177.4 mg/kg bw/day):** ↑ Mortality (2 years): 64 % of males died versus 56 % in controls ↓ Body weight: 12 % in males ↓ Body weight gain: 19.9 % in males Hunched appearance in males Pale eyes in males *Clinical chemistry:* ↑ γ-Glutamyl transferase: 7.2 IU/L** in males versus 2.0 IU/L in controls and 4.8 IU/L** in females versus 1.7 IU/L in controls ↑ Urea nitrogen: 17.5 mmol/L** in males versus 10.6 mmol/L in controls ↑ Inorganic phosphate: 2.36 mmol/L* in males versus 1.7 mmol/L in controls and 1.42 mmol/L** in females versus 1.14 mmol/L in controls *Urinalysis:* ↑ Tubular epithelial cells: 1.0** in males versus 0.2 in controls (at 24 months)	Anon, 1985a Anon, 1991a Anon, 1991b Anon, 1991c DAR: B.6.5.1

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results	Reference
		↑ Casts: 0.3* in males versus 0.1 in controls (24 months) ↑ Urine volume: 12.9 mL* in males versus 9.7 mL in controls (24 months) (paler in colour) ↓Osmolality: 38 % of controls in males ↑ Leukocytes: 17* in males versus 4.2 in controls. *Organ weights:* ↑ Liver: rel. 23.3 % in males and rel. 14.1 % in females ↑ Kidney: rel. 16.8 % in males and rel. 13.1 % in females *Histopathology:* *Liver:* Subscapular dark depressed foci: 40 females versus 29 in controls. Periportal acidophilia: 33** males versus 9 in controls, 22** females versus 2 in controls Periportal chromidial clumping: 9** males and 1 females versus 0 in controls. *Kidney:* Diffuse subcapsular pallor: 23 males versus 14 in controls Subscapular pitting/rough surface (severe/very severe): 27 males versus 12 in controls Chronic nephropathy (increased severity): severe: 19/50 males versus 13/50 in controls and very severe: 14*/50 males versus 4/50 in control *Thyroid:* Focal colloidal basophilia/mineralisation: 21**/50 females versus 8/50 in controls *Lymph nodes:* Renal node haemorrhage/enlargement: 4 males versus 0 in controls *Stomach:* Gastric mucosal thickening: 18 males versus 8 in controls. Fundic glandular mineralisation: 13*/50 versus 3/50 in controls *Parathyroid gland:*	

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results**	**Reference**
		Enlargement: 16 males versus 7 in controls. Diffuse hyperplasia: 29**/50 males versus 15/50 in controls *Blood vessels:* Aortic mucosal thickening: 18 males versus 11 in controls. **NOAEL:** 100 ppm (equivalent to 4.7/5.8 mg/kg bw/day in males/females)	
Repeated dose 28-day oral toxicity study in mice C57BL/6JRj 5/sex/dose OECD 407 (2008) GLP Classification criteria: STOT-RE 1 ≤ 30 mg/kg bw/day STOT-RE 2 30 < C ≤ 300 mg/kg bw/day	Cinmethylin Purity: 96.2 % Enantiomeric ratio: 51:49 (-/+) Route: oral (diet) Dose: 0, 400, 1200 and 4000 ppm (equivalent to 0, 95.1/92.4, 295.9/254 and 791.4/1015.6 mg/kg bw/day males/females) Administered daily in the diet for 28 days.	There were no treatment-related deaths or clinical signs of toxicity at any dose. **400 ppm (95.1/92.4 mg/kg bw/day):** No adverse treatment-related effects. **1200 ppm (295.9/254 mg/kg bw/day):** *Organ weights:* ↑ Liver weight: abs. 15 % and rel. 10 %* in males and abs. 13 % and rel. 11 %* in females **4000 ppm (791.4/1015.6 mg/kg bw/day):** ↓ Body weight gain: 44 %** in males *Clinical chemistry:* ↓ Bilirubin: 30 %** in males ↓ Total protein: 10.9 %** in males ↓ Albumin: 10.1 %** in males and 5.7 %* in females ↓ Globulin: 12.4 %** in males ↓ Cholesterol: 38.7 %** in males and 19.7 %* in females ↓ Triglycerides: 46.7 %** in males and 34.8 % in females *Organ weights:* ↑ Liver weight: abs. 16 % and rel. 19 %** in males and abs. 26 %** and rel. 22 %** in females	Anon, 2016f DAR: B.6.3.1

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results**	**Reference**
		NOAEL: 1200 ppm (equivalent to 296 and 254 mg/kg bw) (males and females)	
Repeated dose 90-day oral toxicity study in mice C57BL/6JRj 10/sex/dose OECD 408 (1998) GLP Classification criteria: STOT-RE 1 ≤ 10 mg/kg bw/day STOT-RE 2 10 < C ≤ 100 mg/kg bw/day	Cinmethylin Purity: 96.2 % Enantiomeric ratio: 51:49 (-/+) Route: oral (diet) Dose: 0, 200, 1000 and 5000 ppm (equivalent to 0, 43/58, 201/285 and 1200/1304 mg/kg bw/day males/females) Administered daily in the diet for 90 days.	There were no treatment-related deaths or clinical signs of toxicity at any dose. **200 ppm (43/58 mg/kg bw/day):** No adverse treatment-related effects. **1000 ppm (201/285 mg/kg bw/day):** *Clinical chemistry:* ↓ Cholesterol: 11.8 %** in males **5000 ppm (1200/1304 mg/kg bw/day):** ↓ Body weight gain: 27.0 %** in males *Clinical chemistry:* ↓ Bilirubin: 19.3 %** in males and 10.6 % in females ↓ Cholesterol: 31.0 %** in males and 19.4 % in females ↓ Triglycerides: 43.0 %** in males and 33.3 %** in females *Organ weights:* ↑ Liver weight: abs. 21.1 %** and rel. 30.5 %** in males and abs. 20.0 %** and rel. 24.2 %** in females ↑ Thymus weight: abs. 13.4 %** and rel. 17.7 % in females NOAEL: 200 ppm (equivalent to 43 and 58 mg/kg bw/day in males and females)	Anon 2018f DAR: B.6.3.2
Repeated dose 90-day oral toxicity study in mice B6C3F1 20/sex/dose (an additional 10/sex/dose were used but	Cinmethylin Purity: not specified Enantiomeric ratio: Not specified Route: oral (diet) Dose: 0, 30, 100, 300 and 1000 ppm (equivalent to 0,	There were no adverse treatment-related effects at any dose.	Anon, 1983b DAR: B.6.3.2

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results**	**Reference**
sacrificed at 7 days) Non-guideline Non-GLP Classification criteria: STOT-RE 1 ≤ 10 mg/kg bw/day STOT-RE 2 10 < C ≤ 100 mg/kg bw/day	3.81/4.36, 11.5/13.85, 39.57/42.57 and 123.11/129.66 mg/kg bw/day males/females) Administered daily in the diet for 90 days.	NOAEL: 1000 ppm (equivalent to 123 and 130 mg/kg bw/day in males and females)	
Carcinogenicity study in mice C57Bl/6J Rj 50/sex/dose OECD 451 (2009) GLP Classification criteria: STOT-RE 1 ≤ 1.67 mg/kg bw/day STOT-RE 2 1.67 < C ≤ 16.7 mg/kg bw/day	Cinmethylin Purity: 93.5 % Enantiomeric ratio: 48:52 (-/+) Route: oral (diet) Dose: 0, 150, 1000 and 5000 ppm (equivalent to 0, 25/27, 162.3/183.8 and 904/939.1 mg/kg bw/day males/females) Administered daily in the diet for 18 months	Non-neoplastic findings presented only (for neoplastic findings, see Section 10.11). There were no treatment-related deaths of clinical findings at any dose. **<u>150 ppm (25/27 mg/kg bw/day):</u>** ↓ Body weight gain: 18.3 %* in males and 14.4 %* in females ↓ Food consumption (Day 0 – week 78): 17.4 % in females **<u>1000 ppm (162.3/183.8 mg/kg bw/day):</u>** ↓ Body weight: 10.7 %** in females ↓ Body weight gain: 9.9 % in males and 21.6 %** in females ↓ Food consumption (Day 0 – week 78): 7 % in males and 16.3 % in females *Organ weights:* Liver: rel. 11.9 %** in males *Histopathology:* *Nasal cavity (level III):* Respiratory metaplasia: 19/50* males versus 8/50 in controls and 13/50 females versus 8/50 in controls	Anon, 2018d DAR: B.6.5.2

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results	Reference
		Degeneration/regeneration of the olfactory epithelium: 6/50 males versus 1/50 in controls and 1/50 females versus 0 in controls **<u>5000 ppm (904/939.1 mg/kg bw/day):</u>** ↓ Body weight: 18.1 %** in males and 22.5 %** in females ↓ Body weight gain: 46.5 %** in males and 45.4 %** in females ↓ Food consumption (Day 0 – week 78): 10 % in males and 24.9 % in females *Organ weights:* Liver: abs. 12.3 %** and rel. 38.3 %** in males and rel. 27.3 %** in females *Histopathology:* *Liver*: Centrilobular hypertrophy: 5/50* males versus 0 in controls Periportal hypertrophy: 34/50* females versus 0/50 in controls Oval cell hyperplasia: 38/50* females versus 2/50 in controls *Nasal cavity (level III):* Respiratory metaplasia: 47/50** males versus 8/50 in controls and 41/50** females versus 8/50 in controls Degeneration/regeneration of the olfactory epithelium: 50/50** males versus 1/50 in controls and 27/50** females versus 0 in controls No NOAEL was established due to effects on body weight in low dose females.	
Carcinogenicity study in mice B6C3F1 50/sex/dose (100/sex/control group)	Cinmethylin Purity: 92 % Enantiomeric ratio: not specified Route: oral (diet) Dose: 0, 300, 100 and 1000 ppm (equivalent to 0, 7.2/8.3, 22.1/26.8 and 231/272 mg/kg	Non-neoplastic findings presented only (for neoplastic findings, see Section 10.11). There were no treatment-related deaths or clinical findings at any dose. **<u>30 ppm (7.2/8.3 mg/kg bw/day):</u>** No treatment-related findings.	Anon, 1986b; Anon, 1991d DAR: B.6.5.2

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results**	**Reference**
Non-guideline Non-GLP Classification criteria: STOT-RE 1 ≤ 1.67 mg/kg bw/day STOT-RE 2 1.2 < C ≤ 12 mg/kg bw/day	bw/day males/females) Administered daily in the diet for 24 months	**<u>100 ppm (22.1/26.8 mg/kg bw/day):</u>** No treatment-related findings. **<u>1000 ppm (231/272 mg/kg bw/day):</u>** *Organ weight:* ↑ Liver weight: abs. 22.02 % and rel. 27.01 % in males and abs. 18.64 % and rel. 13.08 % in females. **<u>NOAEL:</u>** 100 ppm (equivalent to 221./26.8 mg/kg bw/day in males/females)	
Repeated dose 35-day oral toxicity study in dogs Beagle 2/sex/dose Non-guideline Non-GLP	Cinmethylin Purity: 92.4 % Enantiomeric ratio: Not specified Route: oral (diet) Dose: 0, 300, 3000, 10000 and 30000 ppm (equivalent to 0, 8.8/10.5, 131.1/103.6, 338.7/334.2 and 330.0/433.6 mg/kg bw/day males/females) Note – cinmethylin intake was not proportionally increased for the high dose animals; males had a higher intake in the 10000 ppm dose group compared to males in the 30000 ppm dose group.	There were no treatment-related deaths at any dose. **<u>≤ 3000 ppm (131.1/103.6 mg/kg bw/day):</u>** No adverse treatment-related effects **<u>10000 ppm (338.7/334.2 mg/kg bw/day):</u>** *Organ weights*: ↑ Liver weight: abs. 38 % and rel. 35 % in males; abs. 20 % and rel. 27 % in females *Histopathology:* *Liver:* Hepatocytes with clear cytoplasm (midzonal): 2/2 males versus 0 in controls. **<u>30000 ppm (330.0/433.6 mg/kg bw/day):</u>** ↓ Body weight: 38 % in males and 33 % in females Animals lost weight during the study – in males mean body weight change was -2645 g** versus +970 g in controls and in females mean body weight change was -2020 g** versus +515 g in controls. ↓ Food consumption: 22-40 %* in males and 3-61 %* in females	Anon, 1984a DAR: B.6.3.1

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results**	**Reference**
	Administered daily in the diet for 5 weeks	*Urinalysis:* ↓ Urine output ↓ Kidney function (as measured by excretion of phenolsulfonpthalein) in males and females *Organ weights:* ↑ Liver weight: rel. 30 % in males and rel. 50 %** in females *Histopathology:* *Liver:* Hepatocytes with clear cytoplasm (central): 1/2 males versus 0 in controls. Centrilobular congestion: 2/2 females versus 1 in controls. *Kidney:* Pars recta: vacuolated and enlarged epithelium: 1/2 males and 2/2 females versus 0 in controls. Pars recta: vacuolated epithelium: 1/2 males versus 0 in controls. NOAEL: 3000 ppm (equivalent to 131 and 104 mg/kg bw/day in males and females)	
Repeated dose 90-day oral toxicity study in dogs Beagle 6/sex/dose US EPA (similar to OECD 409) GLP	Cinmethylin Purity: not specified Enantiomeric ratio: Not specified Route: oral (diet) Dose: 0, 2, 100, 200, 3000 and 6000 ppm (equivalent to 0, 0.06, 2.9/3.0, 5.6/5.8, 96.5/91.9 and 180.5/192.3 mg/kg bw/day males/females)	There were no treatment-related deaths at any dose. **≤ 200 ppm (5.6/5.8 mg/kg bw/day):** No adverse treatment-related effects. **3000 ppm (96.5/91.9 mg/kg bw/day):** *Organ weights:* Liver weight: abs. 20.6 % and rel. 16.1 % in males; abs. 16.4 % and rel. 22.2 %* in females **6000 ppm (180.5/192.3 mg/kg bw/day):** *Clinical chemistry:* ↑ Alanine phosphatase: 36.9 % in males and 63.5 % in females	Anon, 1987a DAR: B.6.3.2

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results**	**Reference**
	Administered daily in the diet for 90 days	*Organ weights:* Liver weight: abs. 29.5 %* and rel. 23.1 %** in males; abs. 30.7 %** and rel. 44.1 %** in females NOAEL: 200 ppm (5.6 and 5.8 mg/kg bw/day in males and females)	
Repeated dose 1-year oral toxicity study in dogs Beagle 6/sex/dose US EPA (similar to OECD 452) GLP	Cinmethylin Purity: 91 % and 93 % (two batches used) Enantiomeric ratio: Not specified Route: oral (diet) Dose: 0, 300, 3000 and 10000 ppm (equivalent to 0, 7.9, 83.4/81.4 and 235.9/284.8 mg/kg bw/day males/females) Administered daily in the diet for 52 weeks.	**<u>300 ppm (7.9 mg/kg bw/day):</u>** No adverse treatment-related findings **<u>3000 ppm (83.4/81.4 mg/kg bw/day):</u>** ↓ Body weight: 12 %* in females ↓ Body weight gain: 15.7 % in males and 33 % in females *Haematology:* ↑ White blood cell count: 17.5 % in males and 45.4 %* in females ↑ Neutrophil count: 10 % in males and 14 %* in females ↑ Lymphocyte count: 18.5 % in males *Clinical chemistry:* ↑ Alkaline phosphatase: 84 %* in males and 88 %* in females ↓ Albumin: 11 %* in males *Organ weight:* ↑ Liver: abs. 24 %* and rel. 27 %* in males; abs. 11 % and rel. 26 %* in females **<u>10000 ppm (235.9/284.8 mg/kg bw/day):</u>** ↓ Body weight: 21 %* in males and 28 %* in females ↓ Body weight gain: 57 % in males and 73 %** in females ↓ Food consumption: 39 %* in males and 44 %* in females *Haematology:*	Anon, 1985b DAR: B.6.3.3

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results**	**Reference**
		↓ Red blood cell count: 17.7 %* in males and 20.3 %* in females ↓ Haemoglobin: 16.4 %* in males and 19.6 %* in females ↓ Haematocrit: 16.9 %* in males and 17.8 %* in females ↑White blood cell count: 51.5 % in males and 87.4 %* in females ↑ Neutrophil count: 43.5 % in males and 31.4 %* in females ↑ Lymphocyte count: 59.3 %* in males and 86.2 %* in females ↑ Monocyte count:300 % in males *Clinical chemistry:* ↑ Alkaline phosphatase: 350 %* in males and 335 %* in females ↓ Albumin: 13 %* in males and 16 %* in females *Organ weight:* ↑ Liver: abs. 42 %** and rel. 76 %** in males; abs. 30 %** and rel. 84 %** in females NOAEL: 300 ppm (equivalent to 7.9 mg/kg bw/day in males and females)	
Repeated dose 1-year oral toxicity study in dogs Beagle 6/sex/dose US EPA (similar to OECD 452) GLP	Cinmethylin Purity: 92.4 % Enantiomeric ratio: Not specified Route: oral (diet) Dose: 0, 2, 30, 100, 200 and 3000 ppm (equivalent to 0, 0.044/0.048, 0.68/0.74, 2.3/2.4, 4.7/4.3 and 80.8/70.7 mg/kg bw/day males/females)	There were no treatment-related deaths or clinical signs of toxicity at any dose. **<u>3000 ppm (80.8/70.7 mg/kg bw/day):</u>** *Haematology:* ↑ WBC: 24.9 %* in males ↑ Neutrophils: 69.8 %* in males versus 59.0 % in controls ↓ Lymphocytes: 29.7 %* in males versus 40.2 % in controls *Clinical chemistry:* ↑ Alkaline phosphatase: 58.5 %* (week 13), 53.5 %* (week 26) and 50.8 %* (week 52) in females only.	Anon, 1988b DAR: B.6.3.3

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results**	**Reference**
	Administered daily in the diet for 52 weeks.	Organ weight: ↑ Liver: abs. 23.1 % and rel. 26.3 %* in males **≤200 ppm (4.7/4.3 mg/kg bw/day):** No treatment-related adverse effects. NOAEL: 200 ppm (equivalent to 4.7 mg/kg bw/day in males and 4.3 mg/kg bw/day in females)	
Repeated dose 1-year oral toxicity study in dogs Beagle 6/sex/dose US EPA (similar to OECD 452) GLP	Cinmethylin Purity: 92.4 % Enantiomeric ratio: Not specified Route: oral (diet) Dose: 0, 2, 30, 100, 200 and 3000 ppm (equivalent to 0, 0.04, 0.63/0.62, 2.3/2.1, 4.1 and 64.3/71.2 mg/kg bw/day males/females) Administered daily in the diet for 52 weeks, followed by a 26 week recovery period (no treatment).	There were no treatment-related deaths or clinical signs of toxicity at any dose. **3000 ppm (64.3/71.2 mg/kg bw/day):** *Haematology:* ↑ WBC: 30.5 %** in males ↑ Total neutrophils: 41.2 %* in males **≤200 ppm (4.1 mg/kg bw/day):** No treatment-related adverse effects. NOAEL: 200 ppm (equivalent to 4.1 mg/kg bw/day in males and females).	Anon, 1988c DAR: B.6.3.3
DERMAL			
Repeated dose 28-day dermal toxicity study in rats Wistar 10/sex/dose	Cinmethylin Purity: 93.5 % Enantiomeric ratio: 48: 52 (-/+) Route: dermal (semi-occlusive) Dose: 0, 100, 300, and 1000 mg/kg	There were no treatment-related deaths or clinical signs of toxicity at any dose. **100 mg/kg bw/day:** No treatment-related effects observed. **300 mg/kg bw/day:** Local effects:	Anon, 2018i DAR: B.6.3.4

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results	Reference
OECD 410 (1981) GLP Classification criteria: STOT-RE 1 ≤ 60 mg/kg bw/day STOT-RE 2 60 < C ≤ 600 mg/kg bw/day	bw/day in 4 mL/kg bw Administered daily for 4 weeks.	Slight erythema: 1/10 males and 1/10 females **<u>1000 mg/kg bw/day:</u>** Local effects: Slight erythema: 2/10 males and 2/10 females Crust formation: 4/10 males and 2/10 females NOAEL (systemic toxicity): 1000 mg/kg bw/day (males and females)	

↑↓ denote an increase or decrease in a parameter as a proportion of the control value
*Statistical significance: * p ≤ 0.05, ** p ≤ 0.01*
abs. = absolute
rel. = relative

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

Cinmethylin has been studied following repeated oral dosing in rats, mice and dogs and in a repeated dose dermal study in rats. Information is also available from a two generation reproduction study in rats, two carcinogenicity studies in rats and two carcinogenicity studies in mice.

The primary target organs are the liver (in all three species tested), the nasal cavity (level III) in rats and mice and the kidney (in rats only). In general, the adverse effects observed occurred at doses considered above the cut-off criteria for classification. In the Draft Assessment Report (GB, 2020), effects observed in the kidney of rats were attributed to a male rat specific mechanism of action, α2μ-globulin accumulation. Further information relating to that is considered further below.

Oral studies:

Rats, 28-day (one study)

In a 28-day, GLP and OECD test guideline compliant study, cinmethylin was administered to Wistar rats (5/sex/group) in the diet at concentrations of 0, 1500, 5000 and 1500 ppm (equivalent to 0, 137/141, 477, 1522/1331 mg/kg bw/day in males/females) (Anon, 2015a). Only effects at the lowest concentration of 1500 ppm (137/141 mg/kg bw/day) are relevant for classification (STOT-RE 2: 30 > C ≤ 300 mg/kg bw/day). At this dose, there were no adverse treatment-related findings.

At concentrations of ≥ 5000 ppm there were increases in liver weight in males and females (up to 66 % greater than controls) and increases in kidney weight in males only (up to 19 % greater than controls). Clinical chemistry findings such as increased γ-glutamyl transferase activity, increased total proteins, cholesterol and triglycerides and decreased glucose levels were observed in both males and females at the mid dose and above, and were indicative of liver toxicity and alterations in liver metabolism.

Histopathology supported these findings with all females found to have centrilobular hypertrophy (slight - moderate) and all males with diffuse hypertrophy of the liver (minimal to slight) (versus 0 control animals).

Males in all groups (both treated and control) were observed to have eosinophilic droplets in the proximal tubules of the kidney. However, the severity of the finding was found to increase in a dose-dependent manner from the mid dose. In the 90-day study by Anon (2018g), these droplets were shown to be α2μ-globulin (see 90 day study below for further details).

In the thyroid, follicular hypertrophy/hyperplasia was observed in males and females of the mid and top dose groups, and at the top dose only, the colloid was altered in males and females.

There were no toxicologically relevant findings in this study at doses within the guidance value ranges for classification.

Rats, 90-day (two studies)

In a recently performed GLP and OECD test guideline compliant study (Anon, 2018g) Wistar rats (10/sex/group) were administered cinmethylin in the diet at concentrations of 0, 1000, 3000 and 10,000 ppm for 90 days (equivalent to 0, 67/79, 211/240, 792/814 mg/kg bw/day in males/females). Only the lowest concentration of 1000 ppm was relevant for classification (STOT-RE 2: 10 < C ≤ 100 mg/kg bw/day). At this concentration, no adverse treatment-related effects were observed. At concentrations ≥ 3000 ppm the liver, thyroid and nasal cavity appeared to be the target organs in both males and females. In males only, the kidneys were adversely affected.

Liver toxicity was evidenced by an increase in weight in both sexes at the mid dose and above (up to 62 % greater than controls), centrilobular hypertrophy in females from the mid dose, diffuse hypertrophy, fatty changes in males treated with 10,000 ppm and dark brown discolouration and peripheral pigment storage in males and females of the top dose group. Changes to clinical chemistry such as increased γ-glutamyl transferase activity, cholesterol and triglycerides and decreased glucose and prothrombin time corroborated the findings in the liver.

Thyroid weight was increased in females of the top dose group only (relative weight: 25 % greater than controls). From the mid dose and above hypertrophy/hyperplasia was observed in both males and females with a dose-related increase in incidence and severity.

Degeneration followed by regeneration of the olfactory epithelium (nasal cavity) was observed in all males from the mid dose group and in 4/10 and 7/10 females of the mid and top dose groups respectively. The area most affected was the dorsal meatus. Proteinaceous exudation was observed along with granulocytic infiltrates within the epithelium in males and females.

In males, minimal to slight chronic nephropathy was observed in all treatment groups with incidence and severity increasing with dose. Although granular casts were noted in all treated groups in the absence of a dose-response, the severity increased in a dose-related fashion. Eosinophilic droplets in the cytoplasm of the proximal convoluted tubules occurred in males of all treatment groups. Again, the severity of this finding increased with increasing dose. Immunohistochemical staining showed these droplets to be α2μ-globulin, a low molecular weight protein, primarily synthesised in male rats with the capability to bind to certain chemicals. The resultant adducts accumulate as droplets in the kidneys and cause progressive renal toxicity within a few weeks which can ultimately lead to kidney tumours. According to the guidance on the application of the CLP Criteria Version 5.0 (ECHA, 2017), this specific mechanism is unique to male rats and has no relevance to humans.

In mid and high dosed females, vacuolation of the interstitial glands of the ovaries was observed. This effect was not observed in any other studies and was considered a chance finding of limited toxicological relevance by the authors of the Draft Assessment Report (GB, 2020). The Agency agrees with this assessment.

There were no toxicologically relevant findings in this study at doses within the guidance value ranges for classification.

In a non-GLP and non-guideline study published in 1983, F344 rats (20/sex/group) were administered cinmethylin in the diet at concentrations of 0, 30, 100, 300 and 1000 ppm (equivalent to 0, 2.18/2.61, 7.51/8.73, 22.51/26.08 and 75.78/88.56 mg/kg bw/day in males/females) (Anon, 1983a). The study was considered limited due to a number of deviations, these included:

- Purity was not reported.
- Lack of toxicity up to the top dose.
- Details of test substance stability and homogeneity of the preparation were not reported.
- Ophthalmological examination prior to administration of the test substance was not performed.
- In haematology and clinical biochemistry, blood clotting time/potential, creatinine and gamma glutamyl transpeptidase were not determined.
- Protein measurement was not conducted in the urine.
- At gross necropsy, weight of adrenals, ovaries, thymus and spleen were not recorded.
- Histopathology was not performed on parathyroid.

All doses were within the guidance value ranges for classification (STOT RE 1: ≤ 10 mg/kg bw/day, STOT RE 2: STOT-RE 2: 10 < C ≤ 100 mg/kg bw/day).

The results of this study showed there were no adverse treatment-related findings following dietary administration of cinmethylin up to a dose of 75.78/88.56 mg/kg bw/day in F344 rats.

Rats, reproductive toxicity (2 studies – only one of which was considered acceptable and is included in Table 25)

In a well-conducted two-generation test, Wistar rats (25/sex/group) were administered cinmethylin in the diet for 10 weeks prior to mating and throughout mating, gestation and lactation (equivalent to approximately 90 days) (Anon, 2018h). The dose was 0, 250, 1000 and 5000 ppm (equivalent to 0, 19.7-21.8/20.6-23.8, 79.4-87.7/81.3-96.9 and 412-450/394-481 mg/kg bw/day in males/females). In females, the dose was adjusted to 125, 500 and 2500 ppm during lactation to ensure the achieved dose intakes during this period were equivalent to those in the pre-mating phase. Using the guidance values for a standard 90-day repeated dose study (STOT RE 2: STOT-RE 2: 10 < C ≤ 100 mg/kg bw/day), only effects observed following intake of 250/125 and 1000 ppm are relevant for consideration of classification.

At 250/125 ppm (19.7-21.8/20.6-23.8 mg/kg bw/day) there were no adverse treatment-related findings. At the mid dose of 1000/500 ppm (79.4-87.7/81.3-96.9 mg/kg bw/day) there was a small increase in liver weight in males only (absolute: 10.8 % and relative 8 % greater than controls). There was no associated clinical chemistry or histopathological correlates at this dose.

At the top dose of 5000/2500 ppm (412-450/394-481 mg/kg bw/day), the liver, kidney, thyroid and nasal cavity were adversely affected.

Liver weight was increased by up to 26 % in males and females, although there was no concomitant histopathology associated with this finding.

Kidney weights were also statistically-significantly increased at the top dose in males only. This was associated with chronic nephropathy, eosinophilic droplets and granular casts. These effects corroborate

the findings observed in the repeated dose studies in the rat and are considered a consequence of α2μ-globulin accumulation.

The thyroid was found to be enlarged in both males and females of the top dose group, the weight of which was found to be up to 19 % greater than controls. Hypertrophy/hyperplasia of follicular epithelial cells was also observed in both sexes.

All animals of the top dose were found to have degeneration/regeneration of the olfactory epithelium compared to 0 animals in the control groups.

The only finding in this study occurring at a dose relevant for classification with STOT-RE 2 was increased liver weight in males dosed with 79.4-87.7 mg/kg bw/day. At this dose, there were no histopathological correlates. Although the finding might be relevant to humans, it is not considered as a significant toxic effect worthy of classification.

A second two-generation study is available in rats (Anon, 1986c). This study had a large number of deviations from the guideline and is of low reliability (see Section 10.12 for full list of study deviations and limitations). Briefly, cinmethylin was administered in the diet to Sprague Dawley rats (20 male and 30 females/dose for the F_0 parental generation and 20 male and 25-30 females for the F_1 parental generation) for 10 weeks prior to mating and then throughout the mating, gestation and lactation periods. Concentrations administered were 0, 200, 2000 or 20000 ppm (equivalent to 0, 12-17/13-34, 115-170/130-353 and 1290-2125/1434-2893 mg/kg bw/day for males/females respectively. Only the lowest dose in this study was relevant for classification. Clinical signs in parental animals were mainly observed from the mid dose upwards. At the mid dose, increases in liver weight were observed in males and females (with no histopathological correlates). The top dose of 20000 ppm far exceeded the guidance limits for classification. An increase in mortality and more severe liver findings were observed in the top-dose treated animals.

Rats, combined chronic toxicity/carcinogenicity (2 studies)

In a combined chronic toxicity and carcinogenicity study (GLP and OECD test guideline compliant), Wistar rats were administered cinmethylin in the diet for either 12 months (10/sex/group) or 24 months (50/sex/group) (Anon, 2018c). Concentrations administered were 0, 200, 1000 and 5000 ppm.

In the 12 month group (chronic phase), the equivalent doses were 0, 10/13, 51/69 and 265/351 mg/kg bw/day in males/females. Only the low dose group was relevant for classification and at this dose, no adverse treatment-related findings were observed (STOT RE 2: 2.5 < C ≤ 25 mg/kg bw/day). Findings were limited mainly to the top dose group (265/351 mg/kg bw/day). At this dose, the liver, kidney, thyroid and nasal cavity were adversely affected. Females also had reduced body weight (12.6 % of controls) and reduced body weight gain (21.3 % of controls).

Liver weight was increased in males and females (up to 17 % greater than controls) and with this γ-glutamyl transferase was also statistically-significantly increased. Centrilobular hypertrophy was observed in both males and females and there were cytoplasmic alterations in males only (Grade 1: 1/3 males and Grade 2: 2/3 males versus 0 males in control group).

Similar to the previously described studies, kidney effects were observed in males of the top dose group only. These included increased weight (15 % greater than controls) and the presence of eosinophilic droplets. Although the eosinophilic droplets were not specifically analysed, it is assumed they are α2μ-globulin.

Degeneration/regeneration of the olfactory epithelium (level III) in the nasal cavity was observed in all males and females of the top dose group (versus 0 in control groups). Also observed was the presence of a proteinaceous exudate in the lumen in all animals.

There was an increase in incidence of hypertrophy/hyperplasia of the thyroid gland in males and both males and females were observed to have an increased incidence and severity of altered colloid.

Overall, there were no findings relevant for classification following administration of cinmethylin to rats for 12 months.

In the 24 month group (carcinogenicity phase), the dose received was equivalent to 0, 9/11, 45/59 and 242/317 mg/kg bw/day in males/females, administered daily for 24 months. Only the lowest dose of 9/11 mg/kg bw/day is relevant for classification (STOT-RE 2: 1.25 < C ≤ 12.5 mg/kg bw/day). There were no adverse treatment-related findings at this dose. Mortality rates appeared to increase in a dose dependant manner in males (12, 16, 18 and 24 % in 0, 200, 1000 and 5000 ppm groups), however, the numbers of animals dying were not considered excessive and were within the HCD provided (range 0 – 32 %). There were no treatment-related histopathological findings that could account for early death. Body weight was reduced in females of the top dose group (13 % of controls) and body weight gain was reduced in both males and females of the top group (12 % in males and 18 % in females). As with the 12 month phase of the study, the target organs were the liver, kidney, thyroid and nasal cavity.

Findings in the liver included statistically significantly increased weight in males and females of the top dose group (up to 20 %). Centrilobular pigment storage was observed in females of the mid and top dose groups and periportal hypertrophy was seen in females only of the top dose group. Cytoplasmic alterations and multinucleated hepatocytes were found in males only of the top dose group and centrilobular hypertrophy and periportal pigment storage in both sexes of the top dose group.

Kidney weight was statistically significantly increased in top dose group males (18 %) and to a lesser extent in females (11 %), however the latter increase was not statistically significant and there were no histopathological correlates. In males, the increased weight was accompanied by mineralisation (tubular and papilla).

In the nasal cavity, degeneration/regeneration was observed in 2/50 males of the mid dose group and all males and females of the top dose group. Proteinaceous exudate was observed in 20/50 males and 30/50 females, metaplasia of the respiratory epithelium was seen in 14/50 males and 12/50 females versus 0 controls animals. Multifocal inflammation was also observed in males of the mid dose group (5/50 versus 2/50 in controls) and in both sexes at the top dose group (12/50 males versus 2/50 in controls and 8/50 females versus 1/50 in controls). These effects were all deemed treatment-related.

Follicular cell hyperplasia in the thyroid was observed in males of the top dose group only (10/50 versus 2/50 in controls) and altered colloid was seen in both males and females (24/50 males versus 7/50 in controls and 33/50 females versus 5/50 in controls).

After 24 months of administration of cinmethylin to Wistar rats in the diet, there were no adverse treatment-related effects at doses relevant for classification.

In an older study, not conducted according to guidelines or GLP, F344 rats received cinmethylin in the diet daily for periods of 6, 12, 18 or 24 months (10/sex/group for 6 and 12 months, 15/sex/dose for 18 months and 50/sex/group for 24 months) (Anon, 1985a; Anon, 1991a; Anon, 1991b and Anon, 1991c). Animals were administered 0, 30, 100 or 3000 ppm (equivalent to 0, 1.4/1.7, 4.7/5.8 and 144.2/177.4 mg/kg bw/day in males/females). The low and mid dose were within the guidance value range for classification (STOT-RE 2: 1.25 < C ≤ 12.5 mg/kg bw/day). The only adverse finding within this dose range was “slight” focal colloidal basophilia/mineralisation in the thyroid of females receiving 5.8 mg/kg bw/day (18/50 females versus 8/50 in controls). This finding was found to be statistically significant and test substance-related.

No animals of the 6-12 month groups died during the administration period and there was no treatment-related mortality in the 18-month group. At the end of the study, mortality in males was higher than that of females (54-64 % in males versus 30-42 % in females). In males of the top dose group there was an increase

in mortality compared to the controls and lower dose groups (64 % versus 56 % in controls). The increase in mortality at the top dose was concordant with an increase in chronic renal nephropathy (22 males in the top dose with chronic renal disease compared to 12 in controls). Males were also found to have reduced body weight (12 %) and reduced body weight gain (19.9 %). Their appearance was hunched and their eyes pale.

At the top dose, the liver, kidney and thyroid were the primary target organs and effects were also observed in the lymph nodes, stomach, parathyroid gland and blood vessels.

Liver weight was increased in top dose males and females up to (23.3 %) and subscapular dark depressed foci were observed in females only (40/50 females versus 29/50 in controls). In top dose males, periportal chromidial clumping was observed (9 males versus 0 in controls) and in both sexes of the top dose, periportal acidophilia was observed (33/50 males versus 9/50 in controls and 22/50 females versus 2/50 in controls). An increase in γ-glutamyl transferase in males and females supported the findings of liver toxicity.

Kidney weight was increased in both top dose males and females (17 % in males and 13 % in females). However, histopathology and urinalysis only revealed findings in males. Urinalysis revealed an increase in tubular epithelial cells, casts leukocytes and a decrease in osmolarity. Urine volume was increased in males and was noted to be paler in colour. The kidneys were found to be pale (diffuse subcapsular pallor) in 23/50 males (versus 14/50 in controls). Subcapsular pitting/rough surface (severe/very severe) was also noted in 27/50 males (versus 12/50 in controls). Chronic nephropathy with increased severity was apparent in 33/50 males (versus 17/50 in controls).

Findings in the thyroid included statistically significant increase in focal colloidal basophilia/mineralisation in top dose females (21/50 versus 8/50 in controls).

Other potentially treatment-related findings included renal lymph node haemorrhage/enlargement in 4/50 males (versus 0 in controls), gastric mucosal thickening in 18/50 males (versus 8 in controls) and fundic glandular mineralisation (13/50 males versus 3/50 in controls. Enlargement of the parathyroid gland was observed in 16/50 males (versus 7/50 in controls) and a statistically significant increase in incidence of diffuse hyperplasia was noted also in males (29/50 versus 15/50 in controls). In the blood vessels, thickening of the aortic mucosa was seen in 18/50 males versus 11/50 in controls.

Overall, the majority of the findings in this study occurred at doses not relevant for classification. The only finding at a dose relevant for classification was increased incidence of females with “slight” focal colloidal basophilia/mineralisation of the thyroid following 24 months of dosing with 100 ppm cinmethylin (5.8 mg/kg bw/day). However, in the absence of any follicular cell hypertrophy and/or hyperplasia, these mild histopathological findings are not adverse.

Mice, 28 day (1 study)

In a well-conducted study, cinmethylin was administered to C57BL/6JRj mice (5/sex/dose) in the diet for 28 days (Anon, 2016f). Doses were 0, 400, 1200 and 4000 ppm (equivalent to 0, 95.1/92.4, 295.9/254 and 791.4/1015.6 mg/kg bw/day; only effects observed at the low and mid dose are relevant for classification (STOT-SE 2: 30 < C ≤ 300 mg/kg bw/day).

Throughout the study no treatment-related deaths occurred and there were no clinical signs of toxicity. No adverse treatment-related effects were observed at the low dose of 400 ppm (95.1/92.4 mg/kg bw). At the mid dose of 1200 ppm (295.9/254 mg/kg bw/day) an increase in liver weight was observed in males and females (males: abs. 15 % and rel. 10 % and females: abs. 13 % and rel. 11 %). Only the relative liver weight was statistically significantly increased, but at this dose, the increase was considered an adaptive effect as there were no clinical chemistry or histopathology findings to support an adverse effect.

The top dose of 4000 ppm (791.4/1015.6 mg/kg bw) far exceeded the guidance value range for classification. At this dose, body weight gain was affected in males (decrease of 44 % compared to controls). Liver weight was adversely affected with a statistically significant increase in both males and females (males: abs. 16 % and rel. 19 % and females: abs. 26 % and rel. 22 %). Clinical chemistry findings supported liver toxicity (decreases in bilirubin, total proteins, albumin, globulin, cholesterol and triglycerides).

There were no findings in this repeated dose study to support classification for specific target organ toxicity.

Mice, 90 day (2 studies)

In a 90-day study, conducted according to OECD guidelines and GLP, C57BL/6JRj mice (10/sex/dose) received cinmethylin in the diet at doses of 0, 200, 1000 and 5000 ppm (equivalent to 0, 43/58, 201/285 and 1200/1304 mg/kg bw/day) for 90 days (Anon, 2018f). All doses, with the exception of the lowest were considered above the limits for classification (STOT-SE 2: 10 < C ≤ 100 mg/kg bw/day). At the low dose of 200 ppm (43/58 mg/kg bw/day) no adverse effects were observed. No treatment-related clinical signs or mortality were observed at any dose.

At the mid dose of 1000 ppm (201/285 mg/kg bw/day) a decrease in cholesterol was noted in males only. At the top dose of 5000 ppm (1200/1304 mg/kg bw/day) there was evidence of liver toxicity with an increase in liver weight in males and females (20 – 30 % greater than controls) and associated clinical chemistry (decreases in cholesterol, bilirubin and triglycerides). The thymus weight was also found to be in females (abs. 13.4 % and rel. 17.7 %).

Overall, there were no adverse findings at doses relevant for classification in this study.

In an older 90-day study in mice (non-guideline and non-GLP), B6C3F1 mice (20/sex/dose) were fed a diet containing cinmethylin at concentrations of 0, 30, 100, 300 and 1000 ppm (equivalent to 0, 3.81/4.36, 11.5/13.85, 39.57/42.57 and 123.11/129.66 mg/kg bw/day in males and females) (Anon, 1983b). In support of the previous study, no adverse treatment-related effects were observed at any dose level.

Mice, carcinogenicity (2 studies)

In a well-conducted carcinogenicity study in C57B1/6J Rj mice lasting 18 months, animals were fed cinmethylin in their diet at concentrations of 0, 150, 1000 and 5000 ppm (equivalent to 0, 25/27, 162.3/183.8 and 904/939.1 mg/kg bw/day in males/females) (Anon, 2018d). The extrapolated guidance values for this study were: STOT-RE 2 1.67 < C ≤ 16.7 mg/kg bw/day. Therefore, all doses used in this study were above the limits for classification.

A reduction in body weight was noted at all doses with an associated reduction in food consumption (body weight gain was reduced between 14 – 45 % and food consumption decreased between 7 – 25 %, depending on dose). At doses of 1000 ppm and above (≥ 162.3/183.8 mg/kg bw/day) the liver was adversely affected. Increases in liver weight were observed at the mid and top dose (11.9 % - 38.3 %) and hypertrophy and hyperplasia was observed at the top dose only. Histopathology also revealed an effect on the nasal cavity (level III) at the mid and top dose, with respiratory metaplasia and degeneration/regeneration of the olfactory epithelium in males and females (incidence and severity increasing with dose).

Overall, there were no adverse treatment-related effects at doses relevant for classification in this 18-month study in mice.

An older carcinogenicity study in mice is available with a number of limitations and deviations rendering it unsuitable to conclude on carcinogenicity (See Section 10.11 for list of limitations and deviations) (Anon, 1986b; Anon, 1991b). However, the findings in this study were still considered useful in contributing to the weight of evidence for effects on systemic toxicity following repeated dosing. B6C3F1 mice (50/sex/dose in the main carcinogenicity group and 120/sex/controls) were administered cinmethylin in the diet for 24 months at concentrations of 0, 30, 100 and 1000 ppm (equivalent to 0, 7.2/8.3, 22.1/26.8 and 231/272 mg/kg bw/day in males/females). The extrapolated guidance values for this study were: STOT-RE 2 1.25 < C ≤ 12.5 mg/kg bw/day. Therefore, all only effects occurring at the lowest dose of 30 ppm (7.2/8.3 mg/kg bw/day) could be considered relevant for classification. At this dose, there were no treatment-related findings.

There were no effects occurring at the mid-dose of 100 ppm (22.1/26.8 mg/kg bw/day). At the top dose of 1000 ppm (231/272 mg/kg bw/day) an increase in liver weight was observed in both males and females (up to 27 % compared to controls). There was no associated histopathology or clinical chemistry findings.

Overall, there were no adverse treatment-related effects at doses relevant for classification in this 24-month study in mice.

Dogs, 35 days (1 study)

An old, 5 week dietary study in Beagle dogs (2/sex/dose) investigated effects following repeated dosing of cinmethylin (Anon, 1984a). Concentrations administered were 0, 300, 3000, 10000 and 30000 ppm (equivalent to 0, 8.8/10.5, 131.1/103.6, 338.7/334.2 and 330.0/433.6 mg/kg bw/day in males/females). Cinmethylin intake was not increased proportionally for high dose animals; intake in males in the 10000 ppm group was higher than males in the 30000 ppm dose group. Using the guidance value ranges based on a 90-day repeated dosing study in rats, only effects occurring at doses of ≤ 300 mg/kg bw/day can be considered relevant for classification (STOT RE 1: C ≤ 30 mg/kg bw/day and STOT RE 2: STOT-RE 2 30 < C ≤ 300 mg/kg bw/day). Using these ranges as a guide, all doses are relevant for classification for males and in females.

At doses ≤ 3000 ppm (131.1/103.6 mg/kg bw/day) there were no adverse treatment-related effects. At 10000 ppm (338.7/334.2 mg/kg bw/day), an increase in absolute and relative liver weights were observed in both males and females (abs. 38 % and rel. 35 % in males; abs. 20 % and rel. 27 % in females). There was no associated clinical chemistry or histopathology in females but in males, hepatocytes were observed to be enlarged and contain clear cytoplasm (2/2 males versus 0 in controls). Effects in the liver were not considered severe by the study authors.

At 30000 ppm (330/433.6 mg/kg bw/day), animals lost weight during the course of the study. In males, terminal body weight was 38 % lower than controls and in females it was 33 % lower than controls. The mean body weight change in males was -2645 g (versus +970 g in controls) and in females it was -2020 g (versus +515 g in controls). This was observed with a reduction in food consumption (22-40 % in males and 3-61 % in females versus controls). There were no reductions in body weight in males of the 10000 ppm group, despite these animals receiving a higher dose. Animals in the top dose group were found to be dehydrated during weeks 3-5 following treatment with cinmethylin. Urinalysis showed decreased urine output and kidney function was impaired in males and females (as measured by excretion of phenolsulfonpthalein). The liver was the main target organ in this study. In top-dose treated males relative liver weight was 30 % higher than controls; in females, relative weight was 50 % higher than controls. Central hepatocytes were enlarged and had clear cytoplasm in 1/2 males (versus 0 control animals) and centrilobular congestion was noted in both treated females (compared to only 1/2 control animals). In the pars recta of the kidneys, vacuolated and enlarged epithelium were observed in 1/2 males and 2/2 females (versus 0 control animals). Coupled with the urinalysis observations, this finding was most likely linked to the observation of dehydration.

Treatment-related findings at doses that might be considered relevant for classification included weight loss, dehydration and increased liver weight with associated histopathology from a dose of 330 mg/kg bw/day.

Dogs, 90 days (1 study)

In an old, GLP and US EPA-compliant (similar to OECD 409) study in Beagle dogs, males and females (6/sex/dose) received cinmethylin daily in the diet for 90-days (Anon, 1987a). There were a number of deviations to the OECD guideline but overall, the study is considered acceptable in the assessment of the weight of evidence for classification. Deviations from the previous test guideline OECD TG 409 (1981) were:

- Batch and purity were not reported.
- Ophthalmological examination were not conducted.
- Ornithine decarboxylase and gamma glutamyl transpeptidase were not determined.

Compared to the current OECD TG No. 409 (1998) :

- Weekly detailed clinical observations were not performed.
- Cinmethylin purity was not reported.
- Ophthalmological examination were not conducted.
- Additional satellite groups were not included for recovery.
- In haematology and clinical biochemistry, blood clotting potential, ornithine decarboxylase and gamma glutamyl transpeptidase were not determined.
- At gross necropsy, weight of gall bladder, thymus, epididymides and uterus were not recorded.
- Histopathology was not performed on larynx, pharynx, seminal vesicles, coagulating gland, harderian gland and vagina.
- A discussion of the observed significantly changed parameters in relation to historical control data is missing.

Concentrations administered were 0, 2, 100, 200, 3000 and 6000 ppm (equivalent to 0, 0.06, 2.9/3.0, 5.6/5.8, 96.5/91.9 and 180.5/192.3 mg/kg bw/day in males/females). Using the guidance value ranges based on a 90-day repeated dosing study in rats, only effects occurring at doses of ≤ 100 mg/kg bw/day can be considered relevant for classification (STOT RE 1: C ≤ 10 mg/kg bw/day and STOT RE 2: STOT-RE 2 10 < C ≤ 100 mg/kg bw/day). Therefore, all doses with the exception of the top dose are relevant for classification.

There were no treatment-related findings at doses ≤ 200 ppm (5.6/5.8 mg/kg bw/day). At the only other dose relevant for classification (3000 ppm, 96.5/91.9 mg/kg bw/day) absolute and relative liver weights were increased in both males and females (abs. 20.6 % and rel. 16.1 % in males; abs. 16.4 % and rel. 22.2 % in females). Only the increase in relative weight in females was considered statistically significant. There were no histopathology correlates or clinical chemistry to support this finding.

At the top dose (which exceeded the classification guidance value range), liver weight was statistically significantly increased in both sexes (up to 44.1 %) and clinical chemistry revealed increased levels of alanine phosphatase (up to 63.5 %).

The only finding occurring at a dose relevant for classification was increased liver weight in males and females.

Dogs, 1 year (3 studies)

A 1-year repeated dose oral toxicity study was performed by Anon (1985b). Cinmethylin was administered in the diet to Beagle dogs (6/sex/dose) in a GLP and US EPA-compliant study (similar to OECD 452) at concentrations of 0, 300, 3000 and 10000 ppm (equivalent to 0, 7.9, 83.4/81.4 and 235.9/284.8 mg/kg bw/day in males/females). Deviations to the current guideline include:

- Clinical biochemistry: gamma glutamyl transpeptidase was not determined.
- The body weight data were recorded with the precision of 0.1 kg only.
- Humidity was 25-75 % instead of 30–70 %.
- Detailed clinical observations were not performed.
- In haematology and clinical biochemistry : mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, prothrombin time, activated partial thromboplastin time and gamma glutamyl transpeptidase were not determined.
- Osmolality and specific gravity were not recorded.
- At gross necropsy, weight of epididymides and uterus were not recorded.
- Histopathology was not performed on coagulating gland, Harderian gland, lacrimal gland, deep lymph nodes, seminal vesicle, uterus and vagina.
- A discussion of the observed significantly changed parameters in relation to historical control data is missing.

Guidance range values for classification for a 1 year study (based on 90-day oral studies in rats) are STOT RE 1: C ≤ 2.5 mg/kg bw/day and STOT-RE 2: 2.5 < C ≤ 25 mg/kg bw/day. Using these ranges as a guide, only the lowest dose in this study is relevant for classification. At this dose, there were no treatment-related findings.

At the mid-dose and above decreases in body weight (between 12 – 28 %) and body weight gain (15 – 73 %) were observed. The liver was the target organ with decreases in absolute and relative weight at the mid dose and above (decreases of 11 – 84 % of controls). There was no histopathology to support these findings but clinical chemistry indicated liver toxicity with increases in alkaline phosphatase in both males and females and a decrease in albumin in males of the mid dose and males and females of the top dose.

There were some changes to haematology parameters at the mid dose and above. These included a raised white blood cell, neutrophil and lymphocyte count and, at the top dose only, decreases in red blood cell count, haemoglobin and haematocrit. These findings are considered treatment-related and adverse.

An older one year feeding study in Beagle dogs, carried out according to US EPA guidelines and GLP is also available (Anon, 1988b). Deviations from 1981 OECD guidelines are minimal and the study is considered acceptable with regards to hazard assessment. Guidance range values for classification for a 1 year study (based on 90-day oral studies in rats) are STOT RE 1: C ≤ 2.5 mg/kg bw/day and STOT-RE 2: 2.5 < C ≤ 25 mg/kg bw/day. Using these ranges as a guide, all doses aside from the top dose are relevant for classification. There were no treatment-related findings at doses relevant for classification.

Males and females (6/dose) were fed daily on a diet containing cinmethylin (0, 2, 30, 100, 200 and 3000 ppm – equivalent to 0.044/0.048, 0.68/0.74, 2.3/2.4, 4.7/4.3 and 80.8/70.7 mg/kg bw/day) for 52 weeks. There were no treatment-related deaths and no clinical signs of toxicity. There were no effects on food consumption, body weight or body weight gain at any dose.

There were no adverse findings at doses ≤ 200 ppm. At the top dose of 3000 ppm (80.8/70.7 mg/kg bw/day) haematology revealed changes in white blood cell parameters in males only. These changes included a statistically significant increase in white blood cells (WBC), an increase in neutrophils and a decrease in lymphocytes. Altered alkaline phosphate levels were observed in top dose females only at weeks 13, 26 and 52 (approximately 50 % increase compared to controls).

In males, liver weight was increased (abs. 23.1 % and rel. 26.3 %). Only the relative weight was statistically significantly increased. There were no other findings in the liver. The prostate was found to be small in one control, one 200 ppm and two 3000 ppm males. There were no histopathological correlates.

A second one year feeding study in Beagle dogs was carried out by the same study author under the exact same conditions with the addition of a 26 week recovery period (Anon, 1988c). Guidance range values for classification for a 1 year study (based on 90-day oral studies in rats) are STOT RE 1: C ≤ 2.5 mg/kg bw/day and STOT-RE 2: 2.5 < C ≤ 25 mg/kg bw/day. Using these ranges as a guide, all doses aside from the top dose are relevant for classification. There were no treatment-related findings at doses relevant for classification.

Males and females (6/dose) were fed daily on a diet containing cinmethylin (0, 2, 30, 100, 200 and 3000 ppm – equivalent to 0.04, 0.63/0.62, 2.3/2.1, 4.1 and 64.3/71.2 mg/kg bw/day) for 52 weeks. For the 26 week period after this, animals received a normal diet with no cinmethylin present. There were no treatment-related deaths and no clinical signs of toxicity. There were no effects on food consumption, body weight or body weight gain at any dose.

There were no adverse treatment-related findings at doses ≤ 200 ppm.

At 3000 ppm (64.3/71.2 mg/kg bw/day) a statistically significant increase in WBC and neutrophils were observed in males only. During the recovery period, these findings were reversed.

There were no treatment-related changes in organ weights. Prostate gland size was within the normal range.

Overall, there were no treatment-related findings at doses relevant for classification in these 1 year studies in dogs.

Dermal studies:

Rats, 28-day (one study)

In a well-conducted repeated dose dermal study in Wistar rats (10/sex/dose), cinmethylin was applied to the skin (semi-occlusively) daily for 28-days. Doses were 0, 100, 300 and 1000 mg/kg bw/day in 4 ml/kg drinking water containing 0.5 % carboxymethyl cellulose and Tween 80. The guidance value range for classification following dermal exposure for 28 days is: STOT-RE 1 ≤ 60 mg/kg bw/day and STOT-RE 2 60 < C ≤ 600 mg/kg bw/day. Only effects at the low and mid dose were relevant for classification.

At the low dose of 100 mg/kg bw/day, no treatment-related effects were observed. At the mid dose of 300 mg/kg bw/day slight erythema was observed in 1 male and 1 female. At the top dose, the incidence and severity of these localised skin effects increased.

Overall, there were no systemic effects observed at any dose following topical administration of cinmethylin to rats for 28 days.

10.9.2 Comparison with the GB CLP criteria

STOT RE is assigned on the basis of a substance demonstrating evidence of significant or severe toxicity, generally at or below the oral guidance value of 100 mg/kg/d (for a classification in category 2) obtained in a 90-day rat study.

'Significant' toxicity is taken to mean changes that clearly indicate functional disturbance or morphological changes that are toxicologically relevant. 'Severe' toxicity is considered to be more profound or serious and indicates changes that are of a considerably adverse nature with a significant impact on health.

Cinmethylin has been adequately tested in a number of dietary studies in rats, mice (ranging from 28 days to 2 years) and in dogs (28 days to 1 year). In rats, cinmethylin was also tested in a repeated dose dermal study in which no systemic effects were observed up to a maximum dose of 1000 mg/kg bw/day (21 days).

There were no adverse findings at doses relevant for classification with STOT-RE 1 in any study. Therefore, classification in this category is not required.

In all three species tested, a target organ was the liver. At doses relevant for classification with STOT-RE 2, liver findings were limited to increases in organ weight in male rats of a 2-generation study, male and female mice in a 28-day study and male and female dogs in a 35- and 90-day study (effects summarised in Table 26).

In rats and mice, there was no relevant histopathology or clinical chemistry associated with these findings. In dogs, the hepatocytes of 1/2 males were observed to contain clear cytoplasm. The small sample size and lack of effect in animals treated with a slightly higher dose diminishes the toxicological relevance of this finding. Overall, the findings in the liver of rats, mice and dogs are not considered significant or severe.

Other findings in dogs in a 35-day study included dehydration and body weight loss. Again, the sample size was limited and the same signs were not observed in animals treated with a similar but slightly higher dose. Therefore, the toxicological relevance is questionable and the findings are not considered further for classification.

In rats, effects on the thyroid were also noted, however in the majority of cases, the occurrences were at doses higher than the guidance values for classification. However, in a 2-year carcinogenicity study, an increased incidence of focal colloidal basophilia/mineralisation was observed in females only at a dose relevant for classification. As this finding occurred in a single sex only and in isolation, it does not warrant classification.

Table 26: Adverse effects occurring at doses relevant for classification with STOT RE 2 in rats, mice and dogs following oral administration of cinmethylin in the diet

Study	**(Adjusted) guidance value for STOT RE 2** **mg/kg bw/day**	**Effects at doses below guidance cut-off values**
28-Day study in rats (Wistar)	300	No treatment-related findings at doses ≤ 300 mg/kg bw/day
90-Day study in rats (Wistar)	100	No treatment-related findings at doses ≤ 100 mg/kg bw/day
90-Day study in rats (F344)	100	No treatment-related findings at doses ≤ 100 mg/kg bw/day
2-Generation study in rats (Wistar)	100	**<u>500 ppm (79.4 – 87.7 mg/kg bw/day in males):</u>** ↑ Liver weight: abs. 10.8 % and rel. 8 % in males only.

1-Year combined chronic/carcinogenicity study in rats – chronic phase (Wistar)	25	No treatment-related findings at doses ≤ 25 mg/kg bw/day
2-Year combined chronic/carcinogenicity study in rats– chronic phase (Wistar)	12.5	No treatment-related findings at doses ≤ 12.5 mg/kg bw/day
2-Year carcinogenicity study in rats (F344)	12.5	**100 ppm (5.8 mg/kg bw/day):** Focal colloidal basophilia/mineralisation: 18/50** females versus 8/50 in controls.
28-Day study in mice (C57BL/6JRj)	300	**1200 ppm (295.9/254 mg/kg bw/day in males/females):** ↑ Liver weight: abs. 15% and rel. 10 %* in males and abs. 13 % and rel. 11 %* in females
90-Day study in mice (C57BL/6JRj)	100	No treatment-related findings at doses ≤ 100 mg/kg bw/day
90-Day study in mice (B63CF1)	100	No treatment-related findings at doses ≤ 100 mg/kg bw/day
18-Month carcinogenicity study in mice (C57BL/6JRj)	16.7	All doses were above the guidance values for classification.
2-Year carcinogenicity study in mice (B63Cf1)	12.5	No treatment-related findings at doses ≤ 12.5 mg/kg bw/day
35-Day study in dogs (Beagle)	300	**30000 ppm (330.0 mg/kg bw/day in males):** ↓ Body weight: 38 %lower than controls in males Body weight loss: -2645 g (compared to +970 g in controls) Dehydration: ↓ urine output, ↓ kidney function Vacuolated and enlarged epithelium (pars recta): 1/2 males versus 0 in controls ↑ Liver weight: rel. 30 % in males Hepatocytes with clear cytoplasm: 1/2 males versus 0 in controls **10000 ppm (338.7/334.2 mg/kg bw/day in males/females):** ↑ Liver weight: abs. 38 % and rel. 35 % in males and abs. 20 % and rel. 27 % in females
90-Day study in dogs (Beagle)	100	**3000 ppm (96.5/91.9 mg/kg bw/day in males/females):** ↑ Liver weight: abs. 20.6 % and rel. 16.1 % in males and abs. 16.4 % and rel. 22.2 %* in females
1-Year study in dogs (Beagle)	25	No treatment-related findings at doses ≤ 25 mg/kg bw/day
1-Year study in dogs (Beagle) (1988a)	25	No treatment-related findings at doses ≤ 25 mg/kg bw/day
1-Year study in dogs (Beagle) (1988b)	25	No treatment-related findings at doses ≤ 25 mg/kg bw/day

Therefore, it is concluded that there is no evidence of significant or severe toxicity at doses below the guidance values for classification for specific target organ toxicity following repeated oral or dermal administration of cinmethylin. No classification for this endpoint is warranted.

10.9.3 Conclusion on classification and labelling for STOT RE

Not classified – conclusive but not sufficient for classification

10.10 Germ cell mutagenicity

The genotoxicity of cinmethylin was tested in a range of in vitro and in vivo studies. In vitro studies include two bacterial reverse mutation assays (Ames test), an in vitro mammalian cell gene mutation assay in mouse lymphoma cells and an in vitro micronucleus test in human lymphocytes. In vivo studies consisted of a modern in vivo micronucleus test in mouse bone marrow and an old in vivo chromosome aberration assay in rat bone marrow. All but the in vivo chromosomal aberration assay in the rat were modern, conducted according to current OECD test guidelines and GLP compliant.

Table 27: Summary table of mutagenicity/genotoxicity tests in vitro

Method, guideline, deviations if any	**Test substance,**	**Relevant information about the study including rationale for dose selection (as applicable)**	**Observations**	**Reference**
Reverse mutation assay (Ames test) OECD 471 (1997) GLP	Cinmethylin Purity: 89.6 % Enantiomeric ratio: not specified	Strains used: *S. typhimurium*: TA98, TA100, TA1535 and TA1537 *E. coli*: WP2 uvrA Test concentrations: 0, 33, 100, 333, 1000, 2800, 5600 µg/plate Vehicle: acetone Positive controls: MNNG, AAC, NOPD, 4-NQO or 2-aminoanthracene Standard plate test and pre-incubation test (± S9) 48-72 h incubation	**Negative** No treatment-related increases in the mean number of revertants either in the presence or absence of metabolic activation, up to the limit concentration. Precipitation was observed at the top concentration of 5600 µg/plate.	Woitkowiak C., 2018a DAR: B.6.4.1

Method, guideline, deviations if any	**Test substance,**	**Relevant information about the study including rationale for dose selection (as applicable)**	**Observations**	**Reference**
Reverse mutation assay (Ames test) OECD 471 (1997) GLP	Cinmethylin Purity: 97.5 % Enantiomeric ratio: 50: 50 (-/+)	Strains used: *S. typhimurium*: TA98, TA100, TA1535 and TA1537 *E. coli*: WP2 uvrA Test concentrations: 0, 33, 100, 333, 1000, 2800, 5200 µg/plate Vehicle: acetone Positive controls: MNNG, AAC, NOPD, 4-NQO or 2-aminoanthracene Standard plate test and pre-incubation test (± S9) 48-72 h incubation	**Negative** No treatment-related increases in the mean number of revertants either in the presence or absence of metabolic activation, up to the limit concentration. Precipitation was observed at the top concentration of 5200 µg/plate.	Woitkowiak C., 2018b DAR: B.6.4.1
In vitro forward mutation assay in mouse lymphocytes OECD 490 (2016) GLP	Cinmethylin Purity: 93.5 % Enantiomeric ratio: 48: 52 (-/+)	Mouse L5178Y cells Thymidine kinase locus (TK +/-) **Experiment I:** Incubation time: 4 hours (± S9) Test concentrations: 1.9, 3.8, 7.5, 15.0, 30.0, 60.0, 80.0 100.0, 125.0 µg/mL (+S9) 1.9, 3.8, 7.5, 15.0, 30.0, 45.0, 60.0, 100.0 and	**Negative** Cinmethylin did not cause an increase in mutant frequency to meet the criteria for a positive result either in the presence or absence of metabolic activation. A dose-dependent increase in mutation frequency was noted in the 4 h incubations, in the absence of S9. However, at no timepoint was the threshold (GEF) (a mutation frequency of > 126 of the solvent control) exceeded. Precipitation occurred at concentrations of 60 µg/mL in the absence of S9 and 80 µg/mL in experiment I and at concentrations of 100 µg/mL and above in experiment II.	Sokolowski A., 2018 DAR: B.6.4.1

Method, guideline, deviations if any	Test substance,	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
		125.0 µg/mL (-S9) **<u>Experiment II:</u>** Incubation time: 24 hours (-S9) Test concentrations: 1.9, 3.8, 7.5, 15.0, 30.0, 45.0, 60.0, 100.0 and 125.0 µg/mL		
In vitro micronucleus test in human lymphocytes OECD 487 (2016) GLP	Cinmethylin Purity: 93.2 % Enantiomeric ratio: 48: 52 (-/+)	Human peripheral blood lymphocytes **<u>Experiment I (female 31 years old):</u>** Incubation time: 4 hours Test concentrations: 11.2, 22.3, 44.7, 53.6, 64.3, **77.2, 92.6, 111**, 167, 500 µg/mL (+S9) 11.2, **22.3, 44.7, 53.6**, 64.3, 77.2, 92.6, 111, 167 , 500 µg/mL (-S9) **<u>Experiment II (male 21 years old):</u>** Incubation time: 20 hours Test concentrations: - S9 : 10.9, **21.8, 43.7, 48.1**, 52.9,	**Negative** No biologically-relevant increase in the number of cells carrying micronuclei was observed in experiment I or II in the presence or absence of activation. Cytotoxicity (where cytostasis is ≥ 45 ± 5 %) was observed at the highest evaluated concentrations in experiments I (at 53.6 and 111.0 µg/mL) and II (at 48.1 µg/mL) in the absence and presence of metabolic activation.	Naumann S., 2018 DAR: B.6.4.1

Method, guideline, deviations if any	Test substance,	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
		58.1, 51.1, 64.1, 76.9, 100 µg/mL Concentrations in bold were the evaluated concentrations. Vehicle: acetone (0.5 %) in culture medium Positive controls:MMC (4 h), demecolicin (20 h), CPA (4 h) (+S9)		

Table 28: Summary table of mutagenicity/genotoxicity tests in mammalian somatic or germ cells in vivo

Method, guideline, deviations if any	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
In vivo micronucleus test in mouse bone marrow OECD 474 (2016) GLP	Cinmethylin Purity: 96.3 % Enantiomeric ratio: not specified	Mice Crl:NMRI Oral, gavage Single administration **Preliminary study:** 3/sex/dose 2000 mg/kg bw, 10 mL/kg bw **Main study:** 5 males/dose 0, 500, 1000 and 2000 mg/kg bw, 10 mL/kg bw **Bioavailability study:** 2 males/dose	**Negative** There was no treatment-related or statistically significant increase in the frequency of micronucleated immature erythrocytes (PCE) under any of the test conditions. There was no evidence of cytotoxicity to the bone marrow. However, bioavailability in plasma was clearly demonstrated 2 and 4 hours after administration of 2000 mg/kg bw cinmethylin. Clinical signs at doses ≥ 1000 mg/kg bw/day: Piloerection (5/5 animals 1 h – 2 days post dosing) Hunching (5/5 animals 1 – 4 h post dosing)	Anon, 2018j DAR: B.6.4.2

Method, guideline, deviations if any	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
		2000 mg/kg bw, 10 mL/kg bw Vehicle: corn oil Positive control: CPA in dH_2O (20 mg/kg bw, 10 mL/kg bw)		
In vivo chromosome aberration assay in rat bone marrow Non-OECD GLP	Cinmethylin Purity: 92 % Enantiomeric ratio: not specified	Rats F344 Oral, gavage Single administration **Main study:** 6/sex/dose 0.3, 1.0 and 3.0 mL/kg bw (equivalent to 304, 1014 and 3043 mg/kg bw) Positive control: Triethylenemelamine 0.4 mg/kg bw (i.p. administration)	**Negative** There was no increase in frequency of structural chromosomal aberrations in the bone marrow of rats following administration of cinmethylin. No depression of bone marrow proliferation, as evidence of target tissue cytotoxicity, was observed. Clinical signs at doses ≥ 3043 mg/kg bw/day: Hypoactivity, lacrimation, polyurea, chromadacryorrhea, periocular swelling.	Anon, 1983c DAR: B.6.4.2

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

In vitro studies

Two Ames tests, a mammalian cell gene mutation assay and a micronucleus test are available to determine the potential of cinmethylin to cause genotoxicity in vitro.

The first Ames test was conducted with a batch of cinmethylin considered low purity (89.6 %) (Woitkowiak C., 2018a). Two experiments were conducted, the standard plate test (SPT) and the pre-incubation test (PIT). Both experiments used S.typhimurium strains TA98, TA100, TA1535 and TA1537 and E.coli strain WP2 uvrA and were conducted in the presence and absence of metabolic activation (S9 mix from rat liver) for

48-72 hours. Appropriate positive and negative controls were included in each experiment. Cinmethylin was tested at concentrations of 0, 33, 100, 333, 1000, 2800 and 5600 µg/plate in triplicate.

Precipitation of the test substance was observed at the top concentration both in the presence and absence of S9. This had no influence on the scoring on the plate.

No treatment-related increases in the number of revertants was observed in the SPT and PIT either in the presence or absence of S9. Controls behaved accordingly. Therefore, cinmethylin was tested up to the limit concentration and there was no evidence of a mutagenic effect under the conditions of this good quality study.

The second Ames test was conducted with cinmethylin of higher purity (97.5 %) (Woitkowiak C., 2018b). Two experiments were conducted, the standard plate test (SPT) and the pre-incubation test (PIT). Both experiments used S.typhimurium strains TA98, TA100, TA1535 and TA1537 and E.coli strain WP2 uvrA and were conducted in the presence and absence of metabolic activation (S9 mix from rat liver) for 48-72 hours. Appropriate positive and negative controls were included in each experiment. Cinmethylin was tested at concentrations of 0, 33, 100, 333, 1000, 2600 and 5200 µg/plate.

Precipitation of the test substance was observed at the top concentration both in the presence and absence of S9. This had no influence on the scoring on the plate.

No treatment-related increases in the number of revertants was observed in the SPT and PIT either in the presence or absence of S9. Controls behaved accordingly. Therefore, cinmethylin was tested up to the limit concentration and there was no evidence of a mutagenic effect under the conditions of this well-conducted study.

The mutagenic potential of cinmethylin to mammalian cells was tested in a GLP and OECD compliant in vitro gene mutation assay in mouse lymphoma L5178Y cells (MLA assay) (Sokolowski, 2018). A preliminary cytotoxicity experiment was carried out to determine the concentrations to be used for the main study. Based on this experiment, concentrations of up to 125 µg/mL were chosen for the main study.

Two experiments (I and II) were conducted, each in duplicate (cultures I and II). Experiment I was conducted in the presence and absence of metabolic activation (S9-mix from rat liver) for a period of 4 hours. Test concentrations used were: 1.9, 3.8, 7.5, 15.0, 30.0, 60.0, 80.0 100.0, 125.0 µg/mL (+S9) or 1.9, 3.8, 7.5, 15.0, 30.0, 45.0, 60.0, 100.0 and 125.0 µg/mL (-S9). Experiment II was carried out in the absence of S9 for a period of 24 hours. Test concentrations of cinmethylin were 1.9, 3.8, 7.5, 15.0, 30.0, 45.0, 60.0, 100.0 and 125.0 µg/mL. Positive and solvent controls behaved accordingly.

Phase separation occurred in experiment I at concentrations of ≥ 60 µg/mL in the absence of S9 and at ≥ 80 µg/mL in the presence of S9. In experiment II, phase separation was noted at ≥ 100 µg/mL.

Cytotoxicity (mean relative growth < 50 %) occurred in experiment I, culture II (4 hours) at ≥ 45 µg/mL (-S9) and ≥ 80 µg/mL (+S9). In experiment II (24 hours) mean relative growth was < 10 % at ≥ 100 µg/mL.

No substantial or reproducible increase in mutation frequency (MF) was noted in experiment I or II either in the presence or absence of metabolic activation. A statistically significant dose-dependent increase in MF was determined in the 4 hour experiment without metabolic activation (Table 29). However, at no timepoint did the MF exceed the MF threshold of the solvent control by greater than 126. Therefore, cinmethylin was not considered to be mutagenic under the conditions of this good quality study up to concentrations causing precipitation and/or cytotoxicity.

Table 29: Results of gene mutation assay in mouse lymphoma (L5178Y) cells: Experiment I, culture I – 4 hour incubation without metabolic activation

Test group	Mutant frequency (MF/10^{-6} cells)	Small mutant frequency (/10^{-6} cells)
Vehicle control (acetone)	106	72
MF threshold[¥]	232	
Test item		
1.9	[#]	
3.8	114	77
7.5	90	60
15.0	117	82
30.0	121	87
45.0	182	134
60.0[PS]	173	109
100.0[PS]	[##]	
125.0[PS]	[##]	
Positive control (MMS)	320	265

[#]culture was not continued as minimum number of analysed concentrations was achieved
[##]culture was not continued due to severe cytotoxicity
[¥]$MF_{vehicle\ control\ corr}$ + GEF (126 x 10^{-6})
[PS] = phase separation

Cinmethylin was tested for its ability to induce micronuclei in human lymphocytes in vitro in an OECD and GLP compliant study (Naumann S., 2018). Two independent experiments were conducted (experiments I and II) each with duplicate cultures. Human peripheral blood lymphocytes (taken from a 31 year old female for experiment I and a 21 year old male for experiment II) were incubated for 4 hours (in the presence or absence of metabolic activation (S9-mix from rat livers) or 20 hours in the absence of metabolic activation. A preliminary cytotoxicity test was performed to determine the test concentrations of cinmethylin for the main study. For experiment I (4 h), the concentrations tested were: 11.2, 22.3, 44.7, 53.6, 64.3, **77.2**, **92.6**, **111**, 167, 500 µg/mL (+ S9), 11.2, **22.3**, **44.7**, **53.6**, 64.3, 77.2, 92.6, 11, 167, 500 µg/mL (- S9). For experiment II (20 h), concentrations tested were: 10.9, **21.8**, **43.7**, **48.1**, 52.9, 58.1, 61.1, 64.1, 76.9, 100 µg/mL (- S9). Only the test concentrations highlighted in bold were evaluated. Treatment began after a 48 hour stimulation period of the cells. Cytochalasin B was added to the cultures to arrent cell cycle and the cultures were fixed and stained after a further 20 hours. Cytokinesis-block proliferation index (CBPI) and cytostasis determined in 1000 binucleated cells served as cytotoxicity parameters and the number of micronucleated cells was determined in 2000 binucleated cells for evaluation of mutagenicity.

Phase separation of cinmethylin in the culture medium was observed in experiment I at ≥ 111 µg/mL (-S9) and at ≥ 167 µg/mL (+S9). In experiment II, no phase separation was observed up to the highest test concentration of 100 µg/mL.

Cytotoxicity (cytostasis ≥ 45 ± 5 %) was observed at 53.6 and 111 µg/mL in experiment I (+S9 and -S9 respectively) and at 48.1 µg/mL in experiment II.

The results of the assay showed no biologically-relevant increase in the number of cells carrying micronuclei in either experiment I in the presence or absence of metabolic activation. In experiment II, there was a statistically significant increase of micronucleated cells observed at the two highest concentration groups of experiment II (43.7 and 48.1 µg/mL) following 20 hours exposure in the absence of metabolic activation (Table 30).

Table 30: Results of the in vitro micronucleus test in human lymphocytes with cinmethylin – Experiment II (20 h incubation in the absence of metabolic activation)

Conc. (µg/mL)	Proliferation Index (CBPI)	Cytostasis %	Micronucleated cells %	HCD (95 % ctrl limit)
Without S9 mix				
Solvent control	1.81		0.30	0.05 – 1.11
21.8	1.73	10.2	0.50	
43.7	1.47	41.7	0.80*	
48.1	1.29	63.8	0.80*	
Positive control	1.40	51.2	3.05*	2.1 - 8.80

CBPI: cytokinesis-block proliferation index
HCD: historical control data of the performing laboratory: % of micronucleated cells in human lymphocyte cultures (2017)
n. c. Not calculated as the CBPI is equal or higher than the solvent control value
*: The number of micronucleated cells is statistically significantly higher than corresponding control values (p≤0.05)

The values obtained were 0.80 % for each tested concentration which was within the historical control data for the solvent control (95 % control limit: 0 – 1.11 %). No increase was observed with or without metabolic activation after 4 hours exposure. Therefore, the increase in micronucleated cells was considered to be within normal biological variation. Overall, there was no biologically relevant increase in the number of cells carrying micronuclei following exposure to cinmethylin up to cytotoxic concentrations.

In vivo studies

Two in vivo studies are available, a micronucleus test in mouse bone marrow and a chromosome aberration test in rat bone marrow.

In a guideline and GLP in vivo micronucleus test, cinmethylin was administered once orally to Crl:NMRI mice (5 males/dose) at doses of 0, 500, 1000 and 2000 mg/kg bw (Anon, 2018j). Doses and animals were selected on the basis of a preliminary study in both males and females using a single dose of 2000 mg/kg bw. The vehicle (corn oil) acted as a negative control and cyclophosphamide (CPA) was used as a positive control. All animals were sacrificed at 24 h post-dosing. An additional bioavailability study was carried out in which a further 2 males were dosed with 0 or 2000 mg/kg bw. These additional animals were sacrificed at 48 hours and the bone marrow from each femora was removed and examined.

Samples were prepared and stained and 4000 polychromatic erythrocytes (PCE) were evaluated per animal and examined for micronuclei. The normochromatic erythrocytes (NCE) occurring per 500 PCE were also recorded. Blood samples taken immediately after sacrifice were analysed to verify bioavailability of cinmethylin. Blood samples from 2 animals treated with 2000 mg/kg bw were also taken at 2 and 4 hours post administration.

No deaths occurred in any dose group during this study. Minor signs of toxicity were observed in all animals dosed with 1000 mg/kg bw and above. These included hunching and piloerection during the first 4 hours following administration. Piloerection continued until study termination.

The total percentage micronucleated PCE (in 4000 PCE/animal) in each dose group remained less than the vehicle control at both 24 and 48 hours. The positive control behaved accordingly.

Although there was no evidence of bone marrow cytotoxicity, the bioavailability study clearly demonstrated the presence of cinmethylin in plasma samples taken 2 and 4 hours after dosing. As the bone

marrow is a well perfused tissue, based on these data, exposure of the bone marrow to cinmethylin and or its metabolites is anticipated. The amount of cinmethylin present in plasma samples taken at 24 and 48 hours post administration was below the limits of quantification (LOQ < 100 ng/mL plasma).

Therefore, under the conditions of this guideline study, cinmethylin did not induce an increase in the frequency of micronucleated immature erythrocytes (PCE) in the bone marrow of male mice administered doses up to a limit dose of 2000 mg/kg bw. Clinical signs of toxicity (hunched posture and piloerection) were noted at the mid- and top-dose (1000 and 2000 mg/kg bw). Bone marrow exposure was demonstrated in this assay directly by the presence of cinmethylin and/or its metabolites in plasma and indirectly by the systemic toxicity observed in the study from the mid dose.

A GLP chromosome aberration study in Fischer rats is available (Anon, 1983c). Although not strictly carried out according to OECD test guidelines, the study broadly followed OECD 475 and is considered acceptable. Animals (6/sex/dose) were treated with a single dose of cinmethylin (0, 0.3, 1.0, or 3.0 mL/kg, equivalent to 304, 1014 and 3043 mg/kg bw) by oral gavage. No preliminary dose range-finding study was carried out. Distilled water (3 mL/kg) was used as a negative control and triethylenemelamine (TEM) (0.4 mg/kg bw, i.p.) was employed as a positive control. Animals were sacrificed 6, 16 or 24 hours after administration. Prior to the scheduled sacrifice (2-3 hours), animals were injected i.p. with colchicine (4.0 mL/kg bw) to arrest mitosis. Cells were collected from the femoral bone marrow and processed in order to determine the incidence of chromosomal aberrations, 50 cells per rat were analysed. Cytotoxicity was determined by measuring the mitotic index (MI) by analysis of the metaphases of 500 cells per animal.

No animals died in this study. Clinical signs of toxicity were observed in top dose treated animals only. Signs included hypoactivity, lacrimation, polyurea and chromadacryorrhea.

None of the treated groups exhibited a statistically-significant increase in the frequency of cells with structural chromosomal aberrations (aberrations per cell and % aberrant cells excluding gaps) compared to the concurrent negative control. The positive control demonstrated a clear and statistically significant increase in the frequency of cells with structural chromosomal aberrations.

However, there was no evidence of bone marrow toxicity in treated animals. Despite this, there were general signs of clinical toxicity to indicate the substance had reached the systemic circulation and the findings in animals treated with the positive control indicate that the test system was of appropriate sensitivity to detect known clastogens.

Overall, under the conditions of this old, non-guideline study, cinmethylin did not exhibit clastogenic activity in the bone marrow of rats up to the top dose of 3043 mg/kg bw (at which systemic toxicity occurred).

10.10.2 Comparison with the GB CLP criteria

The potential mutagenicity of cinmethylin has been well investigated in a number of in vitro and in vivo genotoxicity studies. In vitro, negative results were obtained in the presence and absence of S9 in bacterial and in a mammalian cell gene mutation tests. Similarly, a negative result was also obtained in an in vitro micronucleus test using human lymphocytes. In vivo, well conducted tests measuring the induction of micronuclei in the bone marrow of mice and chromosomal aberrations in rats both gave negative results. Overall, it can be concluded that cinmethylin lacks mutagenic potential. No classification is required for this endpoint.

10.10.3 Conclusion on classification and labelling for germ cell mutagenicity

No classification – conclusive but not sufficient for classification

10.11 Carcinogenicity

The long term toxicity and carcinogenesis of cinmethylin has been investigated in both rats and mice. For each species, there are two studies, a modern guideline study conducted according to GLP and an older non-guideline and non-GLP study.

Table 31: Summary table of animal studies on carcinogenicity

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
Combined chronic toxicity/carcinogenicity study in rats OECD 453 (2009) GLP Wistar 50/sex/dose (carcinogenicity phase – 12 months) 10/sex/dose (chronic toxicity phase – 24 months) Findings from the chronic phase of the study are reported in Section 10.9 – Specific target organ toxicity – repeated dosing. Year of study: 2015-2017	Cinmethylin Purity: 93.5 % Enantiomeric ratio: 48/52 (-/+) Concentration: 0, 200, 1000 and 5000 ppm (equivalent to 0, 9/11, 45/59 and 242/317 mg/kg bw/day in males/females) Administered daily via the diet for up to 24 months.	**<u>Non-neoplastic findings (24 months):</u>** **<u>5000 ppm (242/317 mg/kg bw/day):</u>** ↓ Body weight: 13 %* in females ↓ Body weight gain: 12 %** in males and 18 %** in females *Organ weights:* ↑ Liver weight: rel. 13 %** in males and rel. 20 %** in females. ↑ Kidney weight: rel. 18 %** in males and rel. 11 %* in females *Histopathology:* *Liver:* Cytoplasmic alterations: 18/50* males (versus 0 in controls) Centrilobular hypertrophy: 1/50 male and 12/50* females (versus 0 in controls) Periportal hypertrophy: 7/50* females versus 1/50 females in controls Periportal pigment storage: 24/50* males and 15/50* females versus 0 in controls. Centrilobular pigment storage: 27/50** females Multinucleated hepatocytes: 17/50* males versus 8/50 males in control group *Kidney:* Mineralisation (tubular): 8/50* males versus 1/50 in controls Mineralisation (papilla): 7/50* males versus 0/50 in controls *Nasal cavity:*	Anon, 2018c DAR: B.6.5.1

<table>
<tr><th>Method, guideline, deviations if any, species, strain, sex, no/group</th><th>Test substance, dose levels duration of exposure</th><th>Results</th><th>Reference</th></tr>
<tr><td></td><td></td><td>
Degeneration/regeneration of the olfactory epithelium (level III): 50/50** males and 50/50** females versus 0 in controls

Proteinaceous exudate: 20/50** males and 30/50** females versus 0 in controls

Metaplasia of the respiratory epithelium: 14/50** males and 12/50** females versus 0 in controls

Inflammation (multifocal): 12/50** males versus 2/50 in controls and 8/50* females versus 1/50 in controls.

Thyroid gland:

Hyperplasia (follicular cell): 10/50* males versus 2/50 in controls

Altered colloid: 24/50** males versus 7/50 in controls and 33/50** females versus 5/50 in controls

<u>1000 ppm (45/59 mg/kg bw/day):</u>

Histopathology:

Liver:

Centrilobular pigment storage: 22/50** females

Nasal cavity:

Degeneration/regeneration of the olfactory epithelium (level III): 2/50 males versus 0 in controls

Inflammation (multifocal): 5/50 males versus 2/50 in controls

<u>200 ppm (9/11 mg/kg b/day):</u>

No adverse treatment-related findings.

<u>Neoplastic findings:</u>

Tumours were noted in the uterus and liver of females only.

Incidence of neoplastic findings in females treated with cinmethylin in the diet for 24 months.
<table>
<tr><td>Dose level [ppm]</td><td>0</td><td>200</td><td>1000</td><td>5000</td></tr>
<tr><td>Dose [mg/kg bw/day]</td><td>0</td><td>11</td><td>59</td><td>317</td></tr>
<tr><td>UTERUS</td><td colspan="4"></td></tr>
</table>
</td><td></td></tr>
</table>

<table>
<tr><th>Method, guideline, deviations if any, species, strain, sex, no/group</th><th>Test substance, dose levels duration of exposure</th><th>Results</th><th>Reference</th></tr>
<tr><td></td><td></td><td>
<table>
<tr><td>No. animals examined</td><td>50</td><td>50</td><td>50</td><td>50</td></tr>
<tr><td>Endometrial adenocarcinoma</td><td>2 (4 %)</td><td>6 (12 %)</td><td>6 (12 %)</td><td>8* (16 %)</td></tr>
<tr><td>HCD</td><td colspan="4">Mean (14 studies 2001 – 2015): 15.1 % (range: 2-30 %)
Mean (5 studies 2009 – 2015): 13.6 % (range: 2-20 %)</td></tr>
<tr><td>Endometrial stromal polyp</td><td>6 (12 %)</td><td>11 (22 %)</td><td>10 (20 %)</td><td>14* (28 %)</td></tr>
<tr><td>HCD</td><td colspan="4">Mean (11 studies 2001 – 2015): 16 % (range: 4-38 %)
Mean (5 studies 2009 – 2015): 22 % (range: 12-38 %)</td></tr>
<tr><td>LIVER</td><td colspan="4"></td></tr>
<tr><td>Hepatocellular adenoma</td><td>3 (6 %)</td><td>0 (0 %)</td><td>0 (0 %)</td><td>0 (0 %)</td></tr>
<tr><td>Hepatocellular carcinoma</td><td>1 (2 %)</td><td>0 (0 %)</td><td>1 (2 %)</td><td>3 (6 %)</td></tr>
<tr><td>HCD</td><td colspan="4">Mean (13 studies 1999 – 2015): 1.7 % (range: 0-6 %)
Mean (4 studies 2009 – 2015): 1.5 % (range: 0-4 %)</td></tr>
</table>
HCD was provided by the testing facility from two year studies carried out in Wistar rats between 2001-2015 for adenocarcinoma, 2014-2015 for endometrial stromal polyp and 1999-2015 for hepatocellular carcinoma. As the data was from a time period spanning greater than the preferred 5 years from the study date, a second range was determined from the same data within a 5 year timespan (2009 – 2015).
</td><td></td></tr>
<tr><td>A 2 year feeding study in rats

Non-guideline
Non-GLP

F344
50/sex/dose (24 month carcinogenicity group)</td><td>Cinmethylin
Purity: 92 %
Enantiomeric ratio: Not specified.

Dose levels: 0, 30, 100 and 3000 ppm (equivalent to 0, 1.4/1.7, 4.7/5.8 and</td><td><u>Non-neoplastic findings:</u>
Findings presented below relate to the 24 month phase of the study only. Findings related to the chronic phase of the study can be found in Section 10.9.
<u>3000 ppm (144.2/177.4 mg/kg bw/day):</u>
↑ Mortality (2 years): 64 % of males died versus 56 % in controls – due to chronic nephropathy.
↓ Body weight: 12 % in males
↓ Body weight gain: 19.9 % in males
Hunched appearance in males</td><td>Anon, 1985a
Anon, 1991a
Anon, 1991b
Anon, 1991c
DAR: B.6.5.1</td></tr>
</table>

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
15/sex/dose (18 month chronic toxicity group) 10/sex/dose (6 and 12 month chronic toxicity group) Findings from the chronic phase of the study are reported in Section 10.9 – Specific target organ toxicity – repeated dosing. Year of study: 1983 - 1985	144.2/177.4 mg/kg bw/day in males and females) Administered daily in the diet for 6, 12, 18 or 24 months.	Pale eyes in males *Clinical chemistry:* ↑ γ-Glutamyl transferase: 7.2 IU/L** in males versus 2.0 IU/L in controls and 4.8 IU/L** in females versus 1.7 IU/L in controls ↑ Urea nitrogen: 17.5 mmol/L** in males versus 10.6 mmol/L in controls ↑ Inorganic phosphate: 2.36 mmol/L* in males versus 1.7 mmol/L in controls and 1.42 mmol/L** in females versus 1.14 mmol/L in controls *Urinalysis:* ↑ Tubular epithelial cells: 1.0** in males versus 0.2 in controls (at 24 months) ↑ Casts: 0.3* in males versus 0.1 in controls (24 months) ↑ Urine volume: 12.9 mL* in males versus 9.7 mL in controls (24 months) (paler in colour) ↓Osmolality: 38 % of controls in males ↑ Leukocytes: 17* in males versus 4.2 in controls. *Organ weights:* ↑ Liver: rel. 23.3 % in males and rel. 14.1 % in females ↑ Kidney: rel. 16.8 % in males and rel. 13.1 % in females *Histopathology:* *Liver:* Subscapular dark depressed foci: 40 females versus 29 in controls. Periportal acidophilia: 33** males versus 9 in controls, 22** females versus 2 in controls Periportal chromidial clumping: 9** males and 1 females versus 0 in controls. *Kidney:* Diffuse subcapsular pallor: 23 males versus 14 in controls Subscapular pitting/rough surface (severe/very severe): 27 males versus 12 in controls Chronic nephropathy (increased severity): severe: 19/50 males versus 13/50 in controls and very severe: 14*/50 males versus 4/50 in control	

<table>
<tr><th>Method, guideline, deviations if any, species, strain, sex, no/group</th><th>Test substance, dose levels duration of exposure</th><th>Results</th><th>Reference</th></tr>
<tr><td></td><td></td><td>
Thyroid:
Focal colloidal basophilia/mineralisation: 21**/50 females versus 8/50 in controls
Lymph nodes:
Renal node haemorrhage/enlargement: 4 males versus 0 in controls
Stomach:
Gastric mucosal thickening: 18 males versus 8 in controls.
Fundic glandular minerlisation: 13*/50 versus 3/50 in controls
Parathyroid gland:
Enlargement: 16 males versus 7 in controls.
Diffuse hyperplasia: 29**/50 males versus 15/50 in controls
Blood vessels:
Aortic mucosal thickening: 18 males versus 11 in controls.

<u>100 ppm (4.7/5.8 mg/kg bw/day):</u>
Thyroid:
Focal colloidal basophilia/mineralisation: 18**/50 females versus 8/50 in controls

<u>30 ppm (1.4/1.7 mg/kg bw/day):</u>
No adverse treatment-related effects observed.

<u>Neoplastic findings:</u>
There were no treatment-related increases in tumours in any tissues.
Increases in interstitial tumours were observed in all dose groups at 18 and 24 months.
<table>
<tr><td>Dose level [ppm]</td><td>0</td><td>30</td><td>100</td><td>3000</td></tr>
<tr><td>Dose [mg/kg bw/day]</td><td>0</td><td>1.4</td><td>4.7</td><td>144.2</td></tr>
<tr><td>TESTES - Interstitial cell tumours (benign)</td><td colspan="4"></td></tr>
</table>
</td><td></td></tr>
</table>

<table>
<tr><th>Method, guideline, deviations if any, species, strain, sex, no/group</th><th>Test substance, dose levels duration of exposure</th><th>Results</th><th>Reference</th></tr>
<tr><td></td><td></td><td>
<table>
<tr><td>No. animals examined</td><td>50</td><td>50</td><td>50</td><td>50</td></tr>
<tr><td>18 month incidence</td><td>8 (53 %)</td><td>11 (73 %)</td><td>10 (67 %)</td><td>12* (80 %)</td></tr>
<tr><td>24 month incidence</td><td>27 (54 %)</td><td>39 (78 %)</td><td>34 (68 %)</td><td>38* (76 %)</td></tr>
<tr><td>HCD</td><td colspan="4">NTP[1.] Mean (40 studies 1977 – 1987): 89 % (range: 68-98 %)
Laboratory[2.] Mean (3 studies 1983 – 1989): 78 % (range: 72-86 %)</td></tr>
</table>
[1.]National Toxicity Program (NTP) HCD based on 40 carcinogenicity feedings studies each using 50 F344 rats per dose group, performed between 1977 and 1987 at the National Cancer institute(NCI) and the National Toxicology Program in the US.
[2.]Laboratory control data based on three 2 year feeding studies in F344 rats (n=2).
</td><td></td></tr>
<tr><td>Carcinogenicity study in mice
OECD 451 (2009)
GLP

C57BL/6J Rj
50/sex/dose

Year of study: 2015-2016</td><td>Cinmethylin
Purity: 93.5 %
Enantiomeric ratio: 48/52 (-/+)

Dose: 0, 150, 1000 and 5000 ppm (equivalent to 0, 25/27, 162.3/183.8 and 904/939.1 mg/kg bw/day males/females)

Administered daily in the diet for 18 months</td><td>Non-neoplastic findings:
<u>5000 ppm (904/939.1 mg/kg bw/day):</u>
↓ Body weight: 18.1** % in males and 22.5** % in females
↓ Body weight gain: 46.5** % in males and 45.4** % in females
↓ Food consumption (Day 0 – week 78): 10 % in males and 24.9 % in females

Organ weights:
Liver: abs. 12.3 %** and rel. 38.3%** in males and rel. 27.3%** in females

Histopathology:
Liver:
Centrilobular hypertrophy: 5/50* males versus 0 in controls
Periportal hypertrophy: 34/50* females versus 0/50 in controls
Oval cell hyperplasia: 38/50* females versus 2/50 in controls
Nasal cavity (level III):
Respiratory metaplasia: 47/50** males versus 8/50 in controls and 41/50** females versus 8/50 in controls</td><td>Anon, 2018d
DAR: B.6.5.2</td></tr>
</table>

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		Degeneration/regeneration of the olfactory epithelium: 50/50** males versus 1/50 in controls and 27/50** females versus 0 in controls **<u>1000 ppm (162.3/183.8 mg/kg bw/day):</u>** ↓ Body weight: 10.7** % in females ↓ Body weight gain: 9.9 % in males and 21.6** % in females ↓ Food consumption (Day 0 – week 78): 7 % in males and 6.3 % in females *Organ weights:* Liver: rel. 11.9 %** in males *Histopathology:* *Nasal cavity (level III):* Respiratory metaplasia: 19/50* males versus 8/50 in controls and 13/50 females versus 8/50 in controls Degeneration/regeneration of the olfactory epithelium: 6/50 males versus 1/50 in controls and 1/50 females versus 0 in controls **<u>150 ppm (25/27 mg/kg bw/day):</u>** ↓ Body weight gain: 18.3* % in males and 14.4* % in females ↓ Food consumption (Day 0 – week 78): 17.4 % in females **<u>Neoplastic findings:</u>** There were no increases in neoplastic findings observed in mice treated with up to 5000 ppm cinmethylin. The number of animals with neoplasms and the total number of neoplasms were comparable between control and high dose groups.	
Carcinogenicity study in the mouse Non-guideline Non-GLP B6C3F1 60/sex/dose (24-month group)	Cinmethylin Purity: 92 % Enantiomeric ratio: Not specified Dose: 0, 30, 100 and 1000 ppm (equivalent to 0, 7.2/8.3,	**<u>Non-neoplastic findings:</u>** There were no treatment-related deaths or clinical findings at any dose. **<u>1000 ppm (231/272 mg/kg bw/day):</u>** *Organ weight:* ↑ Liver weight: abs. 22.02 % and rel. 27.01 % in males and abs. 18.64 % and rel. 13.08 % in females.	Anon, 1986b Anon, 1991d DAR: B.6.5.2

<table>
<tr><th>Method, guideline, deviations if any, species, strain, sex, no/group</th><th>Test substance, dose levels duration of exposure</th><th>Results</th><th>Reference</th></tr>
<tr><td>10/sex/dose (12 month group)
120/sex/control group

Year of study: 1983</td><td>22.1/26.8 and 231/272 mg/kg bw/day males/females)

Administered daily in the diet for 12 or 24 months</td><td>
<u>100 ppm (22.1/26.8 mg/kg bw/day):</u>
No treatment-related findings.

<u>30 ppm (7.2/8.3 mg/kg bw/day):</u>
No treatment-related findings.

<u>Neoplastic findings:</u>
Incidence of hepatic tumours in male B6C3F1 mice treated with cinmethylin for 24 months
<table>
<tr><td>Dose (ppm)</td><td>0</td><td>30</td><td>100</td><td>1000</td></tr>
<tr><td>mg/kg bw/day</td><td>0</td><td>7.2</td><td>22.1</td><td>231</td></tr>
<tr><td>No. animals examined</td><td>98</td><td>54</td><td>53</td><td>55</td></tr>
<tr><td>Adenoma %</td><td>15.3</td><td>18.5</td><td>26.4+</td><td>23.6+</td></tr>
<tr><td>HCD#</td><td colspan="4">Mean (40 studies 1977-1987): 10 % (range: 0-44)</td></tr>
<tr><td>Carcinoma %</td><td>11.2</td><td>5.6</td><td>15.1</td><td>18.2</td></tr>
<tr><td>HCD</td><td colspan="4">NTP (40 studies between 1977-1987) Range: 8-32, mean 21.1</td></tr>
</table>
Incidence of hepatic tumours in female B6C3F1 mice treated with cinmethylin for 24 months:
<table>
<tr><td>Dose (ppm)</td><td>0</td><td>30</td><td>100</td><td>1000</td></tr>
<tr><td>mg/kg bw/day</td><td>0</td><td>8.3</td><td>26.8</td><td>272</td></tr>
<tr><td>No. animals examined</td><td>98</td><td>56</td><td>54</td><td>53</td></tr>
<tr><td>Adenoma %</td><td>9.2</td><td>21.4*+</td><td>16.7</td><td>18.9*+</td></tr>
<tr><td>HCD#</td><td colspan="4">NTP (40 studies between 1977-1987) Range: 0-18, mean 3.8</td></tr>
</table>
</td><td></td></tr>
</table>

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference

Carcinoma %	**2.0**	**1.8**	**3.7**	**5.7***	
HCD#	NTP (40 studies between 1977-1987) Range: 0-15, mean 4.6				

*P ≤ 0.05, comparison against control, 1-tailed Hoel-Walburg test for 2 groups (prevalence analysis)

+P ≤ 0.05, comparison against control, 1-tailed Cox's test for 2 groups (fatal analysis)

#Historical control data taken from the National Toxicity Program, 40 carcinogenicity studies conducted between 1977-1987 (current study performed in 1983-1985) in BC36F1 mice (Haseman J.K. *et al*., 1986). It is not clear if these studies were performed for 24 months as with the current study or the more standard time period of 18 months.

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

Four studies to inform on the long-term toxicity and carcinogenic potential of cinmethylin are available, two in rats and two in mice. For each species, there is a recently performed guideline study conducted adhering to GLP and an older non-guideline/non-GLP study.

Rats

In a well-conducted OECD test guideline-compliant study, cinmethylin was administered to Wistar rats for up to 24 months (50/sex/dose) (Anon, 2018c). Dietary concentrations were 0, 200, 1000 and 5000 ppm (equivalent to 0, 9/11, 45/59 and 242/317 mg/kg bw/day). An additional sub-group of animals (10/sex/dose) were selected for the chronic phase of the study and received the same dietary concentrations of cinmethylin for a period of 12 months (equivalent doses of 0, 10/13, 51/69 and 265/351 mg/kg bw/day). Details of the effects observed in this phase are discussed in the section of specific target organ toxicity following repeated dosing (Section 10.9).

The main target organs for non-neoplastic effects were the liver, nasal cavity, thyroids and the kidneys of males (the effects in the kidneys were shown by Anon (2018d) to be due to the accumulation of α-2μ-globulin, a male-rat specific phenomenon of no relevance to humans).

Increased liver weight was observed in top dose males and females, with a statistically significant increase of up to 20 % observed. Centrilobular pigment storage (representing lipofuscin) was observed in females of the mid and top dose groups and periportal hypertrophy was seen in females of the top dose group only. Cytoplasmic alterations and multinucleated hepatocytes were found in males only of the top dose group and centrilobular hypertrophy and periportal pigment storage in both sexes of the top dose group.

Kidney weight was statistically significantly increased in top dose group males (18 %) and to a lesser extent in females (11 %), however in females this increase was not statistically significant and there were no histopathological correlates. In males, the increased weight was accompanied by mineralisation (tubular and papilla).

Treatment-related effects on the nasal cavity were observed. Degeneration/regeneration was observed in 2/50 males of the mid dose group and all animals of the top dose group. Proteinaceous exudate was

observed in 20/50 males and 30/50 females, metaplasia of the respiratory epithelium was seen in 14/50 males and 12/50 females versus 0 controls animals. Multifocal inflammation was also observed in males of the mid dose group (5/50 versus 2/50 in controls) and in both sexes at the top dose group (12/50 males versus 2/50 in controls and 8/50 females versus 1/50 in controls).

In the thyroid, an increased incidence of follicular cell hyperplasia was observed in males of the top dose group only (10/50 versus 2/50 in controls) and altered colloid was seen in both males and females (24/50 males versus 7/50 in controls and 33/50 females versus 5/50 in controls).

The only significant neoplastic findings were found in the uterus and liver of females. In the top dose group there was a slight, but statistically significant increase in the incidence of endometrial adenocarcinoma of the uterus (4 %, 12 %, 12 % and 16 % in the control, low, medium and high dose groups respectively). In addition, an increased incidence of endometrial stromal polyps was noted in all dose groups, but a statistically significant increase was observed in top dose group females (12 %, 22 %, 20 % and 28 %* in the control, low, medium and high dose groups respectively).

Historical control data was provided by the performing laboratory from similar studies with the same species and strain of animal. The HCD was from studies carried out between the years 2001 – 2015. As the ideal date range for HCD should be within 5 years of the study date (2015), the Agency has analysed the HCD to provide a range for the incidence of these tumours between 2009-2015. Between these years, incidence of endometrial adenocarcinoma at the performing laboratory in female Wistar rats ranged between 2-20 % (mean 13.6 %). The finding of 16 % of animals with this tumour in the top dose group is clearly well within the expected range for spontaneous formation. HCD was also provided for endometrial stromal polyps in control female Wistar rats from carcinogenicity studies carried out between 2001 – 2015. After performing a similar analysis, the range between the years 2009 – 2015 was 12-38 % (mean 22 %). Again, although there was an increase in polyps observed in females of the top dose, the incidence was well within the expected range for spontaneous formation and is unlikely to be a result of a carcinogenic response of cinmethylin. In support of this, no similar findings were observed in an older, supporting carcinogenicity study in rats or in the two carcinogenicity studies in mice. In addition, the uterus is not a target organ of toxicity of cinmethylin.

There was a small increase in the incidence of hepatocellular carcinoma in top-dose treated females only (2 %, 0 %, 2 % and 6 % in control, low, medium and high dose groups respectively). The increase in the incidence in the top group (3 rats versus 1 in controls) was not considered statistically significant.

Historical control data from the performing laboratory from 24 month studies carried out in female Wistar rats is available. As described previously, the HCD was for studies carried out between 1999-2015. The Agency has taken data from the studies carried out only within a 5 year timeframe of the current study. Therefore the incidence of hepatocellular carcinoma in female Wistar rats in studies carried out between 2009-2015 is 0-4 % (mean 1.5 %). The increase in tumours in the current study was above the more contemporaneous HCD and therefore relation to treatment cannot be excluded. However, the lack of adenoma in the same tissue of treated animals and any other pre-neoplastic lesions indicates a lack of biological plausibility for this finding. No increase in liver tumours were observed in males. There was also no evidence of an increase in liver tumours in an older carcinogenicity study in rats or in a well-conducted study in mice. Liver tumours occurred in an old, none-guideline study in mice, however, the strain of mouse used (B6C3F1) is known to have a high spontaneous liver tumour rate and these tumours did not indicate a carcinogenic response to treatment with cinmethylin. The Agency notes that the carcinogenicity response observed occurred in the presence of significant systemic toxicity, was very weak, sex- and species-specific, and that although the liver is a target organ of toxicity in the rat (causing hypertrophy, pigmentation and multinucleated hepatocytes in both sexes in the study), there was no clear evidence of pre-neoplastic lesions. It is also noted that the incidence of liver carcinoma was within the extended laboratory HCD range and the Rita database HCD. In addition, there were no significant differences between sexes in kinetic and target organs of toxicity to explain a response only in females. Furthermore, cinmethylin is not genotoxic.

A second carcinogenicity study is available in F344 rats, originally split into several reports with several references (Anon, 1985a, Anon, 1991a, Anon, 199b, Anon, 1991c). The study was conducted in 1983 and was not carried out according to OECD guidelines or GLP. When compared to the currently valid OECD 453 (2009) the study has the following deviations:

- The spacing for the top dose was greater than 10-fold, not matching the recommended 2-4-fold interval.
- Detailed clinical observations outside the home cage, preferably in a standard arena, were not performed in this study.
- For clinical pathology (haematology, clinical chemistry, urinalysis), no values were determined prior to treatment initiation.
- The clinical chemistry parameter creatinine was not determined in this study.
- At termination, epididymides, ovaries and thyroids weights were not determined in animals of chronic toxicity groups.
- Generally, except for some neoplastic findings, laboratory historical control data are lacking.

Overall, the study still provides reliable information to add to the weight of evidence towards carcinogenic hazard assessment of cinmethylin. However, as values for haematology, clinical chemistry and urinalysis were not determined prior to treatment, these parameters are difficult to interpret.

Cinmethylin was administered to groups of male and female F344 rats in the diet at dose levels of 0, 30, 100 and 3000 ppm for 24 months (50/sex/dose) (equivalent to 0, 1.4/1.7, 4.7/5.8 and 144.2/177.4 mg/kg bw/day in males and females). Satellite groups of 10/sex/dose were treated for 6 or 12 months and 15/sex/dose for 18 months.

An increase in mortality was observed in top-dose treated males in the last 6 months of the study (64 % morality versus 56 % in controls). The cause of death was deemed to be due to chronic nephropathy caused by α2μ-globulin accumulation. In males treated with 3000 ppm (144.2 mg/kg bw/day) there was a decrease in body weight (12 %) and body weight gain (19.9 %). These animals appeared hunched and had pale eyes.

Target organs in this study were the liver, thyroid and kidneys, with most changes observed in animals of the top dose group (3000 ppm, 144.2/177.4 mg/kg bw/day). Indications of liver damage included a statistically significant increase in γ-glutamyl transferase in males and females of the top dose group (in males: 7.2 IU/L versus 2 IU/L in controls) and in females: 4.8 IU/L versus 1.7 IU/L in controls) and an increase in relative liver weight (23.3 % in males and 14.1 % in females). Histopathology revealed a statistically significant increase in periportal acidophilia in both sexes (33 males versus 9 in controls and 22 females versus 2 in controls), a statistically significant increase in periportal chromidial clumping (9 males and 1 female versus 0 in controls) and an increased incidence of subscapular dark depressed foci in females (40 females versus 29 in controls).

Adverse findings in the kidney occurred in males of the top dose group only and were considered due to α2μ-globulin accumulation. Relative kidney weight was increased by 16.8 % compared to controls (a small increase of 13.1 % was also observed in females treated with 3000 ppm, 177.4 mg/kg bw/day). Clinical chemistry revealed a statistically significant increase in urea nitrogen of 17.5 mmol/L versus 10.6 mmol/L in controls. Urinalysis showed statistically significant increases in tubular epithelial cells (1 versus 0.2 in controls), increases in casts (0.3 versus 0.1 in controls), an increase in urine volume (12.9 mL versus 9.7 mL in controls), a decrease in osmolality (38 % of controls) and an increase in leukocytes (17 versus 4.2 in controls). Chronic nephropathy was observed in all males but increased in severity with increasing dose. Diffuse subcapsular pallor was noted in 23 males versus 14 in controls and pitting/roughness of the subscapular surface (severe/very severe) was observed in 27 males (versus 12 in controls).

In the thyroid, a statistically significant increase in incidence of focal colloidal basophilia/mineralisation was observed in 21/50 females treated with 3000 ppm (177.4 mg/kg bw/day) (versus 8/50 in controls). This was also observed in 18/50 females of the mid dose of 100 ppm (5.8 mg/kg bw/day).

Other findings observed in top dose treated animals only included renal lymph node haemorrhage/enlargement (4 males versus 0 in controls), gastric mucosal thickening (18 males versus 8 in controls), enlargement of the parathyroid gland (16 males versus 7 in controls) and diffuse hyperplasia of the parathyroid gland (29/50 versus 15/50 in controls). Aortic mucosal thickening was noted in 18 males versus 11 in controls. All these changes were considered related to treatment. No adverse treatment-related findings were observed at the low dose of 30 ppm (1.4/1.7 mg/kg bw/day in males and females respectively).

There were no treatment-related increases in tumours in any tissues. Increases in benign testicular interstitial tumours (Leydig tumours) were observed in all dose groups at 18 and 24 months. There was a statistically significant increase in the incidence of tumours in the top group, both at 18 and 24 months (18 months: 53, 73, 67 and 80 %; 24 months: 54, 78, 68 and 76 % in the control, low, mid and high dose groups respectively). However, at both timepoints, the finding lacked a dose response. Interstitial tumours in male F344 rats are a common finding, occurring spontaneously and tend not to provide reliable evidence of treatment-related carcinogenicity (Cook et al., 1999, Mati et al., 2002; RIVM, 2004; EU Specialised Expert Report, 2004). Indeed, the laboratory HCD (consisting of three 2 year feeding studies in F344 rats between the years of 1983-1989) showed a range of 72-86 % (mean 78 %) for this finding. This was supported by HCD provided the National Toxicity Program (NTP) HCD based on 40 carcinogenicity feedings studies each using 50 F344 rats per dose group, performed between 1977 and 1987 at the National Cancer institute (NCI) and the National Toxicology Program in the US (range 68-98 %, mean 89 %).

Overall, there was no evidence of a carcinogenic response in this study.

Mice

A recently performed, well-conducted study in C57BL/6J Rj mice is available (Anon, 2018d). Mice (50/sex/dose) were fed a diet containing cinmethylin at dose levels of 0, 150, 1000 and 5000 ppm (equivalent to 0, 25/27, 162.3/183.8 and 904/939.1 mg/kg bw/day) for 18 months.

The main target organs for non-neoplastic effects were the liver and nasal cavity. Treatment-related adverse effects were observed from a dose of 1000 ppm and above.

Body weight and body weight gain were reduced at all doses (up to 22.5 % reduction in body weight and up to 46.5 % reduction in body weight gain). This was associated with a reduction in food consumption.

Relative liver weight was increased in males from the mid dose (11.9 %) and in both males and females from the top dose (38.3 % in males and 24.9 % in females). At this dose, absolute liver weight was also increased in males (12.3 %). Associated histopathological findings were observed at the top dose only. These included centrilobular hypertrophy in males only (5/50 versus 0 in controls), periportal hypertrophy in females only (34/50 females versus 0 in controls) and oval cell hyperplasia in females (38/50 versus 2/50 in controls). These changes were all found to be statistically significant.

In the nasal cavity, statistically significant changes to the olfactory epithelium (level III) were observed from the mid dose and above. At the mid dose of 1000 ppm (162.3/183.8 mg/kg bw/day) respiratory metaplasia was observed in 19/50 males and 13/50 females (versus 8/50 in controls). Degeneration and regeneration of the olfactory epithelium was noted in 6/50 males (versus 1/50 in controls) and 1/50 females (versus 0 in controls). At the top dose of 5000 ppm (904/939.1 mg/kg bw/day) the incidence of these findings increased (respiratory metaplasia: 47/50 males and 41/50 females versus 8/50 in controls and degeneration/regeneration of the olfactory epithelium: 50/50 males versus 1/50 in controls and 27/50 females versus 0 in controls).

There were no increases in neoplastic findings observed in mice treated with up to 5000 ppm (904/939.1 mg/kg bw/day) cinmethylin (at dose at which significant systemic toxicity was observed). The number of animals with neoplasms and the total number of neoplasms were comparable between control and high dose groups.

An older non-guideline and non-GLP study in B6C3F1 mice is also available (Anon, 1986b; Anon, 1991d). The study commenced in 1983 and when compared to the current OECD test guidelines, was found to have a number of deviations and limitations:

- Water was stated to be available ad libitum. However, difficulties in adjusting the automatic watering system occurred at the start of this study, especially for females. Furthermore, inspections of the watering systems revealed that water was inaccessible for most low-dose males (40/44) at week 74 and most control males (61/73) at week 84. This resulted in thin appearance and mortalities of five control males due to dehydration.
- Due to technical error, pre-necropsy (fasted weights) were not taken on animals sacrificed during the first 3 days of the scheduled terminal necropsy. Therefore, at terminal sacrifice (104 week) non-fasted weights were used to calculate relative organ weights (organ to body weights) for all animals.
- Compared with the currently valid OECD TG 453 (2009):
- The spacing for the top dose was 10-fold, not matching the recommended 2-4-fold interval.
- Detailed clinical observations outside the home cage, preferably in a standard arena, were not performed in this study.
- Ophthalmoscopy were not performed in this study.
- Urinalysis was not performed for animals of the chronic toxicity group.
- For haematology, the following parameters were not determined in animals of chronic toxicity groups: mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), prothrombin time, and activated partial thromboplastin time.
- The clinical chemistry parameter creatinine was not determined in this study.
- Due to insufficient blood volume collected at the time of the 52-week interim sacrifice, the following parameters were not determined for all animals: direct bilirubin, globulin, phosphorous, and sodium.
- At termination, epididymides, ovaries and thyroids weights were not determined in animals of chronic toxicity groups.
- Generally, except for some neoplastic findings, laboratory historical control data are lacking.

Limitations:

- Results of testing for mouse hepatitis virus (MHV) at week 52 revealed positive findings in 28/42 males (56 %, 44 %, 86 % and 90 % for 0, 30, 100 and 100 ppm groups, respectively) and 39/42 females (86 %, 100 %, 89 % and 100 % for 0, 30, 100 and 100 ppm groups, respectively). The data showed an active infection between weeks 40 to 52, seen in all treated groups, including the controls, without a dose response.
- Due to a lack of generalised toxicity at the top dose, including depression of body weight gain, the study lacked evidence to indicate the MTD was achieved.

Due to these limitations and deviations, particularly the lack of evidence of achieved MTD, the study is considered as supportive, adding only to the weight of evidence.

Mice (60/sex/dose) were administered cinmethylin daily in the diet for 24 months at dose levels of 30, 100 and 1000 ppm (equivalent to 7.2/8.3, 22.1/26.8 and 231/272 mg/kg bw/day). Controls were used (120/sex) and a satellite group of 10 animals/sex/dose received cinmethylin for only 12 months.

The only treatment-related finding was an increase in liver weight in males and females of the top dose (1000 ppm, 231/272 mg/kg bw/day). Absolute weight was increased by 22.02 % of controls in males and 18.64 % in females and relative weight was increased 27.01 % in males and 13.08 % in females. There were no histopathological correlates.

With regards to neoplastic findings, hepatic adenoma and carcinoma were observed in all dose groups. In males, there was a slight statistically significant increase in adenoma in the mid and top dose groups (15.3, 18.5, 26.4 and 23.6 % at 0, 30, 100 and 1000 ppm) and a slight (not statistically significant) increase in carcinoma at the top dose group only (11.2, 5.6, 15.1 and 18.2 % at 0, 30, 100 and 1000 ppm). In females, a statistically significant increase in adenoma was observed in the low and top dose groups (9.2, 21.4, 16.7 and 18.9 % at 0, 30, 100 and 1000 ppm respectively) and a small, statistically significant increase of carcinoma was observed in the top dose group only (2.0, 1.8, 3.7 and 5.7 % at 0, 30, 100 and 1000 ppm respectively). Tumours were observed in the absence of a clear dose-response. No laboratory HCD was provided, however all tumours were within range of HCD provided from the National Toxicity Program taken from 40 carcinogenicity studies conducted between 1977-1987 (current study performed in 1983-1985) in BC36F1 mice (Haseman, J.K. et al. 1986). The relevance of this HCD is not clear as it is unknown whether these studies were performed for 24 months as the current study was or the more standard time period of 18 months. Regardless of this, spontaneous formation of liver tumours in this strain of mouse are known to be very common and, according to the guidance on the application of the CLP criteria (Version 5.0 – July 2017), do not provide reliable evidence of a carcinogenic response following treatment.

No other neoplastic findings were observed in this study. Overall, there was no clear evidence of a carcinogenic response in this study.

10.11.2 Comparison with the GB CLP criteria

The carcinogenicity of cinmethylin has been assessed in four long-term studies, two in rats and two in mice. Although increases in various neoplasms were observed, the data available do not support these being related directly to treatment with cinmethylin.

In a single, well conducted study in Wistar rats, liver carcinomas were observed in all treatment groups in females, with a slight increase in incidence observed in the top dose group only (2 %, 0 %, 2 % and 6 % in control, low, medium and high dose groups respectively). In the same study, uterine endometrial adenocarcinomas were observed in all treatment groups with a statistically significant increase in incidence noted in top dose females only (4 %, 12 %, 12 % and 16* % in the control, low, medium and high dose groups respectively).

There is no evidence in humans to suggest that cinmethylin is a known human carcinogen. Therefore, classification in hazard category 1A is not appropriate.

Classification with category 1B is based on sufficient evidence demonstrating a causal relationship between an agent and an increased incidence of neoplasms. The evidence presented in rats and mice does not demonstrate a causal relationship with cinmethylin and an increase in neoplasms.

Classification with category 2 is reserved for when there is limited evidence of carcinogenicity. Both the increase in liver carcinoma and the increase in endometrial adenocarcinoma in female Wistar rats could be considered limited evidence of carcinogenicity. However, the increase in liver tumours occurred in a single sex and species and lacked statistical significance. The tumours occurred in the presence of significant

systemic toxicity. Although the liver was clearly a target organ of cinmethylin (hypertrophy, pigmentation and multinucleated hepatocytes in both sexes), there were no dose-related pre-neoplastic lesions observed in this organ. There are no significant differences in kinetics or target organs of toxicity between sexes to explain a female-only response. Further, cinmethylin is not considered genotoxic. In a second study, in F344 rats, which also noted similar liver toxicity, no liver tumours were observed. Thus, the biological plausibility of this finding being related to treatment is lacking. Finally, the finding was within the range of the extended HCD provided and the Rita database HCD, indicating the increase in tumours observed is likely to be spontaneous and unrelated to treatment.

The statistically significant increase in endometrial adenocarcinoma in female rats were again observed in one study and in a single species. An increased incidence of uterine polyps were observed in this treatment group, however there was no evidence of hyperplasia or any other pre-neoplastic changes. This again brings into question the biological plausibility of the finding. The incidence of adenocarcinoma was well within the range of the contemporaneous HCD provided and is considered spontaneous and unrelated to treatment. The uterus is not considered a target organ of cinmethylin.

Therefore, from the available data presented, cinmethylin is not considered to meet the criteria for classification as a carcinogen. No classification for this endpoint is warranted.

10.11.3 Conclusion on classification and labelling for carcinogenicity

No classification – conclusive but not sufficient for classification

10.12 Reproductive toxicity

10.12.1 Adverse effects on sexual function and fertility

The reproductive toxicity of cinmethylin has been investigated in two 2-generation studies in rats – one recently performed and considered well conducted, and a second older, less reliable study.

Table 32: 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, dose levels duration of exposure**	**Results**	**Ref.**
Two generation reproduction toxicity study in rats OECD 416 (2001) GLP	Cinmethylin Purity: 93.5 % Enantiomeric ratio: 48:52 (-/+) Concentrations: 0, 125/250, 500/1000 and 2500/5000 ppm	**<u>Parental Toxicity</u>** There were no treatment-related deaths or clinical signs of toxicity at any dose. **<u>F0 generation:</u>** **<u>2500/5000 ppm (equivalent to 412-450 and 394-481 mg/kg bw/day in males and females respectively):</u>** ↓ Body weight gain: 20 % (GD 0-7) and 10 % (GD 0-20) in females ↓ Food consumption: 8% (GD 0-20) in females	Anon, 2018h DAR: B.6.6.1

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Ref.**
Wistar 25/sex/dose (F_0 and F_1)	in males/females (equivalent to approximately: 0, 19.7-21.8/20.6-23.8, 79.4-87.7/81.3-96.9 and 412-450/394-481 mg/kg bw/day in males/females – please see table 33 in text)	*Organ weights:* ↑ Liver weight: abs. 23.5 % and rel. 26 % in males and abs. 20.2 % and rel. 24.5 % in females. ↑ Kidney weight: abs. 11.7 % and rel. 14.1 % in males only ↑ Thyroid weight: abs. 15.2 % and rel. 17 % in males and abs. 14.9 % and rel. 19 % in females. *Histopathology:* *Liver:* Enlargement: 6/25 males and 5/25 females *Kidney:* Enlargement: 6/25 males Chronic nephropathy: 25/25 males versus 10/25 in controls Eosinophilic droplets: 22/25 males versus 0 in controls Granular casts: 17/25 males versus 0 in controls *Thyroid gland:* Hypertrophy/hyperplasia of follicular epithelial cells: 10/25 males and 16/25 females versus 0 in controls *Nasal cavity:* Degeneration/regeneration of the olfactory epithelium: 25/25 males and 25/25 females versus 0 in controls **<u>500/1000 ppm (equivalent to 79.4-87.7 and 81.3-96.9 mg/kg bw/day in males and females respectively):</u>** Organ weights: ↑ Liver weight: abs. 10.8 % and rel. 8 % in males only **<u>125/250 ppm (equivalent to 19.7-21.8 and 20.6-23.8 mg/kg bw/day in males and females respectively):</u>** No adverse treatment-related findings. **<u>F1 generation:</u>** **<u>2500/5000 ppm (equivalent to 412-450 and 394-481 mg/kg bw/day in males and females respectively):</u>** *Organ weights:*	

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Ref.
		↑ Liver weight: abs. 19 % and rel. 22 % in males and abs. 19 % and rel. 21 % in females. ↑ Kidney weight: abs. 11.7 % and rel. 14.3 % in males only ↑ Thyroid weight: abs. 21 % and rel. 23 % in males and abs. 22 % and rel. 24 % in females. *Histopathology:* *Liver:* Enlargement: 4/25 males *Kidney:* Enlargement: 8/25 males Chronic nephropathy: 25/25 males versus 11/25 in controls Eosinophilic droplets: 23/25 males versus 0 in controls Granular casts: 18/25 males versus 0 in controls *Thyroid gland:* Hypertrophy/hyperplasia of follicular epithelial cells: 15/25 males and 8/25 females versus 0 in controls *Nasal cavity:* Degeneration/regeneration of the olfactory epithelium: 25/25 males and 25/25 females versus 0 in controls **500/1000 ppm (equivalent to 79.4-87.7 and 81.3-96.9 mg/kg bw/day in males and females respectively):** No adverse treatment-related findings. **125/250 ppm (equivalent to 19.7-21.8 and 20.6-23.8 mg/kg bw/day in males and females respectively):** No adverse treatment-related findings. **Pups:** No treatment-related findings (gross necropsy only) **Reproductive effects:** No treatment-related effects on oestrus cyclicity, mating performance, fertility and sperm parameters, differential ovarian follicle count, pup	

<table>
<tr><th>Method, guideline, deviations if any, species, strain, sex, no/group</th><th>Test substance, dose levels duration of exposure</th><th>Results</th><th>Ref.</th></tr>
<tr><td></td><td></td><td>
survival, sex ratio, nipple development, anogenital parameters or sexual maturation of pups.

<u>5000 ppm (equivalent to 394-481 mg/kg bw/day):</u>
↑ No. of females giving birth to stillborn pups: 5/24 (versus 1/25 in controls) in F_0 generation

<table>
<tr><td></td><td colspan="4">F0</td><td colspan="4">F1</td></tr>
<tr><td>Dose [ppm]</td><td>0</td><td>250</td><td>1000</td><td>5000</td><td>0</td><td>250</td><td>1000</td><td>5000</td></tr>
<tr><td>Females with liveborn</td><td>25</td><td>25</td><td>25</td><td>24</td><td>25</td><td>23</td><td>24</td><td>24</td></tr>
<tr><td>Females with stillborn</td><td>1</td><td>2</td><td>1</td><td>5</td><td>1</td><td>0</td><td>1</td><td>1</td></tr>
<tr><td>HCD</td><td colspan="8">Females with stillborn pups [N]: 0-4</td></tr>
<tr><td>Gestation index [%]</td><td>100</td><td>100</td><td>100</td><td>100</td><td>100</td><td>100</td><td>100</td><td>100</td></tr>
<tr><td>No. pups delivered</td><td>313</td><td>304</td><td>303</td><td>283</td><td>295</td><td>256</td><td>284</td><td>276</td></tr>
<tr><td>- per dam</td><td>12.5</td><td>12.2</td><td>12.1</td><td>11.8</td><td>11.8</td><td>11.1</td><td>11.8</td><td>11.5</td></tr>
<tr><td>HCD</td><td colspan="8">Pups delivered/dam: 9.9-12.7</td></tr>
<tr><td>-liveborn</td><td>312</td><td>300</td><td>298</td><td>273</td><td>294</td><td>256</td><td>282</td><td>274</td></tr>
<tr><td>-stillborn</td><td>1</td><td>4</td><td>5</td><td>10</td><td>1</td><td>0</td><td>2</td><td>2</td></tr>
<tr><td>-stillborn %</td><td>0.3</td><td>1.3</td><td>1.7</td><td>3.5</td><td>0.3</td><td>0</td><td>0.7</td><td>0.7</td></tr>
<tr><td>HCD</td><td colspan="8">Stillborn pups [%]: 0 – 4.2 %</td></tr>
<tr><td>- cannibalised/dead</td><td>4</td><td>1</td><td>0</td><td>1</td><td>0</td><td>0</td><td>0</td><td>2</td></tr>
<tr><td>- cannibalised/dead [%]</td><td>1.3</td><td>0.3</td><td>0</td><td>0.4</td><td>0</td><td>0</td><td>0</td><td>0.7</td></tr>
<tr><td>Live birth index [%]</td><td>99.7</td><td>98.7</td><td>98.3</td><td>96.5</td><td>99.7</td><td>100</td><td>99.3</td><td>99.3</td></tr>
<tr><td>HCD</td><td colspan="8">Live birth index [mean %]: 95.8-100 %</td></tr>
</table>
</td><td></td></tr>
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Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Ref.**
		HCD: 27 studies run 2008-2015 at the test facility with Wistar rats **NOAEL (Parental toxicity):** 500/1000 ppm (80 mg/kg bw/day) **NOAEL (Offspring toxicity):** 2500/5000 ppm (394 mg/kg bw/day) **NOAEL (Reproductive toxicity):** 2500/5000 ppm (394 mg/kg bw/day)	
Two generation reproduction toxicity study in rats Non-OECD guideline (Deviations listed in text) GLP Sprague Dawley F0: 20 males and 30 females/dose F1: 20 males and 25-30 females/dose	Cinmethylin Purity: batch 1: 92.4 % and batch 2: 93 % Enantiomeric ratio: not specified Concentrations: 0, 200, 2000 and 20000 ppm in males/females (equivalent to approximately: 0, 12-17/13-34, 115-170/130-353,1290-2125/1434-2893 mg/kg bw/day in males/females – please see table 34 in text)	**<u>Parental Toxicity</u>** **<u>F_0 generation:</u>** **<u>20000 ppm (equivalent to 1290-1367 and 1434-2883 mg/kg bw/day in males and females respectively):</u>** ↑ Mortality: 1/20 males (week 24) and 7/30 females (weeks 2-26) ↓ Body weight: 11-16 %* in males (weeks 5-21 – end of 2nd pre-mating), 14-20 %*in females producing F_{1a} litters (GD 0-20), 16-25 %*in females producing F_{1b} litters (GD 0-20), 17-21 %* in females producing F_{1a} litters (LD 1-21), 19-24 %* in females producing F_{1b}litters (LD 1-21). ↓ Body weight gain: 33 % in males (weeks 1-11 – end of 1st pre-mating), 37 % in males (weeks 1-21 – end of 2nd pre-mating), 35 % in females (weeks 1-11 – end of 1st pre-mating), 24 % in females (weeks 1-21 – 2nd premating), 34 % in females producing F_{1a} pups (GD 0-20), 44 % in females producing F_{1b} pups (GD 0-20). ↑Clinical signs: Chromodacryorrhea, periocular swelling, urine-stained urogenital area, lacrimation and soft stool in males. Unkempt appearance, piloerection, pale, red vaginal discharge, rales, urine-stained urogenital area, lacrimation, hypothermia and small amount of faeces in females. *Organ weights:* ↑ Liver: abs. 64 %* and rel. 94 %* in males and abs. 79.5 %* and rel. 105 %* in females ↑ Kidneys: rel. 22 %* in males	Anon, 1986c DAR: B.6.6.1

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Ref.
		↑ Testes: rel. 20 %* ↓ Ovaries: abs. 15.7 % *Histopathology:* *Liver:* Enlargement: 15/20 males (minimal) and 23/30 females (minimal-slight) (0 in controls) Periportal increased hepatocellular cytoplasmic eosinophilic density: 18/20** males (0 in controls) and 20/29** females versus 6/30 in controls Periportal hepatocellular cytoplasmic pigment: 19/20** males and 23/29** females (0 in controls) Hepatocellular cytoplasmic vacuolation: 24/29** females (versus 2/30 in controls) Parenchymal mononuclear-cell focus: 13/20** males (versus 4/20 males) Acute parenchymal necrosis (single cell): 2/20 males and 2/29 females (0 in controls) Acute parenchymal necrosis (coagulative): 2/20 males and 2/29 females (0 in controls) *Kidneys:* ↑ Lymphocytic interstitial nephritis: 16/20** males versus 5/20 in controls. *Uterus:* ↑ Haemorrhagic diathesis: 4/30 females (0 in controls) **<u>2000 ppm (equivalent to 115-123 and 134-353 mg/kg bw/day in males and females respectively):</u>** Mortality: 1/20 males (week 20) ↓ Body weight gain: 10 % in females (weeks 1-11 – end of 1st premating), 13 % in females producing F_{1b} pups (GD 0-20). ↑Clinical signs: Viscous salivation, red discharge around penis, hypoactivity, unsteady stance, squinting and mass in males (sporadic) Chromdacryorrhea, periocular swelling, pale, red vaginal discharge, rales, and mass in females.	

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Ref.
		Organ weights: ↑ Liver: abs. 20 %* and rel. 19 %* in males and rel. 13.7 %* in females *Histopathology:* *Liver:* Enlargement: 15/20 males (minimal) and 23/30 females (minimal-slight) (0 in controls) Periportal increased hepatocellular cytoplasmic eosinophilic density: 8/20** males (0 in controls) and 14/30 females versus 6/30 in controls Hepatocellular cytoplasmic vacuolation: 13/30** females (versus 2/30 in controls) Parenchymal mononuclear-cell focus: 13/20** males (versus 4/20 males) Acute parenchymal necrosis (single cell): 1/30 females (no data for controls) *Kidneys:* ↑ Lymphocytic interstitial nephritis: 16/20** males (versus 5/20 in controls). **<u>200 ppm (equivalent to 11.3-12 and 13.7-34 mg/kg bw/day in males and females respectively):</u>** No adverse treatment-related effects. **<u>F_1 generation:</u>** **<u>20000 ppm (equivalent to 1791-2125 and 1575-2893 mg/kg bw/day in males and females respectively):</u>** ↑ Mortality: 5/25 females (weeks 15-25) ↓ Body weight: 34-55 %* in males (weeks 1-21 – pre-mating), 21-54 %* in females (weeks 1-11 – pre-mating), 19-29 %* in females producing F_{2a} pups (GD 0-20), 29-33 %* in females producing F_{2a} pups (LD 1-21), 24-30 %* in females producing F_{2b} pups (GD 0-20), 22-27 %* in females producing F_{2b} pups (LD 1-21). ↓ Body weight gain:	

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Ref.
		27 % in males (weeks 1-11 – end of 1st premating), 24 % in males (weeks 1-21 – end of 2nd premating), 49 % in females producing F_{2a} pups (GD 0-20), 18 % in females producing F_{2a} pups (LD 1-21), 45 % in females producing F_{2b} pups (GD 0-20). ↑ Clinical signs: Periocular swelling, unilateral small testis, red discharge around penis, rales, urine-stained urogenital area and soft stool in males Unkempt appearance, red nasal discharge, rales and urine-stained urogenital area in females. *Organ weights:* ↑ Liver: abs. 65.5 %* and rel. 104 %* in females ↑ Testes:abs. 14 % and rel. 20 %* ↓ Ovaries: abs. 24.7 % *Histopathology:* *Liver:* Enlargement: 20/25 females (minimal-slight) (0 in controls) Periportal increased hepatocellular cytoplasmic eosinophilic density: 12/25** females versus 4/29 in controls Periportal hepatocellular cytoplasmic pigment: 20/20** males and 21/25** females (0 in controls) Hepatocellular cytoplasmic vacuolation: 24/29** females (versus 2/30 in controls) Parenchymal mononuclear-cell focus: 13/20* males (versus 5/20 males in controls) Acute parenchymal necrosis (single cell): 4/20 males (0 in controls) *Kidneys:* ↑ Corticol tubular pigment deposits: 8/20** males and 17/29** females (0 in controls) *Uterus:* ↑ Haemorrhagic diathesis: 1/25 females (0 in controls)	

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Ref.
		↑ Dystocia: 2/25 females (0 in controls) **<u>2000 ppm (equivalent to 138-163 and 129-332 mg/kg bw/day in males and females respectively):</u>** ↑Clinical signs: Red discharge around penis in males and rales in females. *Organ weights:* ↑ Liver: abs. 11.6 %* and rel. 14.7 %* in females *Histopathology:* *Liver:* Enlargement: 5/30 males (minimal/slight) (0 in controls) Hepatocellular cytoplasmic vacuolation: 13/20* males (versus 5/20 in controls) and 11/30* females (versus 2/29 in controls) **<u>200 ppm (equivalent to 13.5-16.1 and 12.2-34 mg/kg bw/day in males and females respectively):</u>** No adverse treatment-related effects. **<u>Pups – F_1 and F_2 generation:</u>** **<u>20000 ppm:</u>** ↑ Clinical signs: 16/36 pups from 4/7 litters (F_{1a}) versus 0 pups in controls and 26/76 pups from 7/17 litters (F_{1b}) versus 5/167 pups from 5/18 litters ↓ Body weight: 15.1 %* on day 1 and 59 %* day 21 ($F1_a$) 10.3 %* on day 1 and 53 %* day 21 ($F1_b$) 15.0%* on day 1 and 55.7 %* day 21 ($F2_a$) 9.8 %* on day 1 and 46.6 %* on day 21 ($F2_b$) *Organ weights (F_{2b} pups):* ↑ Liver: rel. 60.1 %* in males and 44.4 %* in females ↓ Testis: abs. 59.7 %* and rel. 14.6 % ↓ Ovaries: abs. 65.6 %* and rel. 24.8 %	

<table>
<tr><th>Method, guideline, deviations if any, species, strain, sex, no/group</th><th>Test substance, dose levels duration of exposure</th><th>Results</th><th>Ref.</th></tr>
<tr><td></td><td></td><td>
Histopathology (F_{2b} pups):

Liver:

↑ Periportal increased hepatocellular cytoplasmic eosinophilic density:4/10 males and 6/10* females (0 in controls)

↑ Parenchymal hepatocellular cytoplasmic vacuolation: 10/10* females versus 5/10 in controls

Seminal vesicles:

↑ Bilateral immaturity: 9/10** males versus 1/10 in controls

<u>2000 ppm:</u>

↓ Body weight: 11 %* day 21 (F_{1a})
11 %* day 21 (F_{2a})

Organ weights (F_{2b} pups):

↑ Liver: abs. 23.2 % and rel. 19.8 %* in males

↓ Ovaries: abs. 10.9 % and rel. 11.5 %

Histopathology (F_{2b} pups):

Histopathology only available for control and 20000 ppm dose groups.

<u>Reproductive Toxicity:</u>

<u>Table 32a: Reproduction parameters – males:</u>
<table>
<tr><th>Generation</th><th colspan="4">F_0</th><th colspan="4">F_1</th></tr>
<tr><th>Dose (ppm)</th><th>0</th><th>200</th><th>2000</th><th>20000</th><th>0</th><th>200</th><th>2000</th><th>20000</th></tr>
<tr><td>No. males placed with 30 females</td><td>20</td><td>20</td><td>20</td><td>20</td><td>20</td><td>20</td><td>20</td><td>20</td></tr>
<tr><td>Male mating index (%)</td><td colspan="4">ND#</td><td colspan="4">ND#</td></tr>
<tr><td>No. sired at least one litter</td><td>19</td><td>20</td><td>19</td><td>20</td><td>20</td><td>20</td><td>20</td><td>13</td></tr>
</table>
</td><td></td></tr>
</table>

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Ref.

Male fertility index (%)	95	100	100	100	100	100	100	**65***

[#] ND = Not determined. Due to an unequal number of males and females, two subsequent matings for each generation to breed two litters, as well as raw data deficiencies, the number of males that mated with females (defined by females with implant in utero) was not available.

Table 32b: Reproduction and gestational parameters – F_0 females:

Generation	**F_0 producing F_{1a}**				**F_0 producing F_{1b}**			
Dose (ppm)	**0**	**200**	**2000**	**20000**	**0**	**200**	**2000**	**20000**
No. females placed with males	30	30	30	29	30	29	30	27
No. females mated	28	28	28	26	29	29	30	26
Mating index (%)	93.3	93.3	93.3	**89.7**	96.7	100	100	96.3
No. pregnant	15	17	13	**11**	18	19	21	21
Fertility index (%)	53.6	60.7	46.4	42.3	62.1	65.5	70.0	80.8
Pre-coital interval (days)	No data				No data			
Duration of gestation (days)	22.8	22.9	22.5	22.4	22.1	22.2	22.1	22.1
No. females with live-born	12	16	12	**7**	18	19	21	17
Gestation index (%)	80.0	94.1	92.3	**63.6**	100	100	100	80.9
No. with all stillborn	2	1	1	**4**	0	0	0	1
Implantation sites	No data				No data			

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Ref.

(mean no./dam)									
Pups delivered (mean no./dam)	8.57	8.41	9.54	**4.73**	11.4	11.95	10.10	**6.11***
Live pups (mean no./dam)	7.64	7.82	8.46	**3.27***	9.28	11.32	8.19	**4.22***
Post-implantation loss (%)	No data				No data			

Table 32c: Reproduction and gestational parameters – F_1 females:

Generation	**F_1 producing F_{2a}**				**F_1producing F_{2b}**			
Dose (ppm)	**0**	**200**	**2000**	**20000**	**0**	**200**	**2000**	**20000**
No. females placed with males	29	30	30	25	29	29	28	23
No. females mated	28	29	28	**12**	29	29	28	**12**
Mating index (%)	96.6	96.7	93.3	**48.0***	100	96.7	100	**52.2***
No. pregnant	26	29	28	**8**	25	24	27	**11**
Fertility index (%)	92.9	100	100	**66.7#**	86.2	82.8	96.4	91.7
Pre-coital interval (days)	No data				No data			
Duration of gestation (days)	22.1	22.2	22.0	21.5	22.2	22.1	22.1	20.7
No. females with live-born	26	29	28	**5**	25	24	27	**9**

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Ref.

Gestation index (%)	100	100	100	**62.5**	100	100	100	**81.8**
No. with all stillborn	0	0	0	**3**	0	0	0	0
Implantation sites (mean no./dam)	No data				No data			
Pups delivered (mean no./dam)	11.7	12.41	12.07	**5.33***	11.84	13.38	12.19	**6.33***
Live pups (mean no./dam)	11.15	11.28	10.59	**3.00***	11.16	12.38	10.85	**4.44***
Post-implantation loss (%)	No data				No data			

*p≤ 0.05 (one-sided Fisher's Exact Chi-Square test)

p≤ 0.05 (one-sided Dunnett's t-test)

Table 32d: Survival and sex ratio

F_1 pups		**F_0 → F_{1a} pups**				**F_0 → F_{1b} pups**			
Dose level [ppm]		**0**	**200**	**2000**	**20000**	**0**	**200**	**2000**	**20000**
Number of litters delivered		14	17	13	11	18	19	21	18
- with liveborn pups		12	16	12	7	18	19	21	17
- with all stillborn		2	1	1	**4**	0	0	0	1
- with stillborn pups		7	8	8	8	15	7	14	14
Pups delivered	[n]	120	143	124	**52**	205	227	212	110
- liveborn	[n]	107	133	110	36	167	215	172	76
- stillborn	[n]	13	10	14	16	38	12	40	34
Live birth index	**[%]**	78.5	88.4	82.7	**53.6**	79.7	95.4	78.7	**64.9**
Pups found dead	(day 1-4)	6	1	2	2	9	5	8	10
Pups PND 4	(pre-cull)	101	132	108	34	158	210	163	66
Viability index	**[%]**	93.1	99.6	97.6	89.3	90.2	97.1	91.4	**80.4**

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results								Ref.
		Pups culled day 4	23	29	26	1	37	70	34	0
		Pups PND 4 (post-cull)	78	103	82	33	121	140	129	66
		Pups found dead (day 5-21)	0	1	0	**5**	1	8	1	**16**
		Pups PND 21	78	102	82	28	120	132	128	50
		Lactation index **[%]**	100	99.2	100	**87.1**	99.3	94.7	98.8	**79.7***
		Sex ratio [% live males], PND 0	37.9	50.9	37.8	43.7	46.9	44.0	45.5	46.9
		Sex ratio [% live males], PND 21	*No data*				*No data*			
		F_2 pups	**F_1 → F_{2a} pups**				**F_1 → F_{2b} pups**			
		Dose level [ppm]	**0**	**200**	**2000**	**20000**	**0**	**200**	**2000**	**20000**
		Number of litters	26	29	28	8	25	24	27	9
		- with liveborn pups	26	29	28	5	25	24	27	9
		- with all stillborn pups	0	0	0	**3**	0	0	0	0
		- with stillborn pups	9	16	17	6	11	12	16	7
		Pups delivered [n]	306	360	338	**43**	299	321	329	**57**
		- liveborn [n]	290	327	296	24	280	297	293	40
		- stillborn [n]	16	33	42	19	19	24	36	17
		Live birth index [%]	95.2	90.6	87.6	**51.0**	93.4	90.7	89.5	**74.3**
		Pups found dead (day 1-4)	4	6	14	7	15	7	7	13
		Pups PND 4 (pre-cull)	286	321	282	17	265	290	286	27
		Viability index [%]	95.0	97.9	97.7	**71.4**	94.1	94.2	96.9	**67.5**
		Pups culled day 4	91	100	76	0	92	107	80	1
		Pups PND 4 (post-cull)	195	221	206	17	173	183	206	26
		Pups found dead (day 5-21)	0	2	0	1	2	3	1	0
		Pups PND 21	195	219	206	16	171	180	205	26
		Lactation index [%]	100	99.1	100	**80.0**	99.0	98.3	99.5	100
		Sex ratio [% live males], PND 0	48.4	45.2	55.0	**38.0**	50.0	45.1	44.6	48.5

<table>
<tr><th>Method, guideline, deviations if any, species, strain, sex, no/group</th><th>Test substance, dose levels duration of exposure</th><th>Results</th><th>Ref.</th></tr>
<tr><td></td><td></td><td>
<table>
<tr><td>Sex ratio [% live males], PND 21</td><td>No data</td><td>No data</td></tr>
</table>
* p ≤ 0.05 one-tailed Dunnett's t-test

<u>Table 32e: Eye opening of pups</u>
<table>
<tr><th>Dose level [ppm]</th><th></th><th>0</th><th>200</th><th>2000</th><th>20000</th><th>0</th><th>200</th><th>2000</th><th>20000</th></tr>
<tr><td></td><td></td><td colspan="4">F1a</td><td colspan="4">F2a</td></tr>
<tr><td rowspan="2">Time of eye opening (days)</td><td>Mean</td><td>14.5</td><td>15.1</td><td>14.6</td><td>17.0</td><td>15.4</td><td>15.2</td><td>15.7</td><td>15.8</td></tr>
<tr><td>SD</td><td>0.8</td><td>0.9</td><td>0.5</td><td>1.0</td><td>0.6</td><td>0.8</td><td>0.5</td><td>1.0</td></tr>
<tr><td></td><td></td><td colspan="4">F1b</td><td colspan="4">F2b</td></tr>
<tr><td rowspan="2">Time of eye opening (days)</td><td>Mean</td><td>15.1</td><td>15.4</td><td>15.4</td><td>16.9</td><td>15.1</td><td>15.5</td><td>15.4</td><td>17.0</td></tr>
<tr><td>SD</td><td>0.8</td><td>0.9</td><td>0.8</td><td>1.1</td><td>0.8</td><td>0.7</td><td>0.9</td><td>0.9</td></tr>
</table>
Note – sexual maturation (vaginal opening and preputial separation) was not measured in this study.

No NOAELs set due to significant study limitations.
</td><td></td></tr>
</table>

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

A recently-performed guideline 2-generation study was conducted in Wistar rats (25/sex/dose), according to GLP (Anon, 2018h). Animals received cinmethylin in the diet at concentrations of 0, 250, 1000 and 5000 ppm. During lactation, dietary concentrations were adjusted in females to 0, 125, 500 and 2500 ppm to ensure dose levels were equivalent to those in the pre-mating phase (see Table 33 for achieved concentrations of cinmethylin). This adjustment of dose levels was the only deviation to the guideline and is not considered to negatively impact the reliability of the study. Parental animals (F0) were administered cinmethylin for 10 weeks prior to mating and throughout the gestation and lactation periods. After weaning of F1 pups, the F0 generation of animals were sacrificed. Selected F1 pups (25/sex/dose) were then retained post-weaning and mated to produce the F2 generation. F1 females were allowed to litter and rear F2 pups to day 4 before sacrifice.

Parental and offspring toxicity

There were no treatment-related effects on mortality or clinical signs at any dose.

At the top dose (5000 ppm, equivalent to 412-450/394-481 mg/kg bw/day) body weight was reduced in F_0 females by 20 % compared to controls between gestation days (GD) 0-7 and by 10 % between GD 0-20. This was associated with an 8 % reduction in food consumption (compared to controls). At this dose, the liver, kidney, thyroid and nasal cavity were adversely affected.

Table 33: Achieved concentration of cinmethylin in male and female Wistar rats during pre-mating, gestation and lactation phases of the study.

Phase	Generation	Dietary concentration of cinmethylin [ppm]					
		Males			Females		
		250	1000	5000	250 / 125 [a)]	1000 / 500 [a)]	5000 /2500 [a)]
Pre-mating	F_0	19.7	79.4	412	21.4	82.2	417
[mg/kg bw/d]	F_1	21.8	87.7	450	22.8	90.1	460
Gestation	F_0				20.7	81.3	395
[mg/kg bw/d]	F_1				20.6	81.6	394
Lactation	F_0				23.8	93.8	473
[mg/kg bw/d]	F_1				23.5	96.9	481

[a)] Feed concentrations were halved to compensate the increased food intakes during the lactation period

Liver weight was increased by up to 26 % in F_0 males and females and up to 22 % in F_1 males and females. Gross necropsy revealed liver enlargement in 6/25 males and 5/25 females (F_0) and 4/25 F_1 males only (versus 0 in controls) but no histopathology correlates were observed.

Kidney weights were also statistically-significantly increased at the top dose in males only (up to 14.3 % F_0 and F_1). This was associated with chronic nephropathy, eosinophilic droplets and granular casts. These effects are considered a consequence of α2μ-globulin accumulation (please see section 10.12 specific target organ toxicity – repeated dosing).

The thyroid was enlarged in both males and females of the top dose group (up to 19 % higher than controls in F_0 and up to 24 % higher than controls in F_1). Hypertrophy/hyperplasia of follicular epithelial cells was also observed in 10/25 males and 16/25 females (F_0) and 15/25 males and 8/25 females (F_1) (versus 0 in controls).

All animals of the top dose (both F_0 and F_1) were found to have degeneration/regeneration of the olfactory epithelium of the nasal cavity compared to 0 animals in the control groups.

At the mid dose of 1000/500 ppm (79.4-87.7/81.3-96.9 mg/kg bw/day) there was a small increase in liver weight in F_0 males only (absolute: 10.8 % and relative 8 % greater than controls). There were no associated clinical chemistry or histopathological correlates at this dose. No adverse treatment-related findings were observed in F_0 females or F_1 males and females.

At 250/125 ppm (19.7-21.8/20.6-23.8 mg/kg bw/day) there were no adverse treatment-related findings in F_0 or F_1 animals.

There were no treatment-related effects on pup weight or on the organ weight of pups. Only gross necropsy was performed and there were no treatment-related changes observed.

Overall, treatment-related adverse reductions in food consumption (F_0 females during gestation) and mean body weight gain (F_0 females during gestation) were observed at the top dose only. In both generations of adults there were increases in liver weights, thyroid weights, with concomitant thyroid histopathology and adverse findings to the nasal cavity. These findings were all observed at the top dose only (2500/5000 ppm). There were no treatment-related clinical or gross-necropsy findings in pups.

Reproductive toxicity

Following administration of cinmethylin, there were no adverse effects on oestrous cyclicity, mating performance and fertility, sperm parameters, differential ovarian follicle count, pup survival or sex ratio. Examination of the reproductive organs revealed no treatment-related changes.

The mean day of vaginal 2 was increased at 250 ppm and 2500 ppm in F_1 pups (31.3, 32.0, 31.0 and 32.2 in the 0, 250/125, 1000/500 and 5000/2500 ppm groups respectively). At these doses, the values obtained were marginally above the HCD range (day of vaginal opening 29.5 – 31.9 days, taken from 17 studies performed between 2010-2015 at the test facility with Wistar rats). As the difference was less than half a day and showed no dose-response or statistical significance, it was not considered treatment-related. There were no adverse effects to anogenital parameters or preputial separation.

The number of F_0 females with stillborn pups was increased at the top dose (5/24 versus 1/25 in controls). This was slightly above the HCD range of 0-4 (27 studies carried out in Wistar rats between 2008-2015 at the test facility). However, the increase was not statistically significant and was not observed in the F_1 generation. Moreover, the increased number of stillborn pups (10/283 versus 1/313 in controls) occurred mainly in one litter which contained 5 stillborn offspring. Therefore, the finding is not considered related to treatment with cinmethylin.

Overall, there were no treatment-related effects on fertility or sexual function in this study.

An older, less reliable two-generation study is also available (Anon*,* 1986c). This GLP study was carried out according to EPA guidelines but not OECD. There are a number of deviations to current (2001) and previous (1983) OECD 416 test methods:

- Oestrus cycle data analysis was not performed.
- Assessment of sperm parameters was not performed.
- Differential ovarian follicle count was not performed in parental females.
- Organ weight was not measured for uterus, prostate, seminal vesicles, brain, spleen, thymus.
- Assessment of sexual maturation (vaginal opening / preputial separation) was not performed.
- Inappropriate high dose level of 20000 ppm (corresponding to mean intakes in females of up to 2213/1609/2893 mg/kg bw/d during premating/gestation/lactation respectively) associated with mortality rate of 23 % and 17 % in parental females of the F_0 and F_1 generation respectively; exceeding the acceptable upper limit of 10 %.
- Dose levels were spaced by a factor of 10, exceeding guideline recommendations of factor 3 for dietary studies and reducing the sensitivity of the study to detect treatment-related effects and/or a dose-response relationship.
- Male mating index could not be determined.
- Gestation index calculations excluded potential females with only resorptions (not examined).
- Corpora lutea and implantation sites were not recorded for assessment of pre- and post-implantation loss.
- Due to malfunction of the automatic lighting system during the first mating of the F_0 generation animals were continuously exposed to light for an 8-day period. These conditions likely account for the unusually low female fertility indices in control test groups, in both matings of the F_0 generation (F_{1a}: 42-61 %, F_{1b}: 62-80 %).

- As a consequence, the required minimum group size of 20 pregnant females was not reached for control and test groups of the F_0 generation in both matings (except for the mid- and high-dose group of the 2nd mating).
- Numerous instances of poor/insufficient reporting of the study.

Due to this extensive list of deviations, the study is considered to be unreliable and is available only to add to the weight of evidence.

Cinmethylin was administered in the diet to Sprague Dawley rats (20 male and 30 females/dose for the F_0 parental generation and 20 male and 25-30 females for the F_1 parental generation) for 10 weeks prior to mating and then throughout the mating, gestation and lactation periods. Concentrations administered were 0, 200, 2000 or 20000 ppm (equivalent to 0, 12-17/13-34, 115-170/130-353 and 1290-2125/1434-2893 mg/kg bw/day for males/females respectively – see Table 34). Groups were mated to produce a first litter (F_{1a}) and then re-mated 10-14 days after weaning to produce a second litter (F_{1b}). Following weaning of the F_{1b} generation, groups of 20 males and 25-30 females were selected as F_1 breeding animals. These rats were mated to produce two F_2 litters. Treatment of F_1 males was discontinued four weeks before necropsy.

Table 34: Achieved concentration of cinmethylin in male and female Wistar rats during pre-mating, gestation and lactation phases of the study.

Sex / generation / study phase	**Dose [ppm]**					
	F_0			**F_1**		
	200	**2000**	**20000**	**200**	**2000**	**20000**
	Mean cinmethylin intakes [mg/kg bw/d]					
Premating						
Males (up to 1st mating)	12.0	122.5	1366.5	16.1	163.4	2125.4
Males (up to 2nd mating)	11.3	114.6	1288.8	13.5	137.7	1791.5
Females	13.9	138.9	1450.0	17.3	169.9	2213.0
Gestation						
Females (1st mating)	14.7	148.4	1520.7	14.4	142.2	1609.3
Females (2nd mating)	13.7	133.7	1434.0	12.8	129.7	1575.1
Lactation						
Females (1st mating)	34.0	352.8	2859.3	33.9	332.7	2893.3
Females (2nd mating)	30.6	279.9	2283.3	30.4	307.3	2256.0

Parental and offspring toxicity

There was an increased incidence of parental mortality in top dose females (7/30 F_0 and 5/20 F_1 died versus 0 in control groups) and in 1/20 top dose male of the F_0 generation. Of these deaths, 5/30 F_0 and 3/20 F_1 females were attributed to treatment. In the 2000 ppm treatment group, 1/20 F_0 males and 2/30 F_1 females also died, but again, these were not considered related to treatment. In females, deaths generally occurred at the end of pregnancy or shortly after birth. Dystocia was found to be the most common cause of death, sometimes accompanied by fatal haemorrhage from the uterus.

There were no adverse treatment-related findings at the lowest dose of 200 ppm. Clinical signs were observed from the mid dose of 2000 ppm, increasing in incidence at the top dose of 20000 ppm. In F_0

parental females, these included rales, red vaginal discharge, urine-stained urogenital area and reduced defecation. In F_0 parental males, clinical signs included chromodacryorrhea, periocular swelling, urine stained urogenital area, lacrimation and soft stool. Top dose F_1 males showed unilateral small testis, urine-stained urogenital area and an increase in incidence of red discharge around the penis and soft stool. In F_1 females, red nasal discharge, rales and urine-stained urogenital area were recorded.

In the F_0 generation, body weight was statistically significantly decreased compared to controls by 11-16 % in top dosed males during weeks 5-21 (the end of the second pre-mating phase). Body weight was also statistically significantly decreased in females of the top dose group by up to 25 % during gestation and lactation phases. Overall body weight gain was decreased in males by 33-37 % during pre-mating and in females 24-44 % during pre-mating and gestation phases. Similar reductions in body weight and body weight gain were observed in males and females in the top dose group of the F_1 generation. Body weight in males was statistically significantly lower than controls by 34-55 % during the pre-mating phase of the study and in females, body weight was statistically significantly lower than controls during pre-mating, gestation and lactation phases of the study by 19-54 %. Body weight gain was reduced in males by 24-27 % and in females by up to 48 % during gestation and 18 % during the lactation phase. A reduction in body weight gain in F_0 females compared to control animals was also noted in the mid dose group (10 % lower than controls during pre-mating and 13 % lower than controls during gestation).

The main target organs were the liver and kidneys. Statistically significant increased liver weight was observed from the mid dose and above. At 2000 ppm liver weight was increased in F_0 males by 20 % (abs.) and 19 % (rel.) compared to controls, in F_0 females by 13.7 % (rel.) and in F_1 females by 11.6 % (abs.) and 14.7 % (rel.). At 20000 ppm, liver weight was increased in F_0 males (abs. 64 % and rel. 94 %) and females (abs. 79.5 % and rel. 105 %) and in F1 females only (abs. 65.5 % and rel. 104 %). Histopathological correlates at the top dose included liver enlargement (15/20 F_0 males, 23/30 F_0 females and 20/25 F_1 females compared to 0 in controls), increased periportal cytoplasmic eosinophilic density (18/20 F_0 males versus 0 in controls, 20/29 F_0 females versus 6/30 in controls and 12/25 F_1 females versus 4/29 in controls), increased periportal hepatocellular cytoplasmic pigment (9/20 F_0 males, 23/29 F_0 females, 2/20 F_1 males and 21/25 F_1 females versus 0 in controls), hepatocellular cytoplasmic vacuolation (24/29 F_0 females versus 2/30 in controls and 11/25 F_1 females versus 2/29 in controls). Changes to parenchymal cells were also observed, including mononuclear cell focus, observed in 13/20 F_0 males (versus 4/20 controls) and 13/29 F_1 males (versus 5/20 controls); acute single cell necrosis in 2/20 F_0 males, 2/29 F_0 females and 4/20 F_1 males (versus 0 in controls) and acute coagulative necrosis in 2/20 F_0 males and 2/29 F_0 females (versus 0 in controls).

Statistically significant increases in kidney weight were observed in F_0 males only (rel. 22 %). There was an increase in incidence of lymphocytic interstitial nephritis (16/20 males compared to 5/20 in controls) and haemorrhagic diathesis in F_0 females only (4/30 females versus 0 in controls). In the F_1 generation treated with 20000 ppm, there was no change in organ weight but there was an increased incidence of corticol tubular pigment deposits in males and females (8/20 males and 17/29 females versus 0 in controls).

Other findings at the top dose included an increase in testes weight in males (F_0: 20 % (rel.) and F_1: 14 % (abs.) and 20 % (rel.) and a decrease in ovaries weight in females (F_0: 15.7 (abs.) and F_1: 24.7 (abs.) with no histopathological correlates associated with either finding. Whilst the findings in testes were considered related to treatment, the findings in females were not as there was no increase in relative weight. In top dosed females of both generations, there was an increased incidence of haemorrhagic diathesis of the uterus in 4/30 females (F_0) and 1/25 females (F_1) (versus 0 in control animals) and also dystocia (5/30 F_0 and 3/20 F_1 females versus 0 in controls).

In pups, there was an increase in clinical signs at the top dose of 20000 ppm only. Body weight was statistically significantly decreased by 11 % on day 21 at 2000 ppm and by 10-15 % on day 1 and 47-59 % on day 21 at 20000 ppm in F_{1a}, F_{1b}, F_{2a} and F_{2b} pups.

Organ weights were measured and histopathology was performed only on selected F_{2b} pups. In particular, histopathology was only performed on control and top dose groups. Increased liver weight was observed

from the mid dose and above. At 2000 ppm, absolute weight was increased by 23.2 % and relative by 19.8 % in males only. At the top dose, an increase in relative weight only was observed in males and females (60.1 % males and 44.4 % females). Histopathological correlates showed a statistically significant increase in periportal hepatocellular cytoplasmic eosinophilic density in 4/10 males and 6/10 females (versus 0 on controls). Parenchymal hepatocellular cytoplasmic vacuolation was observed in all 10 females (versus 5/10 controls). This was also found to be statistically significant.

There appeared to be a treatment-related effect on ovaries, with decreased weight compared to controls from 2000 ppm (abs. 10.9 % and rel. 11.5 % at 2000 ppm and abs. 65.6 % and rel. 24.8 % at 20000 ppm). This finding was only statistically significant at the top dose for the absolute reading only. There was no associated histopathology. Testis weight was decreased in males of the top dose group only (abs. 65.6 % and rel. 24.8 % - statistically significant for the absolute weight only). Bilateral immaturity of the testis was noted for 9/10 males (versus 1/10 in controls).

Reproductive toxicity

F_0 generation – mating one (F_{1a}) and mating two (F_{1b})

The number of F_0 females found to be pregnant was lower at the top dose than in controls. This resulted in a lower mating index for F_0 females producing the F_{1a} generation (89.7 % versus 93.3 % in controls). The number of females with live-born was also lower, as was the gestation index (63.6 % versus 80.0 % in controls). Gestation index was also slightly lower in top dose treated females during the second mating (80.9 % versus 100 % in controls). Female fertility index was in general much lower in the first mating (42-61 % versus 62-81 % in the second mating), however there was no obvious dose response and controls were also unusually low. This was considered to be due to a fault in the lighting system, meaning animals were exposed to continuous light for 8 days, rather than related to treatment. There was an increase in the number of females with all stillborns in the top dose group (4 versus 2 in controls during mating 1). The number of pups delivered (mean number/dam) was lower in the top dosed animals during both matings (4.73 versus 8.57 in controls during mating one and 6.11 versus 11.4 in controls during mating two). The live number of pups (mean number/dam) was also statistically significantly lower during both matings (3.27 versus 7.64 in controls during mating one and 4.22 versus 9.28 during mating two). The number of litters with all stillborn pups was 4/11 at the top dose during mating one (compared to 2/14 in controls). It is not clear if any of these observations resulted from pre- or post-implantation loss as these parameters were not investigated. The live birth index appeared to be slightly lower during the first mating (53.6 % versus 78.5 % in controls) and during the second mating 64.9 % versus 79.7 % in controls). The lactation index was lower in top dose treated animals during mating one (87.1 % versus 100 % in controls) and statistically significantly lower during mating two (79.7 % versus 99.3 5 in controls). Mean time for eye opening appeared to be increased during mating one (17 days versus 14.5 days in controls) but not mating two. Sexual maturation was not measured in this study. There was no evidence of any reproductive toxicity at lower doses.

F_1 generation – mating one (F_{2a}) and mating two (F_{2b})

Male fertility index was statistically significantly decreased in F_1 males (65 % versus 100 % in controls). This change was not observed in F_0 males.

All of the following findings relate to animals treated with 20000 ppm only, there were no treatment-related findings to reproduction at lower doses. The number of F_1 females that mated was 12/25 in the first mating (versus 28/29 in controls) and 12/23 in the second mating (versus 29/29 in controls). This resulted in a statistically significant decreased mating index (48 % versus 96.6 % in mating one and 52.2 % versus 100 % in mating two). Fertility index was decreased during mating one only (66.7 % versus 92.9 % in controls). The number of females with live-born pups was lower than controls (5/25 versus 26/29 during mating one and 9/23 versus 25/29 during mating two). This resulted in a lower gestation index of 62.5 %

(versus 100 %) during mating one and 81.8 % (versus 100 %) during mating two. The number of dams with all stillborn pups was 3/25 during mating one (versus 0 in controls). The mean number of pups delivered/dam was statistically significantly lower in both matings (5.33 versus 11.7 in controls in mating one and 6.33 versus 11.84 in controls in mating two) and the mean live number of pups/dam was also statistically significantly lower than controls (3 versus 11.15 in controls during mating one and 4.44 versus 11.16 in controls during mating two).

Again, the number of litters with all stillborn pups was increased during the first mating (3/8 versus 0/26), however, this was not observed in the second mating. Live birth index was lower at the top dose during both matings (51 % versus 95.2 % during mating one and 74.3 % versus 93.4 % during mating two). The viability index was found to be lower in this dose group also (71.4 % versus 95 % in controls during mating one and 67.5 % versus 94.1 % in controls during mating two). The lactation index was lower during the first mating (80 % versus 100 % in controls), however this was not observed during the second mating. Sex ratio appeared to be lower than controls during the first mating (38 % live males on post-natal day 0 versus 48.4 % in controls). This was not observed during the second mating or during either mating of the F_0 generation. The mean time for eye opening of pups was higher during both matings (16.9 days versus 15.1 days in controls during mating one and 17 days versus 15.1 days during mating two).

Overall, there appeared to be treatment-related adverse effects on fertility and mating indices, litter size, live birth index, pup survival and lactation index at the top dose of 20000 ppm (equivalent to 2125/1434-2893 mg/kg bw/day in males/females) (a dose far exceeding the limit dose for this type of study). However, the study was carried out with a number of significant deviations from the OECD test guideline and conclusions on these endpoints (particularly fertility index) are not robust. Pups died mainly between the first and second weeks of lactation with deaths associated with severe generalised toxicity of the mothers (mortality, reduced body weight and body weight gain, clinical signs and liver toxicity from doses of 2000 ppm and above) leading to reduced nursing. There was no evidence to suggest a direct effect on or via lactation. General toxicity was also observed in pups. Decreases in body weight compared to controls and increases in liver weight were observed from 2000 ppm and above. At the top dose of 20000 ppm, clinical signs of toxicity, increased liver weights with concomitant histopathology and changes in testes and ovaries weight were observed. Eye opening of top dosed pups was delayed and this was associated with the lower body weights in this group as compared to controls. There were no treatment-related adverse effects in parental animals or pups at the low dose at which no general toxicity occurred.

It is concluded that the adverse effects observed in this study in relation to fertility, reproduction and pup development were an unspecific secondary consequence of the severe generalised toxicity observed at the top dose group.

10.12.3 Comparison with the GB CLP criteria

Cinmethylin has been tested in a guideline-compliant two-generation study in Wistar rats. An additional, unreliable two generation study in Sprague-Dawley rats is also available for completeness. A specific effect on fertility, reproduction and pregnancy outcome was not demonstrated by these studies. In the old non-guideline study, at the top dose of 20000 ppm (equivalent to 2125/1434-2893 mg/kg bw/day in males/females) a number of adverse effects were seen in both adults and pups. These adverse effects included lower fertility and mating indices, changes in litter size, live birth index, pup survival, lactation index and delayed eye opening in pups. However, it must be noted that the dose administered was more than twice that of the recommended limit dose and caused severe toxicity in both parents and pups (mortality, body weight and body weight gain changes, clinical signs, increased liver weight and liver histopathology). The effects on fertility and pups are therefore deemed to be non-specific secondary consequences of the general toxicity observed at the top dose. No such effects were observed in the well-conducted guideline study or at doses at which there was no toxicity.

Cinmethylin is not known as a human reproductive toxicant; therefore classification in Category 1A is not necessary.

From the animal data available, there was no clear evidence to suggest that cinmethylin should be presumed to be a human reproductive toxicant; therefore classification in Category 1B is not appropriate.

Classification in Category 2 is reserved for substances where there is some evidence from human or experimental animals of an adverse effect on sexual function and fertility. Such effects should be observed in the absence of other toxic effects. On the basis that there is no evidence that cinmethylin causes any adverse effects to sexual function or fertility in the absence of severe parental toxicity, it should not be classified in this category. Therefore no classification for this endpoint is required.

10.12.4 Adverse effects on development

There are four developmental toxicity studies available. One older, non-guideline study in the rat, and three studies in rabbits. The first rabbit study is modern, conducted according to recent guidelines and GLP and the other two are older, non-guideline and more limited.

Table 3519: Summary table of animal studies on adverse effects on development

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Ref.**
Developmental Toxicity Study in rats Non-guideline (similar to OECD 414) GLP Sprague Dawley 25/females/dose	Cinmethylin Purity: 92.4 % Enantiomeric ratio: not specified Doses: 0, 30, 300, 1000 and 2000 mg/kg bw/day in corn oil (5mL/kg bw/day) Administered on gestation days (GD) 6-15	**<u>Maternal toxicity:</u>** **<u>2000 mg/kg bw/day:</u>** ↑ Mortality: 2/25 dams on GD 15 ↓ Body weight: approx. 10 % GD 12-20 ↓ Body weight gain: GD 6-7: lost weight (-4.3 g** versus +2.2 g in controls) GD 6-9: lost weight (-8.6 g** versus +7.3 g in controls) GD 6-12: lost weight (-0.5 g** versus +24.7 g in controls) GD 6-16: 58.4 %** GD 6-20: 29 %** GD 0-20: 25.9 %** ↑ Clinical signs: excess salivation, urine-stained abdominal fur, chromorrhinorrhea, vocalisation, hypersensitivity, thin appearance and tip-toe walk. *Organ weights:* ↑ Liver: abs. 24 % and rel. 37 % **<u>1000 mg/kg bw/day:</u>** ↓ Body weight gain: GD 6-7: lost weight (-0.9 g* versus +2.2 g in controls)	Anon 1984 b DAR: B.6.6.2

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Ref.
		GD 6-9: 58.9 %* GD 6-12: 23.9 %* GD 6-16: 15.9 % ↑ Clinical signs: excess salivation, urine-stained abdominal fur *Organ weights:* ↑ Liver: abs. 12 % and rel.16 % **300 mg/kg bw/day:** ↓ Body weight gain: GD 6-7: lost weight (-0.5 g* versus +2.2 g in controls) GD 6-9: 26 % GD 6-12: 24.7 %* GD 6-16: 13.2 % ↑ Clinical signs: excess salivation **30 mg/kg bw/day:** No adverse treatment-related findings. **Developmental toxicity:** **2000 mg/kg bw/day:** ↓ No. litters with viable fetuses: 21 versus 25 in controls ↑ No. litters with only resorptions: 2 versus 0 in controls ↑ Post-implantation loss: 17.1 % versus 4.7 % in controls ↑ Mean no. resorptions: 2.2 versus 0.7 in controls ↑ Total no. resorptions: 51 versus 17 in controls (all early) ↓ Live fetuses: 295 versus 339 in controls ↓ Mean fetal weight: 3.2 g** versus 3.51 g in controls (8.6 % reduction) **≤ 1000 mg/kg bw/day:** No adverse treatment-related findings to pregnancy status	

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Ref.

Table 35a: Fetal and litter incidences of malformations and variations observed in pups after treatment with cinmethylin during GD 6-15.

Dose (mg/kg bw/day)	**0**	**30**	**300**	**1000**	**2000**
No. litters evaluated	25	25	25	25	21
No. fetuses evaluated	164	171	166	161	145
Total **visceral malformations**					
Fetal incidence (%)	2 (1.22)	0	0	1 (0.62)	3 (2.07)
Litter incidence (%)	2 (8.0)	0	0	1 (4.0)	3 (14.3)
Affected fetuses/litter (%)	1.14 ± 3.96	0	0	0.57 ± 2.86	1.96 ± 4.92
Fetuses with multiple **visceral malformations and variations**					
Fetal incidence (%)	0	0	0	1 (0.62)	1 (0.69)
Litter incidence (%)	0	0	0	1 (4.0)	1 (4.76)
Affected fetuses/litter (%)	0	0	0	0.57 ± 2.86	0.60 ± 2.73
Malpositioned heart					
Fetal incidence (%)	0	0	0	0	1 (0.69)
Litter incidence (%)	0	0	0	0	1 (4.76)
Affected fetuses/litter (%)	0	0	0	0	0.68 ± 3.12
HCD	Fetal incidence: 1 (0.02); litter incidence 1 (0.28)				
Situs inversus					
Fetal incidence (%)	0	0	0	0	1 (0.69)

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results						Ref.
		Litter incidence (%)	0	0	0	0	1 (4.76)	
		Affected fetuses/litter (%)	0	0	0	0	0.68 ± 3.12	
		HCD	Fetal incidence: 1 (0.05); litter incidence 1 (0.29)					
		Total **visceral variations**						
		Fetal incidence (%)	0	0	0	4 (2.48)	**18 (12.4)**	
		Litter incidence (%)	0	0	0	4 (16.0)	**7 (33.3)**	
		Affected fetuses/litter (%)	0	0	0	2.61 ± 6.18	13.66 ± 27.63	
		Fetuses with multiple **visceral malformations and variations**						
		Fetal incidence (%)	0	0	0	1 (0.62)	1 (0.69)	
		Litter incidence (%)	0	0	0	1 (4.0)	1 (4.76)	
		Affected fetuses/litter (%)	0	0	0	0.57 ± 2.86	0.60 ± 2.73	
		Dilated renal pelvis						
		Fetal incidence (%)	0	0	0	2 (1.24)	0	
		Litter incidence (%)	0	0	0	2 (8.0)	0	
		Affected fetuses/litter (%)	0	0	0	1.24 ± 4.30	0	
		HCD	Fetal incidence: 27 (1.44); litter incidence: 25 (7.29)					
		Lateral ventricular dilation in brain (slight to moderate severity)						
		Fetal incidence (%)	0	0	0	1 (0.62)	**17 (11.7)****	
		Litter incidence (%)	0	0	0	1 (4.0)	**6 (28.6)****	

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Ref.
		Affected fetuses/litter (%): 0 / 0 / 0 / 0.80 ± 4.0 / 13.07 ± 27.79 HCD: Fetal incidence: 36/1871 (1.92); litter incidence 26/343 (7.58) **NOAEL (maternal toxicity): 30 mg/kg bw/day** **NOAEL (developmental toxicity): 300 mg/kg/bw day**	
Developmental toxicity study in rabbits OECD 414 (2001) GLP New Zealand White 25 females/dose	Cinmethylin Purity: 96.3 % Enantiomeric ratio: not specified Doses: 0, 25, 80 and 250 mg/kg bw/day in 0.5-1 % sodium carboxymethylcellulose suspension in drinking water with 3 drops of Tween80/1000 mL An additional dose group and control group was set up to clarify relevance of skeletal findings at 250 mg/kg bw/day: 0 and 320 mg/kg bw/day (10 mL/kg bw) Administered by gavage on GD 6-28	**<u>Maternal toxicity:</u>** **<u>320 mg/kg bw/day:</u>** ↓ Body weight gain: 22 % of controls (GD 0-29) *Clinical chemistry* ↑ GGT: 86.5 %** *Organ weights* ↑ Liver: abs. 23 %** and rel. 26 %** **<u>250 mg/kg bw/day:</u>** ↓ Body weight gain: 22 % of controls (GD 0-29) 69 %* of controls (GD 6-9) 200 %* of controls (animals lost an average of 68.8 g versus an average loss of 22.9 g in controls) (GD 16-19) *Clinical chemistry* ↑ GGT: 50.7 %* *Organ weights* ↑ Liver: abs. 18 %** and rel. 21 %** **<u>80 mg/kg bw/day:</u>** *Organ weights*	Anon, 2018 k DAR: B.6.6.2

<table>
<tr><th>Method, guideline, deviations if any, species, strain, sex, no/group</th><th>Test substance, dose levels duration of exposure</th><th>Results</th><th>Ref.</th></tr>
<tr><td></td><td></td><td>↑ Liver: abs. 13 %** and rel. 12 %**

<u>25 mg/kg bw/day:</u>
No treatment-related adverse effects.

<u>Developmental toxicity:</u>
<u>320 mg/kg bw/day:</u>
↓ Mean fetal weight: 11.2 %** of controls

<u>250 mg/kg bw/day:</u>
↓ Mean fetal weight: 14.4 %** of controls

No visceral malformations or variations were observed.
Table 35b: Fetal and litter incidences of relevant skeletal malformations observed in pups following treatment with cinmethylin during GD 6-28.
<table>
<tr><th>Dose (mg/kg bw/day)</th><th>0</th><th>25</th><th>80</th><th>250</th><th>0</th><th>320</th></tr>
<tr><td>Litters evaluated</td><td>21</td><td>24</td><td>24</td><td>19</td><td>23</td><td>25</td></tr>
<tr><td>Fetuses evaluated</td><td>181</td><td>206</td><td>194</td><td>182</td><td>205</td><td>235</td></tr>
<tr><td>Live</td><td>181</td><td>206</td><td>193</td><td>182</td><td>204</td><td>235</td></tr>
<tr><td>Dead</td><td>0</td><td>0</td><td>1</td><td>0</td><td>1</td><td>0</td></tr>
<tr><td colspan="7">Total skeletal malformations</td></tr>
<tr><td>Fetal incidence # (%)</td><td>4 (2.2)</td><td>2 (1.0)</td><td>1 (0.5)</td><td>5 (2.7)</td><td>1 (0.5)</td><td>1 (0.4)</td></tr>
<tr><td>Litter incidence # (%)</td><td>3 (14)</td><td>2 (8.3)</td><td>1 (4.2)</td><td>3 (16)</td><td>1 (4.3)</td><td>1 (4.0)</td></tr>
<tr><td>Affected fetuses/litter %</td><td>1.8</td><td>0.7</td><td>0.5</td><td>2.9</td><td>0.4</td><td>0.4</td></tr>
<tr><td colspan="7">Selected individual skeletal malformations</td></tr>
<tr><td colspan="7">Misshapen thoracic vertebra</td></tr>
<tr><td>Fetal incidence # (%)</td><td>0</td><td>0</td><td>0</td><td>3 (1.6)</td><td>0</td><td>0</td></tr>
<tr><td>Litter incidence # (%)</td><td>0</td><td>0</td><td>0</td><td>3 (16)</td><td>0</td><td>0</td></tr>
</table></td><td></td></tr>
</table>

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Ref.

Affected fetuses/litter %	0	0	0	**1.7***	0	0
HCD[$]: Fetuses: 1 (0.05 %), range 0.0 – 0.8 %; litters: 1 (0.4 %), range 0.0 – 5.6 %; affected fetuses/litter: 0.0 – 0.6 %						
Absent lumbar vertebra						
Fetal incidence # (%)	0	0	0	2 (1.1)	0	0
Litter incidence # (%)	0	0	0	2 (11)	0	0
Affected fetuses/litter %	0	0	0	1.1	0	0
HCD[$]: Fetuses: 1 (0.05 %), range 0.0 – 0.5 %; litters: 1 (0.4 %), range 0.0 – 4.3 %; affected fetuses/litter: 0.0 – 0.4 %						
Intercostal rib; cartilage present						
Fetal incidence # (%)	0	0	0	2 (1.1)	0	0
Litter incidence # (%)	0	0	0	2 (11)	0	0
Affected fetuses/litter %	0	0	0	1.1	0	0
No HCD provided.						

[$]HCD = Historical control data – Taken from 12 studies carried out at the performing laboratory between Jan 2010 – Apr 2015 using New Zealand White rabbits

Statistics: litter incidence: Fisher's Exact Test (1-sided); affected fetuses/litter: Wilcoxon-Test (1-sided). *p < 0.05, **P < 0.01

Table 35c: Fetal and litter incidences of relevant skeletal variations observed in pups following treatment with cinmethylin during GD 6-28.

Dose (mg/kg bw/day)	**0**	**25**	**80**	**250**	**0**	**320**
Litters evaluated	21	24	24	19	23	25
Fetuses evaluated	181	206	194	182	205	235
Live	181	206	193	182	204	235
Dead	0	0	1	0	1	0
Total skeletal variations						

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Ref.

Fetal incidence # (%)	173 (96)	199 (97)	188 (97)	173 (95)	182 (89)	223 (95)	
Litter incidence # (%)	21 (100)	24 (100)	24 (100)	19 (100)	23 (100)	25 (100)	
Affected fetuses/litter %	95.8	96.6	97.0	95.1	89.4	94.7	
Selected individual skeletal variations							
Incomplete ossification of cervical centrum; unchanged cartilage							
Fetal incidence # (%)	28 (15)	40 (19)	31 (16)	44 (24)	7 (3.4)	25 (11)	
Litter incidence # (%)	12 (57)	16 (67)	14 (58)	15 (79)	5 (22)	**14* (56)**	
Affected fetuses/litter %	14.0	17.0	15.4	**23.1**	3.4	**10.8***	
HCD[$]: Fetuses: 371 (17.3 %), range 2.7 – 37.6 %; litters: 151 (57.2 %), range 13.6 – 77.8 %; affected fetuses/litter: 1.9 – 33.4 %							
Incomplete ossification of thoracic centrum; unchanged cartilage							
Fetal incidence # (%)	0	1 (0.5)	0	4 (2.2)	1 (0.5)	1 (0.4)	
Litter incidence # (%)	0	1 (4.2)	0	3 (16)	1 (4.3)	1 (4)	
Affected fetuses/litter %	0	0.4	0	**2.2***	0.3	**4.5***	
HCD[$]: Fetuses: 10 (0.5 %), range 0.0 – 1.6 %; litters: 7 (2.7 %), range 0.0 – 8.7 %; affected fetuses/litter: 0.0 – 2.2 %							
Unilateral ossification of sternebra; unchanged cartilage							
Fetal incidence # (%)	6 (3.3)	6 (2.9)	7 (3.6)	14 (7.7)	2 (2.0)	2 (1.7)	
Litter incidence # (%)	5 (24)	6 (25)	6 (25)	10 (53)	4 (17)	4 (16)	
Affected fetuses/litter %	2.8	2.6	3.0	**8.3***	1.8	1.8	
HCD[$]: Fetuses: 50 (2.3 %), range 0.0 – 4.5 %; litters: 38 (14.4 %), range 0.0 – 30.0 %; affected fetuses/litter: 0.0 – 5.8 %							

[$]HCD = Historical control data – Taken from 12 studies carried out at the performing laboratory between Jan 2010 – Apr 2015 using New Zealand White rabbits

<table>
<tr><th>Method, guideline, deviations if any, species, strain, sex, no/group</th><th>Test substance, dose levels duration of exposure</th><th>Results</th><th>Ref.</th></tr>
<tr><td></td><td></td><td>Statistics: litter incidence: Fisher’s Exact Test (1-sided); affected fetuses/litter: Wilcoxon-Test (1-sided). *p < 0.05, **P < 0.01

NOAEL (maternal toxicity): 80 mg/kg bw/day

NOAEL (developmental toxicity): 80 mg/kg/bw day</td><td></td></tr>
<tr><td>Teratogenicity study in rabbits

Non-OECD guideline (EPA guidelines followed)

Deviations to current OECD guideline are listed in text.

GLP

New Zealand White

19 inseminated females/control group

20 inseminated females/treatments groups</td><td>Cinmethylin

Purity: 93 %

Enantiomeric ratio: not specified

Doses: 0, 3, 30 and 100 mg/kg bw/day in corn oil

(2 mL/kg bw)

Administered by gavage on GD 6-18</td><td><u>Maternal toxicity:</u>

The study was compromised by the high rates of mortality observed in ALL dose groups:

Table 35d:
<table><tr><th>Dose group (mg/kg bw/day)</th><th>0</th><th>3</th><th>30</th><th>100</th></tr><tr><td>No. of deaths # (%)</td><td>3/19 (16)</td><td>2/20 (10)</td><td>2/20 (10)</td><td>4/20 (20)</td></tr></table>
<u>Developmental toxicity:</u>

There were no adverse treatment-related effects on pregnancy status or outcome. There were no treatment-related external, visceral or skeletal malformations or variations.

NOAEL: Due to the limitations of this study, no robust NOAELs has been set.</td><td>Anon, 1985c

DAR: B.6.6.2</td></tr>
<tr><td>Teratogenicity study in rabbits

Non-OECD (EPA guidelines followed)

Deviations to current OECD guideline are listed in text.

GLP</td><td>Cinmethylin

Purity: 92.4 %

Enantiomeric ratio: not specified

Doses: 0, 30, 200, 500 and 750 mg/kg bw/day in 0.5 % methylcellulose

(4mL/kg bw)</td><td><u>Maternal toxicity:</u>

The number of females with implantation sites at necropsy was insufficient for all groups. This compromised the reliability and validity of the following data. Table 35e:
<table><tr><th>Dose group (mg/kg bw/day)</th><th>0</th><th>30</th><th>200</th><th>500</th><th>750</th></tr><tr><td>No. females with implantation sites</td><td>13/20</td><td>15/20</td><td>15/20</td><td>15/20</td><td>7/20</td></tr></table>
<u>750 mg/kg bw/day:</u>

↓ Food consumption: 24 % during GD 7-20</td><td>Anon, 1987b

DAR: B.6.6.2</td></tr>
</table>

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Ref.
New Zealand White 20 inseminated females/dose	Administered by gavage on GD 7-19	↓ Body weight gain: body weight loss during GD 7-20 (-136 g* versus +101 g in controls) **500 mg/kg bw/day:** ↓ Food consumption: 10 % during GD 7-20 ↓ Body weight gain: 73.7 % of controls during GD 0-29 (body weight loss during GD 7-20) **≤ 200 mg/kg bw/day:** No adverse treatment-related effects. **Developmental toxicity:** There were no treatment-related effects on pregnancy at any dose group. There were no treatment-related external, visceral or skeletal malformations. **NOAEL:** Due to the limitations of this study, no robust NOAELs has been set.	

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

Study in rats

One developmental toxicity study is available in rats (Anon, 1984b). This study was conducted according to GLP and although it pre-dated test guidelines, it is broadly similar to OECD 414. There were a small number of deviations to current guidelines but these were not considered to compromise the validity of the study. Deviations to the current OECD test method (2018) were:

- Cinmethylin was administered only during organogenesis (GD 6-15). However, this dosing regime was considered acceptable according to the old test guideline (1981).
- Food consumption was not recorded
- Gravid uteri, including the cervix, were not weighed
- The top dose of 2000 mg/kg bw/day was much higher than the limit dose of 1000 mg/kg bw/day.

Cinmethylin was administered by oral gavage to pregnant Sprague Dawley rats (25/dose) on GD 6-15 at doses of 0, 30, 300, 1000 and 2000 mg/kg bw/day. Animals were checked for general health and signs of mortality several times each day during administration, then once daily until scheduled sacrifice. Body weights were recorded prior to mating, on GD 0, during administration and at study termination. Food

consumption was not recorded. On GD 20, all females were sacrificed and assessed by gross pathology (spleen and liver weights were determined). For each dam, corpora lutea were counted and the number and distribution of implantation sites, early and late resorptions, and live and dead fetuses were determined. The fetuses were removed, sexed, weighed and further investigated for external findings. Half of the fetuses of each litter was examined for soft tissue findings and the remaining fetuses for skeletal findings.

Maternal toxicity

There was an increase in treatment-related mortality at the top dose (2/25 dams died on GD 15 – 8 % mortality), both dams were still pregnant. A range of clinical signs were observed with increased incidence with increasing dose. At the top dose, these included excess salivation, urine-stained abdominal fur, chromorrhinorrhea, vocalisation, hypersensitivity, thin appearance and tip-toe walk.

Body weight gain was affected from a dose of 300 mg/kg bw/day. At 2000 mg/kg bw/day an overall statistically significant reduction in body weight gain was noted (25.9 % GD 0-20) with dams losing weight between GD 6-12. Overall body weight was about 10 % lower than controls at this dose only. Similar, but less severe findings were observed at 300 and 1000 mg/kg bw/day with overall body weight gain reduced by 13 and 16 % respectively.

Absolute and relative liver weights were statistically significantly increased at 1000 and 2000 mg/kg bw/day (abs. 12 % and rel. 16 % at 1000 mg/kg bw/day and abs. 24 % and rel. 37 % at 2000 mg/kg bw/day). The two rats that died on GD 15 were found to have stomach lesions, enlargement of the liver and a decrease in spleen size. At necropsy, no treatment-related gross necropsy findings were observed in dams.

Developmental toxicity

At the top dose of 2000 mg/kg bw/day there was a small increase in post-implantation loss (17.1 % versus 4.7 % in controls), an increase in resorptions (2.2 versus 0.7 in controls) and a decrease in the number of live fetuses (295 versus 339 in controls). These findings were mainly due to two dams who suffered total resorptions – one of which suffered total resorptions from 20 implantation sites. Mean fetal weight at this dose was statistically significantly reduced by 8.6 % (3.2 g versus 3.51 g in controls). No such findings were observed at doses of 1000 mg/kg bw/day and below.

One fetus in each of the highest dose groups showed multiple external malformations and variations, however incidences were isolated and lacked a dose response. Three fetuses of the top dose were found to have visceral malformations, compared with 1 fetus in the 1000 mg/kg bw/day group and 2 in the control group. The lack of dose-response and isolated occurrence of each malformation indicated this finding was not related to treatment.

There were no treatment-related skeletal malformations observed. Total incidences of skeletal variations were increased across all treated groups, particularly at the top two doses. No statistical significance was reached and no clear-dose response was evident for the fetal and litter incidences. However, when considering the affected fetuses/litter, there was evidence of a slight dose response with 9.4 % of affected fetuses/ litter in the 1000 mg/kg bw/day dose group and 9.5 % of affected fetuses/litter in the 2000 mg/kg bw/day dose group (versus 6.2 % in controls). At the top dose, the fetal and litter incidences of wavy ribs were increased compared to controls (fetal incidence: 2.65 % versus 0.57 % in controls; litter incidence: 14.3 % versus 4.0 % in controls). The HCD for this finding was 0.06 % (fetal incidence) and 0.55 % (litter incidence), which was far lower than the control values, indicating that there was a higher spontaneous incidence of this finding overall in this study. As the increases in incidence of wavy ribs at the top dose were above the concurrent control, it is possible they were related to treatment.

Soft tissue variations and malformations were recorded at the top two doses, in four foetuses at 1000 mg/kg bw/d, rising to 18 foetuses at 2000 mg/kg bw/day. This was predominantly caused by a

statistically-significant increase in the foetal (11.7 % vs 0 % in controls) and litter incidence (28.6 % vs 0 % in controls) of lateral ventricle dilation in the brain at the top dose (severity: slight to moderate) (a dose twice that of the limit dose specified in the current guideline). The slight-moderate dilation of brain ventricles was described as being caused by the accumulation of small volumes of cerebrospinal fluid with negligible increase in pressure inside the skull. Historical control data (HCD) taken from the performing laboratory between the years 1980 – 1982, based on 343 litters and 1871 fetuses showed that this finding was not a common spontaneous occurrence (fetal incidence: 36/1871 (1.92 %) and litter incidence: 26/243 (7.58 %)). Skeletal and visceral grey zone anomalies can be upgraded (to malformation) or downgraded (to variation) depending on their severities (Solecki R. *et al.* 2013). At the more severe end of the scale, dilation of the ventricles would be hydrocephalus, characterised by a bulbous frontal region of the cranium, concave profile of the face, domed shape head, flattened cerebral tissues and further affected structures in the brain. Clinical symptoms related to hydrocephalus are described as enlargement of the head during the post-natal phase that progress in combination with uncertain movements and locomotion until death. On the basis that structural abnormalities such as these were not observed in this study and that the finding was described as slight-moderate, the ventricular dilation is considered to be a consequence of delayed fetal development. Therefore it is considered a variation rather than a malformation. Whilst slight to moderate dilation involves accumulation of small volumes of cerebrospinal fluid (CSF) with negligible increase in pressure inside the skull and no permanent structural changes to the brain, marked dilation (hydrocephalus) involves accumulation of large volumes of CSF within the brain, leading to marked increased pressure inside the skull and permanent neurological impairment. This is supported by the fact that litters with this finding were found to have a lower mean fetal body weight when compared to controls. Indeed, where the severity of the finding was increased to moderate, the reduction in mean fetal body weight was greater. The finding is also presented with clear indications of maternal toxicity (mortality, reduced body weight gain and body weight gain and clinical signs of toxicity). Body weights of dams that produced these fetuses were reduced by 10 % and on certain days of gestation were found to have lost weight compared to control animals. Where the severity of the finding was increased from slight to moderate, dams were found to have greater reductions in body weight. To further support the reduced concern for these findings, a study by Fox *et al.* (2018) concluded that, in humans, the isolated finding of mild enlargement of the lateral ventricles is found to correlate with a normal postnatal evaluation in > 90 % of cases and moderate enlargement is found to correlate with a normal postnatal evaluation in 75 – 93 %. The authors also concluded that mild ventriculomegaly is likely to represent a normal variant if no other structural abnormalities are noted. In a recent meta-analysis, the rate of neurodevelopmental delay in truly isolated mild ventriculomegaly was 7.9 %, which is similar to the background rate. Therefore, the effect of slight to moderate dilation of lateral brain ventricles can be considered a consequence of a delay in development, which is reversible rather than detrimental to the foetuses.

Overall, there were indications of treatment-related increases in variations at the top dose only. These included an increased incidence of wavy ribs and slight to moderate dilation of lateral ventricles in the brain – a variation likely to represent a developmental delay with no detrimental or irreversible consequences for the fetus. Also observed was a statistically significant decrease in mean fetal body weight and an increase in post-implantation loss (due to two whole resorptions), also at the top dose. These effects were observed at a dose twice that of the recommended limit dose, in the presence of severe maternal toxicity (mortality, body weight loss and clinical signs of toxicity) and are therefore not thought to warrant classification. There was no evidence of specific developmental toxicity in the rat.

Studies in rabbits

Three developmental toxicity studies are available in rabbits, one well-performed modern study and two older studies, performed according to GLP but not OECD guidelines. The two older studies were found to have a number of limitations rendering the results unreliable. They are included for completeness only.

The first study was performed between 2014-2017 by (Anon, 2018k). Cinmethylin was administered to New Zealand White rabbits (25 pregnant females/dose), once daily, by oral gavage on gestation days (GD) 6-28. Initial doses were 0, 25, 80 and 250 mg/kg bw/day. At the top dose, slight maternal toxicity and marginal increases in the incidence of skeletal findings were noted. On that basis, a further higher dose of 320 mg/kg bw/day was tested and compared with an additional control group.

Maternal toxicity

There were no treatment-related deaths or clinical signs of toxicity. From a dose of 250 mg/kg bw and above, a decrease in body weight gain was observed (between GD 0-29 body weight gain was approximately 22 % less than controls in both the 250 mg/kg bw/day and the 320 mg/kg bw/day dose groups). For animals in the 250 mg/kg bw/day dose group, the decrease in body weight gain was particularly (and statistically significantly) low between GD 6-9 (68.8 % lower than controls) and GD 16-19 (average body weight loss of 69 g versus a smaller loss of 23 g in controls animals).

Statistically significantly increased serum-γ-glutamyltransferase (GGT) was observed at both 250 mg/kg bw/day and 320 mg/kg bw/day (50.7 and 86.5 % higher than controls respectively). This finding was indicative of liver toxicity. Indeed, statistically significantly higher liver weights were observed from doses of 80 mg/kg bw/day (abs. liver weight 13 % and rel. liver weight 12 % higher than controls at 80 mg/kg bw/day, rising to abs. liver weight 23 % and rel. liver weight 26 % higher than controls at 320 mg/kg bw/day).

Developmental toxicity

Statistically significant reductions in mean fetal weight were observed at doses of 250 mg/kg bw/day and above. At 250 mg/kg bw/day, mean fetal weight was 14.4 % lower than controls (a similar decrease was observed at 320 mg/kg bw/day).

There were no treatment-related effects on external or visceral malformations and variations. Skeletal malformations were observed in fetuses of all treatment groups, including controls. At a dose of 250 mg/kg bw/day, three fetuses (1.6 %) were found to have misshapen thoracic vertebra (litter incidence 3 (16 %) versus 0 in controls). This finding was not observed in controls animals and was above the HCD range provided by the performing laboratory (data taken from a 5 year period of the current study). Statistical significance was only reached when considering the percentage of affected fetuses/litter (1.7 %). However, in the additional group of rabbits dosed with 320 mg/kg bw/day no such finding was observed, thus, in the absence of a dose-response, it is unlikely that the finding was related to treatment. Two other malformations; absent lumbar vertebra and intercostal rib (with cartilage present) were observed with increased incidence compared to controls (2 fetuses from 2 litters affected versus 0 in controls) at 250 mg/kg bw/day. No HCD was provided for the finding of intercostal rib (with cartilage present) but the finding of absent lumbar vertebra was above the HCD for both fetal and litter incidence. No animals in the 320 mg/kg bw/day group exhibited these findings, again, diminishing the likelihood that they were anything but a chance finding due to the lack of dose-response.

Statistically significant increases in certain skeletal variations were observed. These included: incomplete ossification of cervical centrum; unchanged cartilage, incomplete ossification of thoracic centrum; unchanged cartilage and unilateral ossification of sternebra; unchanged cartilage. In all cases, a clear and consistent dose response was not evident and/or values were within the HCD range provided.

Overall, no treatment-related malformations or variations were observed in this study, up to a dose of 320 mg/kg bw/day. However, fetal weight was reduced from a dose of 250 mg/kg bw/day. This was observed in the presence of modest maternal toxicity (reduced body weight gain and body weight loss), and on its own, is not considered sufficient for classification.

An old teratology study conducted in New Zealand White rabbits is available (Anon, 1985c). Whilst this study was conducted according to GLP, it did not follow OECD guidelines and had a number of serious deviations that limited the reliability of the study. The information has been presented for completeness and to add to the weight of evidence regarding the finding of dilation of lateral ventricles in the brain of rats, following administration of high doses of cinmethylin. The following deviations from the current OECD 414 (2018) guideline were found:

- Cinmethylin was administered during the period of organogenesis only (GD 6-18). The preferred dosing period is GD 6-29.
- Maternal mortality exceeded 10 %, even in control animals (16 %).
- Premature or natural delivery was defined in this study as expulsion of conceptus on GD28 or 29, while abortion was defined as expulsion of conceptus on GD27 or earlier. This differentiation is outdated and differs from the OECD test guideline, which defines abortion as the premature expulsion from the uterus of the products of conception, irrespective of date.
- There were high incidences of spontaneous malformations and variations, mainly in control animals. These may have masked the effects of treatment-related findings.

Prior to the current study, a dose ranging study with cinmethylin was carried out using test groups of 4 females/dose. Dose levels of 0, 30, 100, 300, 1000 and 2000 mg/kg bw/day in corn oil were administered. Pregnancy rates were low and mortalities occurred in some animals, however no dose-response was evident. All decedents, except one top dose female, had stomach ulceration and/or white spots on the stomach mucosa, however a dose response was lacking for this finding. Administration of cinmethylin generally led to reduced maternal weight gain and feed consumption. A slight increase in the incidence of resorptions was reported at the top two dose groups. Based on these data, dose levels of 3, 30 and 100 mg/kg bw/day were selected for the main study. No further details were provided in the DAR.

For the main study, cinmethylin was administered to inseminated females (presumed pregnant) by oral gavage at doses of 0, 3, 30 and 100 mg/kg bw in corn oil (2 mL/kg bw). Caesarean section was performed on gestation day (GD) 29, followed by gross necropsy, including determinations of maternal liver and uterus weight, corpora lutea, number and location of implantation, resorptions and the number of live and dead fetuses. Live fetuses were sexed, weighed and external, visceral and skeletal abnormalities recorded.

Maternal toxicity

There were no treatment-related effects on mortality, clinical signs, food consumption, body weight, body weight gain, gravid uterus weight, carcass weight or liver weight observed in this study. However, high mortality rates were seen across all dose groups (16 %, 10 %, 10 % and 20 % in the control, 3, 30 and 100 mg/kg bw/day dose groups respectively). Similar findings were observed in the dose range-finding study but the lack of dose-response indicated the effect was not substance-related; however, it was likely to be *treatment*-related. The study authors attributed the increased mortality to the corn oil vehicle and also to the method of administration (gavage). It is noted that a similar finding was not observed in the pre-natal developmental study carried out in rats, which also used corn oil as the vehicle and gavage as the means of administration. Stomach ulceration or haemorrhagic areas of mucosa were observed in decedents of all doses which is likely to be treatment-related.

Developmental toxicity

At caesarean section, the percentage of pre- and post-implantation loss, mean number of early and late resorptions, mean number of fetuses per litter, sex ratio and mean fetal body weight were comparable between treated and control groups. Although there were high incidences of spontaneous malformations and variations, mainly in controls, there were no treatment-related external, visceral or skeletal malformations or variations observed. There were no incidences of dilated lateral ventricle in the brain.

Overall, findings in this study were limited to increased incidence of stomach ulceration in dams of the top dose group. No findings of developmental toxicity were observed up to a dose of 100 mg/kg bw/day. However, the conditions of this study mean that no robust conclusions can be drawn.

A second old teratogenicity study in New Zealand White rabbits is available (Anon., 1987b). Similarly to the previous study, this study was conducted according to GLP but was not carried out according to OECD guidelines. The following lists the deviations from the current guideline (OECD 414 2018):

- Cinmethylin was administered during the period of organogenesis (GD 7-19). The preferred dosing period is GD 6-29.
- Room temperature was set to maintain a range of 19-23 °C. Housing at elevated temperatures can induce stress to rabbits leading to pregnancy impairment, particularly increased occurrences of abortions/early deliveries and decreased ovulation and nidation rates.
- The study consisted of 7-15 females/dose group with verified implantation sites and 6-13 dams with live litters at study termination. The current guideline states there should be 20 females with implantation sites at necropsy.
- No historical control data was provided.

These limitations are considered significant, rendering the results of the study unreliable. However, the study has been included for completeness and to provide further reassurance relating to the finding of dilated lateral ventricle of the brain (observed in rats only).

A dose range-finding study was conducted in order to determine the dose levels to be used for the main study. In the range-finding study, doses of 0, 100, 500, 1000 and 2000 mg/kg bw/day of cinmethylin in methyl cellulose (0.5 %) were administered to groups of 8 artificially inseminated female New Zealand White rabbits by oral gavage (GD 7-19). High mortality rates were observed in the top two dose groups as well as significant body weight loss and reduced food consumption. All top dose females and 4/8 females from the 1000 mg/kg bw/day dose group were found to have stomach ulceration (as seen in the study by Anon, 1985c). Surviving females of the 1000 mg/kg bw/day group showed increased resorptions. At the next highest dose of 500 mg/kg bw/day, reductions in food consumption and body weight gain was observed. There were reductions in fetal weight but no alterations in fetuses were observed during external examination. Based on these findings, the maximum dose for the main study was determined to fall between 500-1000 mg/kg bw/day.

In the main study, groups of inseminated (presumed pregnant) female rabbits (20/dose) were administered cinmethylin in methyl cellulose (0.5 %) at doses of 0, 30, 200, 500 and 750 mg/kg bw/day via oral gavage (GD 7-19). Caesarean section was performed on GD 29, followed by gross necropsy, including determinations of maternal liver and uterus weight, number of corpora lutea, number and location of

implantations, resorptions and number of live and dead fetuses. Live fetuses were sexed, weighed and assessed for external, visceral and skeletal anomalies.

Maternal toxicity

There was no treatment-related mortality in this study, although two females of the low dose group and one from the top dose died during the study. There were no treatment-related clinical signs observed. Food consumption was reduced dose-dependently from a dose of 500 mg/kg bw/day. A reduction of 10 % and 24 % was recorded in the 500 mg/kg bw/day and 750 mg/kg bw/day dose groups respectively. In those animals that did not fall pregnant, comparable reductions in food consumption was observed.

Small reductions in body weight (< 10 %) were observed in treated animals, compared to controls. Statistically significant reductions in body weight gain were observed at doses of 500 mg/kg bw/day and above. At the top dose of 750 mg/kg bw/day, body weight loss was observed during GD 7-20 (an average of 136 g was lost compared to a gain of 101 g in controls), resulting in an overall bodyweight gain 31 % lower than controls. At the next highest dose of 500 mg/kg bw/day, animals also lost weight, during GD 16-20 (75.7 g versus a gain of 18.4 g in controls) and had an overall bodyweight gain 74 % lower than controls.

Developmental toxicity

The number of females with implantation sites at necropsy was insufficient in all treatment groups (13/20, 15/20, 15/20, 15/20 and 7/20 for control, 30, 20, 500 and 750 mg/kg bw/day dose groups respectively). This rendered the conception rates low; however, the finding was due to the insemination procedure rather than an effect of treatment with cinmethylin. This finding limits the reliability and validity of the study.

There were no treatment-related effects on abortions and/or premature deliveries. No treatment-related adverse effects were observed for the mean number of corpora lutea or implantation sites. There was a slight increase in early resorptions at the top dose only, mainly due one litter with only four implantation sites, of which two were resorbed. There was no treatment-related adverse effects on fetal sex ratio and no treatment-related external, visceral or skeletal malformations or variations.

Under the limited conditions of this study, no developmental toxicity was observed up to a dose of 750 mg/kg bw/day of cinmethylin.

10.12.6 Comparison with the GB CLP criteria

Cinmethylin has been tested in a non-guideline (but broadly similar to guideline) developmental study in Sprague Dawley rats, in a guideline developmental study in New Zealand White rabbits and in two unreliable non-guideline studies, also in New Zealand White rabbits.

In rats, a specific effect on the development of the unborn fetus was not observed. However, marked increased incidences of variations, indicative of delayed development and a significant decrease in mean fetal body weight were observed at a dose of 2000 mg/kg bw/day and above. At this dose, which is twice that of the limit dose, marked maternal toxicity was observed (body weight loss, mortality and increased clinical signs). At doses at which no maternal toxicity was noted, there were no adverse developmental effects. Therefore, the effects on fetuses are considered non-specific secondary consequences of the general toxicity observed in the dams.

In the guideline study in rabbits, a reduction in mean fetal weight was observed from a dose of 250 mg/kg bw/day. This was observed in the presence of modest maternal toxicity (body weight loss and liver toxicity). It is thought that the maternal toxicity observed caused a depression in fetal weight via a non-specific

secondary mechanism. Such findings were not observed at lower doses in the absence of maternal toxicity. Although the two other studies in rabbits were deemed unreliable, due to the various limitations associated with the study protocol, reduced fetal weight was not observed up to a dose of 750 mg/kg bw/day.

Cinmethylin is not a known human developmental toxicant; therefore classification in Category 1A is not necessary.

From the animal data available, there was no clear evidence to suggest that cinmethylin should be presumed to be a human developmental toxicant; therefore classification in Category 1B is not appropriate.

Classification in Category 2 is reserved for substances where there is some evidence from human or experimental animals of an adverse effect on development. Such effects should be observed in the absence of other toxic effects. On the basis that there is no evidence that cinmethylin causes any adverse effects to development in the absence of maternal toxicity, it should not be classified in this category. Therefore no classification for this endpoint is required.

10.12.7 Adverse effects on or via lactation

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

There were no adverse effects on or via lactation observed in the two-generation studies in rats following administration of cinmethylin.

10.12.9 Comparison with the GB CLP criteria

There is no evidence from humans or animals to suggest that cinmethylin has an adverse effect on lactation or via lactation. The results from absorption, distribution, metabolism and excretion studies do not indicate a likelihood of cinmethylin accumulating to potentially toxic levels in breastmilk. Therefore classification for this endpoint is not appropriate.

10.12.10 Conclusion on classification and labelling for reproductive toxicity

No classification – conclusive but not sufficient for classification

10.13 Aspiration hazard

10.13.1 Comparison with the GB CLP criteria

There are no data available for this endpoint.

10.13.2 Conclusion on classification and labelling for aspiration hazard

Not classified – data lacking

11 EVALUATION OF ENVIRONMENTAL HAZARDS

Cinmethylin (referred to in test reports as BAS 684 H) is a herbicide intended for use against annual weeds including black grass and Italian rye grass in cereals and oilseed rape. Available environmental fate and hazard studies have been considered under the GB CLP Regulation; these are summarised in the Draft Assessment Report (DAR), GB 2020.

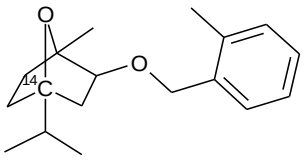
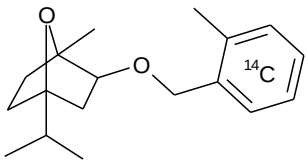
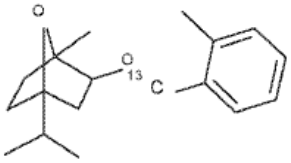
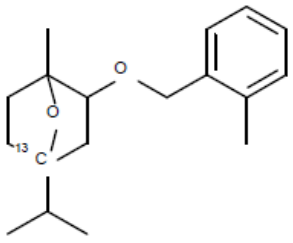
The key information pertinent to determining a classification is presented in the following sections.

The water solubility of cinmethylin in deionised water has been experimentally determined to be 0.069 g/L at 20°C and pH 8.9 (OECD 105; HPLC-UV; GLP). Based on the available data, the solubility of cinmethylin in water is not pH dependant.

A dissociation constant is not available as no dissociation of cinmethylin was observed in water in the pH range 3.2 to 10.9 (OECD 112; GLP). Therefore, no pKa value could be determined.

Radiolabelled environmental fate studies used a combination of several ^{14}C-radiolabelled and ^{13}C-labelled test items. Table 36 presents a summary of these test items. All radiolabelled studies utilised ^{14}C-radiolabelled test items; the ^{13}C-labelled test items were typically used in photolysis studies in combination with the ^{14}C-radiolabelled test items.

Table 36: ^{14}C-radiolabelled and ^{13}C-labelled test items used to study the environmental fate of cinmethylin

Substance name (plus synonyms)	Reg No.	Chemical purity	Structure
[cyclohexane-4-^{14}C]- cinmethylin	900202	99.3%	
[phenyl-U-^{14}C]- cinmethylin (also referred to as benzyl-U-^{14}C])	900202	90.4 – 97.0% (depending upon batch used)	
[benzyl-^{13}C]-cinmethylin	900202	99.6%	
[cyclohexane-4-^{13}C]- cinmethylin	900202	98.1%	

A summary of reliable valid information on the aquatic fate of cinmethylin is presented below. Available soil data have not been presented as suitable aquatic data are available.

11.1 Rapid degradability of organic substances

Table 20: Summary of relevant information on rapid degradability

Method	Study conduct and results	Remarks	Reference
Aqueous Hydrolysis			
Aquatic hydrolysis OECD 111 JMAFF No 12 Nosan No 8147 EPA 835.2120 GLP	25°C. 31 day study at pH 4, 5, 7 and 9. Cinmethylin reported to be stable to hydrolysis at all pH tested based on levels not declining by more than 5% over the study duration. DT_{50} could not be derived.		Hassink, J. (2017a)
Photolysis (Aqueous, Direct)			
Phototransformation in water OECD 316 JMAFF No 12 Nosan No 8147 GLP	25°C, continuous illumination for 15 days with xenon lamp, equivalent to 17.4 days natural sunlight at 40°N. DT_{50} = 41.8 d (artificial light); 48.5 d (natural light) Cinmethylin did not decline by more than 5% in dark controls. Quantum yield could not be calculated as the UV spectrum of cinmethylin showed no absorption above 290 nm.	Photolysis-only degradation rate could not be derived due to a lack of reliable endpoints for dark control. However, when combined with the hydrolysis study results, the degradation of cinmethylin observed here has been attributed to photolytic processes.	Hassink, J. (2017d)
Photolysis (Aqueous, Indirect)			
Aquatic photolysis JMAFF No 12 Nosan No 8147 GLP	25°C, continuous illumination for 15 days with xenon lamp, equivalent to 17.4 days natural sunlight at 40°N. DT_{50} = 30.0 d (artificial light); 34.8 d (natural light) Cinmethylin did not decline by more than 5% in dark controls.	Photolysis-only degradation rate could not be derived based on no reliable endpoints for dark control. However, when combined with the hydrolysis study results, the degradation of cinmethylin observed here has been attributed to photolytic processes.	Hassink, J. (2017f)
Ready biodegradability			

Method	Study conduct and results	Remarks	Reference
Ready biodegradability: CO_2 evolution test OECD No. 301B GLP	Biodegradation after 28 days (based on formed CO_2): Cinmethylin: < 1% Reference (aniline): 98% Inhibition control: 44%	Not readily biodegradable.	Schwarz, H. (2017)
Biodegradation (in simulated water or water/sediment systems)			
Aerobic mineralisation study OECD 309 GLP	Fresh water system, dark, duration 63 days at 20°C Two doses: high (50 µg/L) and low (10 µg/L) Cinmethylin DT_{50}s (SFO): 138 d (low conc) 334 d (high conc) Correction of degradation rates to more environmentally realistic temperatures, e.g. 12°C, has not been undertaken since this would only increase the existing DT_{50}s and so would not change the overall 'rapid degradability' determination.	Reference substance (benzoic acid) degraded to 6.2% at 64 days, with 80.3 – 85.9% evolved to CO_2.	Mueller-Werthwein, M., Hegler, F. (2018a)
Water sediment study OECD 308 EPA 835.4300 GLP	Two natural water/sediment systems, 20°C, dark, 100 days duration. Up to 51.1 – 55.9% of applied radioactivity as cinmethylin partitioned to sediment, with the peak occurring after 14 or 56 days after treatment. At the study end, 0.7-2.1% of applied radioactivity remained as cinmethylin in the water phase. Cinmethylin in the sediment accounted for 16.2 – 30.3% of total applied radioactivity at the study end. Decline of cinmethylin in the whole system (SFO kinetics; modelling endpoints):		Mueller-Werthwein, M., Hegler, F. (2017a)

Method	Study conduct and results	Remarks	Reference
	DT_{50}: 39.2 d DT_{90}: 130.1 d		

11.1.1 Ready biodegradability

One study investigating the ready biodegradability of cinmethylin is available (Schwarz, H., 2017). This was conducted to GLP standards in adherence to the OECD 301B guidelines for the CO2 evolution test and was considered to be reliable. Cinmethylin showed little biodegradation after 28 days, with < 1% of the applied active substance biodegrading to CO2. In contrast, the reference item, aniline, exhibited 98% biodegradation in the same time period, indicating that the systems were microbially viable. The study concluded that cinmethylin is not readily biodegradable.

11.1.2 BOD_5/COD

No BOD_5/COD studies were submitted for cinmethylin.

11.1.3 Hydrolysis

One study investigating the aqueous hydrolysis of cinmethylin is available (Hassink, J., 2017a). The study was well-conducted and to GLP standards and is therefore considered reliable. The study investigated hydrolytic degradation at four pH levels: 4, 5, 7 and 9 over 31 days at 25°C. Cinmethylin was reported to be hydrolytically stable at all pH, with cinmethylin levels remaining at > 95% over the study duration in all cases.

11.1.4 Other convincing scientific evidence

No information available.

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

No information on field investigations. There are no monitoring data for cinmethylin in Europe as this a new active substance to the European market.

11.1.4.2 Inherent and enhanced ready biodegradability tests

No information available.

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

Two studies are available to consider the behaviour of cinmethylin in surface water: one aerobic mineralisation study and one water/sediment study. Both studies were conducted to appropriate guidelines (OECD 309 and OECD 308 respectively) and GLP and are considered reliable.

Aerobic mineralisation in surface water (Mueller-Werthwein, M., Hegler, F. (2018a))

The aerobic mineralisation of cinmethylin was studied in one fresh water system at 20ºC in the dark for 63 days. Two cinmethylin concentrations were investigated: high (50 µg/L) and low (10 µg/L). Benzoic acid was used as a reference standard and 80.3 – 85.9% was mineralised after 64 days, demonstrating microbial viability of the test system. Cinmethylin demonstrated low mineralisation, with up to 4.8% of 14CO2

collected from traps after 63 days at both the low and high concentrations. The kinetics for both low and high concentrations were best described by SFO model fits, with a DT50 of 138 d in the low concentration study and 334 d in the high concentration study.

One major metabolite was identified in the study, M684H001, with a peak formation of 13.1% in the total system after 63 days. The metabolite did not decline by the study end so a default DT¬50 of 1000 days has been applied.

Water/sediment study (Mueller-Werthwein, M., Hegler, F. (2017a))

The behaviour of cinmethylin was studied in two fresh water/sediment systems incubated at 20ºC in the dark for 100 days. Up to 51.1 – 55.9% of the applied cinmethylin partitioned to sediment, with the peak sediment levels occurring either 14 or 56 days after treatment, depending upon the test system. This declined to 16.2 – 30.3% TAR at the study end. Both systems demonstrated significant partitioning of cinmethylin to the sediment: after 100 days, 0.7-2.1% TAR remained as cinmethylin in the water phase, while 16.2 – 30.3% TAR remained as cinmethylin in the sediment phase. The whole system DT50 for cinmethylin was 39.2 d (geomean; n = 2).

One major water metabolite was identified in the study, M684H001, with water levels peaking at 6.5 – 11.4% TAR after 28 d, and sediment levels peaking at 1.8 – 3.8% TAR after 28-56 d. A DT50 value was not derived.

11.1.4.4 Photochemical degradation

Two guideline studies are available investigating the photochemical degradation of cinmethylin in surface water, one investigating direct photolysis (OECD 316) and one investigating indirect photolysis (Japanese JMAFF No 12 Nosan 8147 guidelines). Both studies were also conducted in compliance with GLP and are considered reliable.

Direct photolysis study (Hassink, J., 2017d)

The direct photolysis of cinmethylin was studied in an aqueous buffer solution (pH 7) incubated at 25°C with 15 days of continuous artificial irradiation (xenon lamp), providing an equivalent of 17.4 days of natural sunlight at 40°N. After 15 days cinmethylin dropped to 76 – 78% of applied radioactivity in the photolysis samples and did not drop by more than 5% in dark controls. The DT50 was calculated to be 41.8 days based on artificial light, or 48.5 days based on natural light at 40°N. It was not possible to derive reliable endpoints for the dark control samples; therefore, photolysis-only degradation rates could not be derived.

One major photometabolite formed in the study, M684H003, with formation peaking at 6.8% after 11 d. The metabolite did not decline by the study end so a default DT¬50 of 1000 days has been applied.

The UV spectrum of cinmethylin shows no absorption above 290 nm and therefore no overlap with the spectrum of sunlight. Therefore, the quantum yield of cinmethylin is zero. The quantum yield could not be derived for M684H003.

Indirect photolysis study (Hassink, J., 2017f)

The indirect photolysis of cinmethylin was studied in sterile natural water incubated at 25°C with 15 days of continuous artificial irradiation (xenon lamp), providing an equivalent of 17.4 days of natural sunlight at 40°N. After 15 days cinmethylin dropped to 66.3 – 69.7% of applied radioactivity in the photolysis samples and did not drop by more than 5% in dark controls. The DT50 was calculated to be 30.0 days based on

artificial light, or 34.8 days based on natural light at 40°N. It was not possible to derive reliable endpoints for the dark control samples; therefore, photolysis-only degradation rates could not be derived.

One major photometabolite formed in the study, M684H003, with formation peaking at 11.0% after 15 d. The metabolite did not decline by the study end so a default DT¬50 of 1000 days has been applied.

When considering the results from both photolysis studies in combination with the hydrolysis study results, it was concluded that the degradation observed in the photolysis samples for both studies can be attributed to indirect photolysis processes.

Overall, cinmethylin does not meet the CLP criteria to be classed as “rapidly degradable” in the environment as there is no evidence to suggest that the active substance is at least 70% degraded in the aquatic environment within 28 days. The active substance is stable to hydrolysis and there was only limited photolytic degradation, it is also not readily biodegraded. Slow degradation was seen in higher tier testing: In an aerobic mineralisation study DT_{50} values of 138 d and 334 d were reported at low and high concentrations respectively; in a water/sediment simulation study the mean whole system DT_{50} for cinmethylin was 39.2 d. Where degradation occurred, two significant degradants of ecotoxicological concern were formed. M684H001 formed in the aerobic mineralisation and water-sediment studies, with peak formation observed at 13.1% and 11.4% respectively. M684H003 formed in the direct and indirect photolysis studies, with peak formation observed at 6.8% and 11% respectively. For both degradants, default DT_{50} values of 1000 d have been applied for risk assessment; therefore, the two degradants are themselves also not classed as “rapidly degradable”.

11.2 Environmental transformation of metals or inorganic metals compounds

Not applicable to cinmethylin.

11.3 Environmental fate and other relevant information

Soil adsorption and desorption

One study is available investigating the adsorption behaviour of cinmethylin in soil (Harder, U. and Hegler, F., 2017). The study was conducted to OECD 106 guidelines using the direct method and was undertaken in adherence to GLP; therefore, the study was deemed reliable. The adsorption behaviour was investigated in eight soils (five from Europe, two from the US and one from Japan) with a pH ($CaCl_2$) range from 4.4 – 8.1 and organic carbon content ranging 0.66 – 4.34%. Of these soils, five were deemed appropriate for calculating sorption parameters. The adsorption coefficient KFOC ranged 266.5 – 510.1 mL/g (geomean 317.8 mL/g) and the calculated Freundlich exponent 1/n ranged 0.94 – 1.00 (arithmetic mean 0.97). The sorption of cinmethylin did not show pH dependence, though there was a strong dependence on organic carbon content (linear regression; R2 = 0.95; p = 0.004).

Desorption of cinmethylin could not be investigated during the sorption studies due to the tendency of cinmethylin to volatilise.

Volatilisation and short term transport potential

Cinmethylin has a vapour pressure of 8.1 x 10-3 Pa at 20°C, which suggests that cinmethylin is a moderately volatile substance. Two studies were submitted to further consider the volatilisation and short range transport potential of cinmethylin. The first investigated volatilisation of cinmethylin in formulation from soil and plant surfaces (Hassink, J., 2017b); this study was conducted to German BBA IV 6-1 guidelines and in adherence to GLP. Therefore, the study was considered reliable. Volatilisation of cinmethylin from soil

and plant surfaces was significant in the first 24 hours following application, with volatilisation rates from soil and plant surfaces of 73% and 89% respectively.

The second study followed on from the first and investigated the deposition of cinmethylin into water trays (acting as surface water bodies) following volatilisation of the active substance in a wind tunnel (Wallace, D., 2017). This was a non-guideline study; however, the study adhered to GLP, was clearly reported and the deposition behaviour of the reference substance, lindane, was within the expected range; therefore the study was deemed reliable.

Cinmethylin was applied in formulation to spring barley within a wind tunnel and the deposition rates following volatilisation were measured along with the concentrations in air at 12 hour intervals for 96 hours following application. Deposition of cinmethylin into water downwind of the application site peaked 48 hours after application and ranged 0.14 – 0.82% of the applied amount, with the peak observed 1 m downwind. At 5 m downwind, 0.43% of the applied amount was deposited following volatilisation.

Air concentrations of cinmethylin ranged 0.01 – 3.01 µg/cm^3 during the 96 hour study with the highest concentration observed at 1 m downwind 12 hours after application. Rapid declines were observed both in terms of time following application and distance downwind, with concentrations 1 m downwind decreasing to 0.07 µg/cm^3 72-96 hours after application.

It was concluded that the deposition values following volatilisation were significant when placed into the context of pesticide spray drift values used within the risk assessment of an active substance to surface waters in the UK. When considering entry into surface water via spray drift, the standard (Rautmann) spray drift value at 5 m downwind is 0.57% of the applied amount of active substance. When considering 0.43% of the applied amount is deposited 5 m downwind 48 hours after application, this deposition following volatilisation accounts for an additional 75% of cinmethylin being deposited into a surface water.

Long range transport potential

A calculation study was conducted to consider the photochemical oxidative degradation of cinmethylin in the air (Hassink, J., 2015a). The study was not a laboratory study and therefore was not conducted to GLP, though the methods for calculation via QSAR estimation were transparently reported and deemed reliable. Cinmethylin has a calculated half-life in the air of 0.178 days (assuming a 12 hour day).

In conclusion, when considering the volatilisation studies and the half-life of cinmethylin in air, cinmethylin is a volatile substance but does not display a tendency for long-range transport.

11.4 Bioaccumulation

Studies have been performed to measure the bioaccumulation of cinmethylin in fish, these studies are summarised in Table 38 and further discussion is included in Section 11.4.2.

Table 38: Summary of relevant information on bioaccumulation

Method	Results	Remarks	Reference
Bioaccumulation in fish OECD 305 GLP	707 L kg^{-1} (whole fish at 0.5 µg a.s./L) 688 L kg^{-1} (whole fish at 5 µg a.s./L) Values are lipid normalized Geometric mean of 697 L kg^{-1}	Study considered suitable for use in hazard classification.	Anon (2017c)

Method	Results	Remarks	Reference
17-day exposure phase, 7-day depuration phase, flow-through exposure			
Bioaccumulation in fish OECD 305 GLP 17-day exposure phase, 7-day depuration phase, flow-through exposure	170 L kg^{-1} (whole fish at 0.5 µg a.s./L) 59 L kg^{-1} (whole fish at 5 µg a.s./L) Values are lipid normalized Geometric mean (whole fish 0.5 and 5 µg a.s./L) recalculated based on cinmethylin content to **100.4*** using data from Anon 2017c	Study considered suitable for use in hazard classification.	Anon (2017c) & Anon (2018m)

** BCF value recalculated based on metabolism study (Anon 2018m). See detailed summary in section 11.4.2 below.*

11.4.1 Estimated bioaccumulation

Not applicable, measured partition coefficient and bioaccumulation test data available (see Section 11.4.2).

11.4.2 Measured partition coefficient and bioaccumulation test data

The measured Log Kow for cinmethylin is 4.5 at 20°C; this is greater than the CLP Log Kow trigger of ≥ 4 and indicates a potential for bioaccumulation. Under the requirements of the GB CLP Regulation measured estimates of bioconcentration in fish were provided in relation to cinmethylin.

Two valid studies (Anon 2017c; Anon 2018m) are available estimating the BCF for fish (see Table 38). Both studies are considered reliable and can be considered in relation to estimating the bioaccumulation potential, noting Anon, 2018m uses data from Anon 2017c. Brief summaries are included below.

^{14}C-Cinmethylin: Bioconcentration study in bluegill sunfish (Lepomis macrochirus) (Anon, 2017c).

Bioaccumulation of cinmethylin was investigated using bluegill sunfish (Lepomis macrochirus), according to the OECD 305 guideline and according to GLP. The test item was radio labelled [^{14}C]-cinmethylin. The fish were exposed to a continual flow of dilution water for 17 days, at nominal treatment concentrations of 0.5 or 5.0 µg a.s./L (exposure/uptake phase). The fish were then exposed to a continuous flow of dilution water alone for a further 7 days (depuration period). A control was performed in parallel testing the dilution water used to prepare the treatment solutions. The study met the relevant validity criteria according to OECD 305.

Daily water samples were examined and demonstrated that the test item was maintained within ± 20 % of nominal concentrations for both treatment groups; mean 0.49 ± 0.03 µg a.s./L in the 0.5 µg a.s./L (nominal) treatment group and 4.87 ± 0.16 µg a.s./L in the 5.0 µg a.s./L (nominal) treatment group.

The measured data from both concentration groups from whole fish as well as edible and nonedible portions fit well to a first order kinetic model allowing an estimation of the uptake and depuration rate constants based on simultaneous curve fitting. The data illustrate that steady state was quickly reached during the uptake period, after the sampling on day 3. After the start of depuration, the concentrations in

fish progressively declined. After 7 days in clean water the whole-body residues in fish from both concentration groups had declined to 2 % of the mean steady state concentration (CFss), see Figure 11.4.2-1.

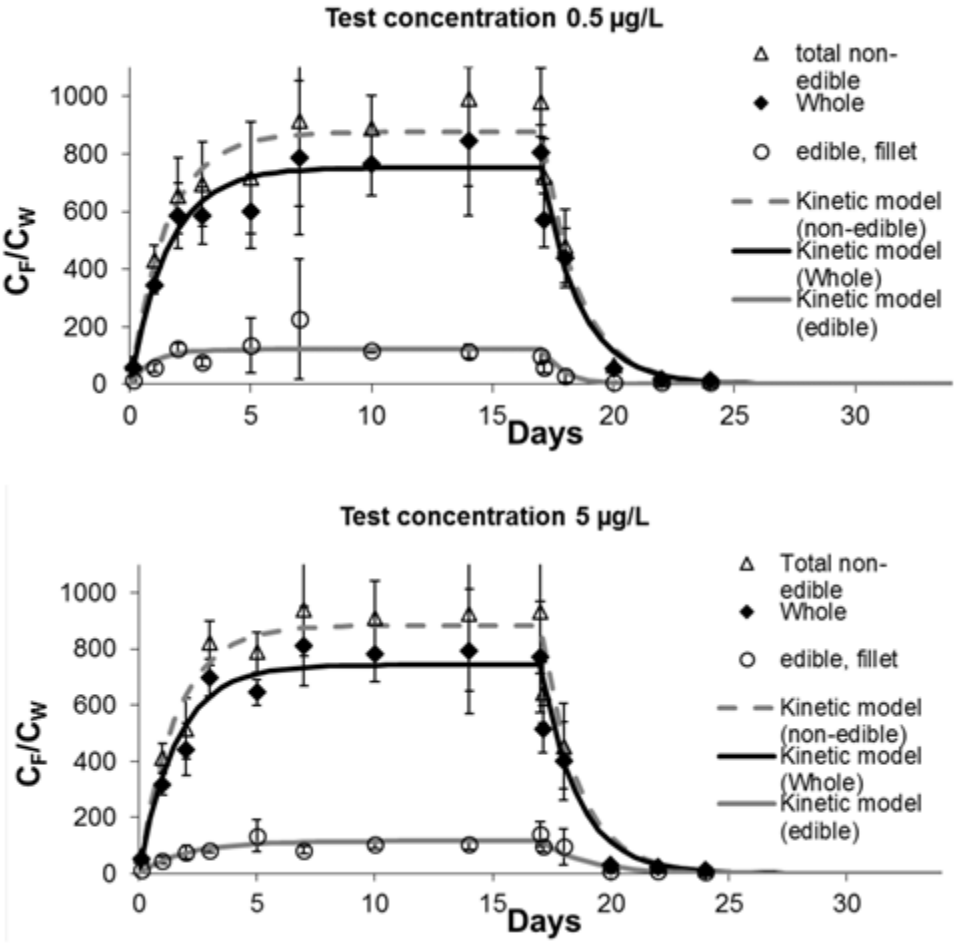


Figure 11.4.2-1: Plot of the uptake and depuration curves as BCF (CF(t)/CW) in whole fish, fillet and non-edible portions. Not corrected for growth or lipid content.

Based on the kinetic model, the calculated uptake rate constants (k_1) were 474 and 476 day-1 from the low and high exposure concentrations respectively. The calculated depuration rate constants (k_2) were 0.63 and 0.65 day-1 from the low and high exposure concentrations respectively. The OECD 305 guideline suggests that the variation in uptake and depuration rate constants derived from exposure at two concentrations should not differ by more than 20 % between the test groups, otherwise concentration dependence may be indicated. The results are summarized in Table 39 below.

Overall the measured BCF_{ss} values were very similar to the calculated BCF_K values indicating that steady state was reached, and that uptake and depuration follow first order kinetics. The most relevant BCF is the BCF_K normalized to 5 % lipid content (BCF_{KL}) because it incorporates all measurements during uptake and depuration and since it removes the influence of the test fish lipid content.

In conclusion, the bioconcentration factor BCF_{KL} was 697 L/kg for the whole fish based on total radioactive residues of ^{14}C-cinmethylin, this is the lipid-corrected geometric mean of the whole fish values at 0.5 and 5 µg a.s./L of 688 and 707 L kg^{-1} respectively.

Table 39: Uptake and depuration rate constants and bioconcentration factors (BCF) for the whole fish based on measured and calculated data.

Parameter	0.5 µg a.s./L	5 µg a.s./L
k_g (growth rate constant; day^{-1}) (standard error)	0.0092 (0.0009)	0.0092 (0.0009)
k_1, (overall uptake rate constant, L/kg/day) (95% confidence interval)	474 (367 – 582)	476 (374 – 578)
k_2, (overall depuration rate constant, day^{-1}) (95% confidence interval)	0.63 (0.48 – 0.78)	0.65 (0.51 – 0.79)
k_{2g} (growth-corrected depuration rate constant, day^{-1})	0.62	0.64
$C_F$ss, (concentration in fish at steady-state, µg a.s./kg) (mean (days 2 – 14) ± standard deviation)	360 ± 54	3648 ± 310
C_W (concentration in the water, µg/L) (mean (days 0 – 14) ±standard deviation)	0.49 ± 0.03	4.78 ± 0.16
L_n (lipid normalization factor) (mean during uptake)	0.054	0.054
BCFss (steady-state BCF; L/kg) (mean (days 2 – 14) ± standard deviation)	731 ± 110	749 ± 64
BCF_{SSL} (lipid normalized steady-state BCF; L/kg)	677	694
BCF_K (kinetic BCF; L/kg)	752	732
BCF_{Kg} (growth-corrected kinetic BCF; L/kg)	764	743
BCF_{KLg} (lipid-normalized kinetic BCF_{Kg}; L/kg)	707	688
Geometric mean BCF_{KLg} [a]	**697**	
$t_{1/2}$, (depuration half-life; day)	1.10	1.07
$t_{1/2g}$ (growth-corrected half-life, day)	1.12	1.08
Time to 95 % steady state (growth-corrected, day)	4.8	4.6

[a]The most relevant BCF in this study is the growth corrected kinetic BCF normalized to 5 % lipid content, BCF_{KLG}.

The study was considered suitable for use in hazard assessment of cinmethylin under the GB CLP Regulation.

Metabolism of cinmethylin in bluegill sunfish (bioconcentration after exposure in a flow through system). (Anon, 2018m).

The in-life part was conducted within the BCF study Anon (2017c) (described in detail above). The study Anon (2018m) was conducted to GLP and investigated the metabolism of cinmethylin during the study Anon (2017c).

Extraction, characterization and identification of residues

Only cinmethylin (BAS 684 H) was present in water samples at the end of the exposure period. When considering whole fish at end of exposure period (day 17) cinmethylin (BAS 684 H) accounted for 24.1 % TRR/0.085 mg a.s./kg (Total Radioactive Residue) at 0.5 µg a.s./L and 8.6 % TRR/0.045 mg a.s./kg at 5 µg a.s./L. The metabolite M684H012 accounted for 24.1 % TRR, 14.7 % TRR, M684H022 (isomer 1) for 7.7 % TRR, 4.2 % TRR, M684H022 (isomer 2) for 8.0 % TRR, 10.6 % TRR, M684H026 for 8.2 % TRR, 5.2 % TRR at 0.5 and 5 µg a.s./L.

In terms of fish carcass cinmethylin, M684H026 were present at 65.4, 27.2 % TRR in 0.5 µg a.s./L treatment group and 24.7, 19.5 % TRR at 5 µg a.s./L. In addition, a maximum of 25 unidentified peaks were found in carcass and viscera however, none accounted for > 7.2 % TRR.

Cinmethylin (48.3, 42.1 % TRR at 0.5 µg a.s./L and 5 µg a.s./L respectively) and the metabolite M684H026 (46.0, 55.7 % TRR at 0.5 µg a.s./L and 5 µg a.s./L respectively) were present in edible fish. It is noted that none of these fish metabolites are considered to be significant products of typical environmental degradation (see Section 11.1 and Annex I).

A summary of the results for each treatment group are presented in Table 40.

Table 40: Summary of identified/characterized components in fish

Designation	Fish fillet		Fish carcass		Fish viscera		Edible tissue		Inedible tissue		Whole fish	
	mg a.s./kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR
Treatment group 1 (0.5 µg a.s./L)												
Cinmethylin	0.045	48.3	0.070	65.4	0.257	10.9	0.045	48.3	0.097	22.5	0.085	24.1
M684H012	n.d.	n.d.	n.d.	n.d.	0.402	17.1	n.d.	n.d.	0.058	13.4	0.044	12.6
M684H022 (isomer 1)	n.d.	n.d.	n.d.	n.d.	0.245	10.4	n.d.	n.d.	0.035	8.2	0.027	7.7
M684H022 (isomer 2)	n.d.	n.d.	n.d.	n.d.	0.255	10.8	n.d.	n.d.	0.037	8.5	0.028	8.0
M684H026	0.043	46.0	0.029	27.2	n.d.	n.d.	0.043	46.0	0.025	5.8	0.029	8.2
Total identified	**0.087**	**94.3**	**0.099**	**92.6**	**1.159**	**49.2**	**0.087**	**94.3**	**0.251**	**58.5**	**0.213**	**60.6**
Characterized by HPLC analysis	n.d.	n.d.	n.d.	n.d.	1.148	48.8	n.d.	n.d.	0.164	38.4	0.127	36.1
Combined Water Extract (LSC)	0.001	1.6	0.001	1.2	0.027	1.1	0.001	1.6	0.005	1.1	0.004	1.2
Total Characterized	0.001	1.6	0.001	1.2	1.175	49.9	0.001	1.6	0.169	39.5	0.131	37.2
Total identified and/ or	**0.089**	**95.8**	**0.100**	**93.8**	**2.334**	**99.1**	**0.089**	**95.8**	**0.420**	**98.0**	**0.344**	**97.9**

Designation	Fish fillet		Fish carcass		Fish viscera		Edible tissue		Inedible tissue		Whole fish	
	mg a.s./kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR
characterized												
Unextracted residue (RRR, by LSC)	0.006	6.7	0.004	3.4	0.043	1.8	0.006	6.7	0.009	2.2	0.009	2.4
Total	**0.095**	**102.5**	**0.104**	**97.2**	**2.377**	**100.9**	**0.095**	**102.5**	**0.429**	**100.1**	**0.352**	**100.3**
Treatment group 2 (5 µg a.s./L)												
Cinmethylin	0.208	42.1	0.242	24.7	1.017	3.6	0.208	42.1	0.343	7.6	0.313	8.6
M684H012	n.d.	n.d.	n.d.	n.d.	5.239	18.6	n.d.	n.d.	0.687	15.1	0.535	14.7
M684H022 (isomer 1)	n.d.	n.d.	n.d.	n.d.	1.515	5.4	n.d.	n.d.	0.199	4.4	0.155	4.2
M684H022 (isomer 2)	n.d.	n.d.	n.d.	n.d.	3.778	13.4	n.d.	n.d.	0.496	10.9	0.385	10.6
M684H026	0.276	55.7	0.191	19.5	n.d.	n.d.	0.276	55.7	0.166	3.7	0.191	5.2
Total identified	**0.484**	**98.0**	**0.433**	**44.2**	**11.548**	**41.0**	**0.484**	**97.8**	**1.892**	**41.6**	**1.578**	**43.3**
Characterized by HPLC analysis	n.d.	n.d.	0.464	47.4	16.271	57.8	n.d.	n.d.	2.539	55.8	1.974	54.1
Combined Water Extract (LSC)	0.001	0.2	0.009	0.9	n.a.	n.a.	0.001	0.2	0.008	0.2	0.006	0.2
Total characterized	**0.001**	**0.2**	**0.474**	**48.4**	**16.271**	**57.8**	0.001	0.2	2.547	56.0	1.980	54.3
Total identified and/ or characterized	0.485	98.0	0.907	92.6	27.819	98.8	**0.485**	**98.0**	**4.438**	**97.6**	**3.559**	**97.6**
Unextracted residue (RRR, by LSC)	0.007	1.5	0.010	1.1	0.205	0.7	0.007	1.5	0.036	0.8	0.030	0.8
Total	**0.492**	**99.5**	**0.917**	**93.7**	**28.024**	**99.5**	**0.492**	**99.5**	**4.474**	**98.4**	**3.588**	**98.4**

In this study at end of exposure period cinmethylin (BAS 684 H) accounted for 24.1 % TRR/0.085 mg a.s./kg (Total Radioactive Residue) at 0.5 µg a.s./L and 8.6 % TRR/0.045 mg a.s./kg at 5 µg a.s./L. In order to

account for the metabolites present using this study the Agency has re-calculated the BCF endpoints based on cinmethylin to 170 and 59 L kg^{-1} for 0.5 and 5 µg a.s./L, corresponding to a geometric mean of 100.4. Annex III, Section III.2.1.2 of ECHA's Guidance on the Application of the CLP Criteria (ECHA, 2017) states that the BCF from radio-labelled studies should, preferentially, be based on the parent compound, so it is appropriate to correct the original values based on TRR when able.

The study was considered suitable for use in hazard assessment according to the GB CLP Regulation.

Overall, the measured Log K_{ow} for cinmethylin (at 4.5) is greater than the CLP Log K_{ow} trigger of ≥ 4 and the experimentally determined whole-fish, lipid-normalised BCF geometric value (considering both studies Anon 2017c and Anon 2018m) is 100.4 (individual BCF values are 170 and 59 L kg^{-1} at 0.5 and 5 µg a.s./L respectively. Therefore, the BCF values are below the CLP trigger of ≥ 500. The information available on cinmethylin therefore indicates that there is not a potential for bioaccumulation according to CLP criteria.

11.5 Acute aquatic hazard

Studies available during the registration of cinmethylin as a pesticide active substance, circa 2020, under Regulation 1107/2009 are summarised in Table 41. All the listed studies have been conducted according to GLP. The studies have been evaluated, considered reliable and deemed suitable for hazard classification purposes, unless otherwise stated (some studies were considered suitable as supporting information). Only studies submitted testing the technical substance, cinmethylin, have been summarised below (formulation studies submitted in the context of the active substance renewal under Regulation 1107/2009 have not been considered further here).

(Note that the available acute toxicity data available for the degradants of cinmethylin (M684H001 and M684H003) considered relevant under Regulation 1107/2009 are summarised in Annex I. This information is included for information only, none of the degradants exhibit equivalent toxicity to cinmethylin or would require aquatic hazard classification in isolation - and so are not considered to impact the hazard classification of cinmethylin).

Table 4121: Summary of relevant information on acute aquatic toxicity

Method	Species	Test material	Results[1]	Reference
Fish				
Acute toxicity to fish OECD 203; GLP 96-hours, static exposure	*Oncorhynchus mykiss*	Batch COD-002038 Cinmethylin (purity 93.5 %)	LC_{50} 8.49 mg /L (m.m.)[a]	Anon (2017d)
Acute toxicity to fish **OECD 203** **GLP** **96-hours, static exposure**	***Cyprinus carpio***	**Batch** **COD-002038** **Cinmethylin** **(purity 93.5 %)**	**LC_{50} 5.75** mg /L **(g.m.)**	**Anon (2017e) & (2018n)**

Acute toxicity to fish OECD 203 GLP 96-hours, static exposure	*Pimephales promelas*	Batch COD-002038 Cinmethylin (purity 93.5 %)	LC_{50} 5.84 mg /L (m.m)[a]	Anon (2017f) & (2018o)
Aquatic invertebrates				
Acute immobilization OECD 202 GLP 48-hours, static exposure	*Daphnia magna*	Batch COD-002038 Cinmethylin (purity 93.5 %)	**EC_{50} 7.26 mg /L (nom.)**	Haerthe (2016a)
EPA 540/9-82-024 **GLP** **48 - 96 hour, static**	*Gammarus pulex*	**Batch WL95481** **Cinmethylin** **(purity 92 %)**	EC_{50} 6.6 mg /L (g.m.)	Pearson & Stephenson (1987a)[b]
	Lymnaea stagnalis		EC_{50} 7.0 mg /L (g.m.)	
	Tubifex		EC_{50} 5.4 mg /L (g.m.)	
	Chironomus lugubris		**EC_{50} > 2.06 mg /L (g.m.)[b]**	
Algae				
Freshwater algal growth inhibition OECD 201 GLP 72-hours, static exposure	*Pseudokirchneriella subcapitata*	Batch COD-002038 Cinmethylin (purity 93.5 %)	E_rC_{50} 23.04 mg /L (g.m.)	Kauf (2017a)
Freshwater algal growth inhibition OECD 201, EPA 850.4500, EPA 850.4550 GLP	*Anabaena flos-aquae*	Batch COD-002038 Cinmethylin (purity 93.5 %)	E_rC_{50} 51.34 mg /L (g.m.)	Kauf (2017b)

96-hours, static exposure				
Other aquatic plants				
Growth inhibition test **OECD 221** **GLP** **7-days, static, water only**	***Lemna gibba***	**Batch** **COD-002038** **Cinmethylin** **(purity 93.5 %)**	**E_rC_{50} 0.0888 mg /L (g.m.)**	**Vlechev (2017a)**
Growth inhibition test Water-Sediment Toxicity Test OECD 239 GLP 14-days, static renewal of water during study	*Glyceria maxima*	Batch COD-002038 Cinmethylin (purity 93.5 %)	E_rC_{50} 0.137 mg /L (g.m.)	Vlechev (2017b)
Water-Sediment Toxicity Test OECD 239 GLP 14-days, static, water/sediment	*Myriophyllum spicatum*	Batch COD-002038 Cinmethylin (purity 93.5 %)	E_rC_{50} 0.414 mg /L (g.m.)[#]	Rzodeczko (2017c) and addendum (Kubitza, 2019a)
Water-Sediment Toxicity Test OECD 239 GLP 14-days, static, water/sediment	*Elodea canadensis*	Batch COD-002038 Cinmethylin (purity 93.5 %)	E_rC_{50} 0.247 mg /L (g.m.)[#]	Rzodeczko (2018a) and addendum (Kubitza, 2019b)

Water-Sediment Toxicity Test OECD 239 GLP 14-days, static, water/sediment	*Egeria densa*	Batch COD-002038 Cinmethylin (purity 93.5 %)	E_rC_{50} 0.116 mg /L (g.m.)[#]	Rzodeczko (2017d) and addendum (Kubitza, 2019c)

Bold entries are the endpoints considered most suitable to set the hazard classification for the active substance for each group of organisms.

[a] Endpoint should have been based on geometric mean measured. However, the geometric mean test concentrations calculated by the UK are comparable to mean measured concentrations hence the study author values have been accepted.

[b] Study considered suitable as supporting information only by the UK due to uncertainties; not possible to confirm validity criteria were met and lack of control without solvent.

[1] m.m. = mean measured concentration; g.m.= geometric mean measured concentration; nom. = nominal concentration

[#] Applicant 'extrapolated' missing analytical data based on linearized single first order kinetics using measured values for other test concentrations. Hence calculated endpoints are not considered suitable for quantitative use by the UK evaluator.

11.5.1 Acute (short-term) toxicity to fish

Acute fish studies considered acceptable are summarised below.

BAS 684 H (Cinmethylin) - Acute toxicity study in rainbow trout (Oncorhynchus mykiss) under static conditions (Anon, 2017d).

The acute toxicity of cinmethylin to O. *mykiss* was assessed in a study performed to the guideline OECD 203 (1992) and according to GLP. Exposure to the test item was for 96 hours under static conditions, at nominal concentrations of 0 (control), 1.16, 2.33, 4.65, 9.3 and 18.6 mg a.s./L. The test item was not maintained within ± 20 % of the nominal concentration during the study; therefore mean measured treatment concentrations were established by the study author at 0.601, 1.28, 2.65, 5.65 and 12.85 mg a.s./L (based on measurements at the test start and termination). Ideally geometric mean concentrations should have been used but the geometric values are comparable to mean measured, calculated as 0.58, 1.27, 2.64, 5.64 and 12.8 mg a.s./L by the Agency. Therefore, the use of mean measured concentrations was considered acceptable. The study met the relevant validity criteria according to the guideline (OECD, 203). After 96 hours of exposure, no mortality was observed in the control and at test item concentrations of up to and including 5.65 mg a.s./L, whereas, at the highest tested concentration, all fish were dead after 96 hours of exposure. Sub-lethal effects (i.e. swimming at the bottom) were found at 5.65 mg a.s./L after 96 hours.

The resulting LC_{50} was 8.49 mg a.s./L (mean measured). The study was considered suitable for use in hazard assessment according to CLP.

Cinmethylin – Carp, acute toxicity test under static conditions (Anon, 2017e and amendment (Anon 2018n)).

The acute toxicity of cinmethylin to C. *carpio* was assessed in a study performed to the guideline OECD 203 (1992) and according to GLP. Exposure to the test item was for 96 hours under static conditions, at nominal concentrations of 0 (control), 2.0, 3.0, 4.4, 5.6 and 10 mg a.s./L. The test item was not maintained within ± 20 % of the nominal concentration during the study; therefore geometric mean measured treatment concentrations were established at 1.51, 2.22, 3.74, 4.81 and 8.64 mg a.s./L (based on measurements at the test start and termination). The study met the relevant validity criteria according to the guideline

(OECD, 203). After 96 hours of exposure, no mortality was observed in the control and at test item concentrations of up to and including 3.74 mg a.s./L, whereas 10 and 100 % mortality was observed at 4.81 and 8.64 mg a.s./L, respectively. Sub-lethal effects (i.e. unbalanced swimming) was found in the test item concentrations of 4.81 mg a.s./L. A statistically significant difference compared to the control was observed at the highest concentration of 8.64 mg a.s./L (Step-down Cochran-Armitage Test, $\alpha = 0.05$, one-sided greater).

The resulting LC_{50} was 5.75 mg a.s./L (geometric mean measured). The study was considered suitable for use in hazard assessment according to CLP.

Cinmethylin – Acute toxicity study in the fathead minnow (Pimephales promelas) under static conditions (Anon 2017f and amendment (Anon 2017 f)).

The acute toxicity of cinmethylin to P. *promelas* was assessed in a study performed to the guideline OECD 203 (1992) and according to GLP. Exposure to the test item was for 96 hours under static conditions, at nominal concentrations of 0 (control), 1.16, 2.33, 4.65, 9.3 and 18.6 mg a.s./L. The test item was not maintained within ± 20 % of the nominal concentration during the study; therefore mean measured treatment concentrations were established by the study author at 0.50, 1.08, 2.37, 4.88 and 11.74 mg a.s./L (based on measurements at the test start and termination). Ideally geometric mean concentrations should have been used but the geometric values are comparable to mean measured, calculated as 0.5, 1.07, 2.35, 4.85 and 11.70 mg a.s./L by the Agency. Therefore, the use of mean measured concentrations was considered acceptable. The study met the relevant validity criteria according to the guideline (OECD, 203). After 96 hours of exposure, no mortality was observed in the dilution water control and at test item concentrations of up to and including 2.37 mg a.s./L (geometric mean measured concentration), whereas 10 and 100 % mortality was observed at 4.88 and 11.74 mg a.s./L respectively (geometric mean measured). Statistically significant effects were determined at the highest test item concentration of 11.74 mg a.s./L (Fishers Exact Binomial Test with Bonferroni Correction, $\alpha = 0.05$, one-sided greater). At the highest tested concentrations, all fish were dead after 24 hours of exposure. Sub-lethal effects (i.e. swimming at the bottom and tottering) were found at 4.88 mg a.s./L (geometric mean measured concentration) after 24 hours.

The resulting LC_{50} was 5.84 mg a.s./L (mean measured). The study was considered suitable for use in hazard assessment according to CLP.

Overall conclusion

There are sufficient suitable studies to allow classification of the acute hazard to fish from exposure to cinmethylin. **The endpoint selected for use in hazard classification is the 96-hour LC_{50} of 5.75 mg a.s./L for Cyprinus carpio (Anon 2017e & 2018n)**. It is noted that ultimately fish are not the most acutely sensitive taxon and therefore not critical for setting the hazard classification.

11.5.2 Acute (short-term) toxicity to aquatic invertebrates

An acute aquatic invertebrate study was considered acceptable and summarised below along with four other studies considered suitable as supporting information.

Acute toxicity of cinmethylin to Daphnia magna STRAUS under static conditions (Haerthe N., 2016a).

The acute toxicity of cinmethylin to *D. magna* was assessed in a study performed to the guideline OECD 202 (2004) and according to GLP. Exposure to the test item was for 48 hours under static conditions, at nominal concentrations of 0 (control), 1.0, 1.8, 3.2, 5.6 and 10 mg a.s./L. The test item was maintained within ± 20 % of the nominal for all concentrations at all observation points during the study; therefore the nominal

values were used when deriving endpoints. The study met the relevant validity criteria according to the guideline (OECD, 202). No immobilisation or other sub-lethal effects were observed in the control groups. The resulting **EC_{50} was 7.26 mg a.s./L (nominal)**. The study was considered suitable for use in risk assessment during evaluation of cinmethylin and is considered suitable for use in hazard assessment according to Reg. (EC) 1272/2008.

Several other studies (detailed below) testing additional aquatic invertebrates were submitted. It should be noted that these studies were only considered suitable as supporting information during the evaluation of cinmethylin.

Cinmethylin: Acute toxicity of cinmethylin to Gammarus pulex under static conditions (Pearson N., Stephenson R.R., 1987a).

The acute toxicity of cinmethylin to *G. pulex* was assessed in a study performed to the guideline US EPA 850.1020, validity criteria compared to 2016 version and according to GLP. Exposure to the test item was for 96 hours under static conditions, at nominal concentrations of 0 (control), 1.0, 2.0, 5.0, 10 and 20 mg a.s./L. All test concentrations at both study initiation and termination were analytically determined. The test item was maintained within ± 20 % of the nominal for all concentrations at all observation points during the study; therefore the nominal values were used when deriving endpoints. It should be noted this study was considered as supporting information as there was insufficient reporting of the analytical method. Therefore, the analytical method was not sufficiently validated in accordance with SANCO/3029/99. The following deficiencies were noted:

i) No specificity data have been provided and no chromatograms have been submitted to check interferences.

ii) No linearity data have been provided.

iii) To assess accuracy and precision, at least 5 recoveries should be provided for at least 2 fortification levels.

iv) The LOQ is not supported by 5 recovery determinations.

v) Matrix effects have not been investigated.

vi) Procedural recoveries have not been completed.

The study met the relevant validity criteria according to the guideline (US EPA 850.1020, 2016). Only a single mortality was observed in the control group (3.3 %). The resulting **LC_{50} was 6.6 mg a.s./L (nominal)**. The study was considered suitable for use as supporting information in hazard assessment according to the GB CLP Regulation.

Cinmethylin: Acute toxicity of cinmethylin to Lymnaea stagnalis under static conditions (Pearson N., Stephenson R.R., 1987a).

The acute toxicity of cinmethylin to *L. stagnalis* was assessed in a study performed according to GLP. The study was not conducted to any international test guideline. Exposure to the test item was for 96 hours under static conditions, at nominal concentrations of 0 (control), 1.0, 2.0, 5.0, 10 and 20 mg a.s./L. All test concentrations at both study initiation and termination were analytically determined. The test item was maintained within ± 20 % of the nominal for all concentrations at all observation points during the study; therefore the nominal values were used when deriving endpoints. It should be noted this study was considered as supporting information as there was insufficient reporting of the analytical method. Therefore, the analytical method was not sufficiently validated in accordance with SANCO/3029/99. The following deficiencies were noted:

i) No specificity data have been provided and no chromatograms have been submitted to check interferences.

ii) No linearity data have been provided.

iii) To assess accuracy and precision, at least 5 recoveries should be provided for at least 2 fortification levels.

iv) The LOQ is not supported by 5 recovery determinations.

v) Matrix effects have not been investigated.

vi) Procedural recoveries have not been completed.

It was not possible to compare this study to validity criteria as it was not performed to a standard test guideline. However, the UK notes 3.3 % mortality in control occurred which is within 10 %, a typical criterion for acute studies testing aquatic invertebrates. The resulting **LC_{50} was 7.0 mg a.s./L (nominal)**. The study was considered suitable for use as supporting information in hazard assessment according to the GB CLP Regulation.

Cinmethylin: Acute toxicity of cinmethylin to Tubifex tubifex under static conditions (Pearson N., Stephenson R.R., 1987a).

The acute toxicity of cinmethylin to *T. tubifex* was assessed in a study performed according to GLP. The study was not conducted to any international test guideline. Exposure to the test item was for 96 hours under static conditions, at nominal concentrations of 0 (control), 1.0, 2.0, 5.0, 10 and 20 mg a.s./L. All test concentrations at both study initiation and termination were analytically determined. The test item was maintained within ± 20 % of the nominal for all concentrations at all observation points during the study with a single exception. At 1.0 mg a.s./L the initial concentration was 125 % of nominal. The exceedance is relatively low and implies that nominal values are more conservative. Therefore, the UK considers the use of nominal concentrations acceptable to derive endpoints in this study. It should be noted this study was considered as supporting information as there was insufficient reporting of the analytical method. Therefore, the analytical method was not sufficiently validated in accordance with SANCO/3029/99. The following deficiencies were noted:

i) No specificity data have been provided and no chromatograms have been submitted to check interferences.

ii) No linearity data have been provided.

iii) To assess accuracy and precision, at least 5 recoveries should be provided for at least 2 fortification levels.

iv) The LOQ is not supported by 5 recovery determinations.

v) Matrix effects have not been investigated.

vi) Procedural recoveries have not been completed.

It was not possible to compare this study to validity criteria as it was not performed to a standard test guideline. However, the Agency notes 3.3 % mortality in control occurred which is within 10 %, a typical criterion for acute studies testing aquatic invertebrates. The resulting **LC_{50} was 5.4 mg a.s./L (nominal)**. The study was considered suitable for use as supporting information in hazard assessment according to the GB CLP Regulation.

Cinmethylin: Acute toxicity of cinmethylin to Chironomus lugubris under static conditions (Pearson N., Stephenson R.R., 1987a).

The acute toxicity of cinmethylin to *C. lugubris* was assessed in a study performed according to GLP with water only exposure. Although not conducted at the time to a standard test guideline, the study was compared to OECD 235 (2011) i.e. the current OECD acute *Chironomus* test, by the Agency. Exposure to the test item was for 48 hours under static conditions, at nominal concentrations of 0 (control), 1.0, 2.0, 5.0, 10 and 20 mg a.s./L. All test concentrations were analysed at study initiation but not termination. Only the two lowest test concentrations were analysed at study termination as shown in Table 42 below. Furthermore, the concentrations were not maintained within ± 20 % of nominal concentrations. Therefore, where possible the Agency calculated geometric mean measured concentrations.

Table 42: Measured concentrations during study

Nominal (mg a.s./L)	0 hours		48 hours		Geometric mean (mg a.s./L)*
	Mean measured (mg a.s./L)	% of nominal	Mean measured (mg a.s./L)	% of nominal	
0.0	<0.1	--	<0.01	--	--
1.0	1.2	120	0.7	70	**0.92**
2.0	2.5	125	1.7	85	**2.06**
5.0	5.1	102	--	--	--
10	10	100	--	--	--
20	20	100	--	--	--

-- Not tested or not applicable.
* Calculated by UK evaluator.

It should be noted this study was considered as supporting information as there was insufficient reporting of the analytical method. Therefore, the analytical method was not sufficiently validated in accordance with SANCO/3029/99. The following deficiencies were noted:

i) No specificity data have been provided and no chromatograms have been submitted to check interferences.

ii) No linearity data have been provided.

iii) To assess accuracy and precision, at least 5 recoveries should be provided for at least 2 fortification levels.

iv) The LOQ is not supported by 5 recovery determinations.

v) Matrix effects have not been investigated.

vi) Procedural recoveries have not been completed.

The study met the validity criteria of OECD 235 (2011). Given the proposed LC_{50} value is an unbound value the mortality results have been presented below (Table 43).

Table 43: Mortality results

Nominal test concentration (geometric mean measured*) mg a.s./L	Cumulative mortality (48 hours)	% mortality (48 hours)
Control	3	10
1.0 (0.92)	6	20
2.0 (2.06)	9	30
5.0	28	93.3
10	30	100
20	30	100

* Calculated by the Agency

The resulting **LC_{50} was > 2.06 mg a.s./L (geometric mean concentration)**. The study was considered suitable for use as supporting information in the hazard assessment of cinmethylin.

Overall conclusion

There are sufficient suitable studies to allow classification of the acute hazard to aquatic invertebrates from exposure to cinmethylin. The GLP study where the analytical method was sufficiently validated generated an EC_{50} value of 7.26 mg a.s./L for *Daphnia magna*. When considering the other available GLP studies, a lower endpoint was derived at > 2.06 mg a.s./L for *Chironomus lugubris* (Pearson & Stephenson, 1987a). However, there is uncertainty regarding the analytical method used in Pearson & Stephenson, 1987a, meaning this endpoint should be used as supporting information only. Nonetheless, it does suggest that *C. lugubris* may be more sensitive, noting there was 30 % mortality at the highest test concentration that was analytically determined.

It is noted that ultimately aquatic invertebrates are not the most acutely sensitive taxa and therefore not critical for setting the hazard classification. In addition, regardless of which endpoint is used (7.26 or >2.06 mg a.s./L) the same acute classification would be determined given both are > 1 mg a.s./L.

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

Algae and aquatic plant toxicity studies considered acceptable are discussed in this section. It should be noted some studies were only considered as supporting information as explained in the relevant study summaries below.

Algae:

Effect of cinmethylin on the growth of the green alga Pseudokirchneriella subcapitata (Kauf A., 2017a).

The toxicity of cinmethylin to P. *subcapitata* was assessed in a study performed to the guideline OECD 201 (2011) and according to GLP. Exposure to the test item was for 72 hours under static conditions, at nominal concentrations of 0 (control) 0.32, 0.85, 2.24, 5.90, 15.52, 40.9 and 10 mg a.s./L. The test item was not maintained within ± 20 % of the nominal during the study; therefore geometric mean measured treatment concentrations were established at 0.27, 0.651, 1.765, 4.294, 11.447, 36.748 and 63.4 mg a.s./L (based on measurements at test initiation and termination), the geometric mean measured treatment concentrations

were used to establish the relevant endpoints for the study. The study met the relevant validity criteria according to the guideline (OECD, 201). No morphological effects on algae were observed in the control and at all test item concentrations. After 72 h of exposure, statistically significant effects compared to the control were detected at all test item concentrations for yield and at concentrations of 1.765 mg a.s./L (geometric mean measured) and above based on growth rate (α = 0.05, one-sided smaller).

The resulting E_rC_{50} = 23.04 mg a.s./L and E_rC_{10} = >1.765 mg a.s./L, the NOE_rC is 0.651 mg a.s./L (based on growth rate). The study was considered suitable for use in hazard assessment according to the GB CLP Regulation.

Effect of cinmethylin on the growth of the blue-green alga Anabaena flos-aquae (Kauf A., 2017b).

The toxicity of cinmethylin to A. *flos-aquae* was assessed in a study performed to the guideline OECD 201 (2011) and according to GLP. Exposure to the test item was for 96 hours under static conditions, at nominal concentrations of 0 (control) 2.56, 6.4, 16, 40 and 100 mg a.s./L. The test item was not maintained within ± 20 % of the nominal during the study; therefore geometric mean measured treatment concentrations were established at 1.36, 3.24, 8.22, 21.09 and 53.17 mg a.s./L (based on measurements at test initiation and termination), the geometric mean measured treatment concentrations were used to establish the relevant endpoints for the study. No morphological effects on algae were observed in the control and at all test item concentrations. After 72 h of exposure, statistically significant effects compared to the control were detected at the three highest test item concentrations (8.22, 21.09 and 53.17 mg a.s./L based on geometric mean) for yield and at concentrations of 21.09 mg a.s./L (geometric mean) and above based on growth rate (α = 0.05, one-sided smaller).

The resulting E_rC_{50} = 51.34 mg a.s./L and E_rC_{10} = 24.55 mg a.s./L, the NOE_rC is 8.22 mg a.s./L (based on growth rate). The study was considered suitable for use in hazard assessment according to the GB CLP Regulation.

Other Aquatic plants:

Effect of cinmethylin on the growth of Lemna gibba under static conditions (Vlechev S., 2017a and addendum (Kubitza, 2019a)).

The toxicity of cinmethylin to L. *gibba* was assessed in a study performed to the guideline OECD 221 (2006) and according to GLP. Exposure to the test item was for 7 days under static conditions, at nominal concentrations of 0 (control), 1.0, 2.6, 6.7, 17.3, 45, 116 and 300 µg a.s./L. The test was conducted in a water only test system. The test item was not maintained within ± 20 % of the nominal during the study, in the overlying water; therefore geometric mean measured treatment concentrations were established at 0.9, 2.3, 6.0, 14.2, 38, 99 and 258 µg a.s./L (based on measurements at test initiation and termination), the geometric mean measured treatment concentrations were used to establish the relevant endpoints for the study. The study met the relevant validity criteria according to the guideline (OECD, 221).

The following parameters were measured to investigate the effects of the test item, frond number and dry weight. Shorter roots were observed in test item concentration of 6.0 and 14.2 µg a.s./L (geometric mean), very short roots were observed at the three highest test item concentrations (38, 99 and 258 µg a.s./L based on geometric mean), whereas, chlorosis was observed in the two highest test item concentrations at the end of the study. Significant effects compared to control were observed (for at least one measured parameter) at the four highest test concentrations (14.2, 38, 99 and 258 µg a.s./L based on geometric mean). The growth rate results are summarised in Table 44.

Table 44: Inhibition of growth rate for each of the measured parameters

Geometric mean test concentration (mg a.s./L)	% inhibition of growth rate at study termination (7 days)	
	Based on frond number	Based on dry weight
Control	--	--
0.0009	-0.88	-0.53
0.0023	0.39	-1.07
0.006	0.46	0.95
0.0142	2.56	3.66**
0.038	15.1*	12.46**
0.099	57.12*	26.01**
0.258	86.33*	40.13**

-- = not applicable. Negative values indicate an increase compared to control. * Statistically different compared to control (Welch t-test with Bonferroni-Holm adjustment, α = 0.05, one-sided smaller), ** Statistically different compared to control (Multiple sequentially-rejective U-test after Bonferroni-Holm, α = 0.05, one-sided smaller).

The E_rC_{50} values were calculated to be 0.0888 mg a.s./L based on frond number and >0.2580 mg a.s./L based on dry weight. The E_rC_{10} values are 0.0285 and 0.0300 mg a.s./L based on frond number and dry weight respectively. The overall NOE_rC value is 0.006 mg a.s./L.

The study was considered suitable for use in hazard assessment according to the GB CLP Regulation.

Effect of cinmethylin on the growth of the aquatic plant Glyceria maxima under semi-static conditions (Vlechev S., 2017b and addendum (Kubitza, 2019b)).

The toxicity of cinmethylin to *G. maxima* was assessed in a study performed to the guideline OECD 239 (2014) and according to GLP. Exposure to the test item was for 14 days under semi-static conditions, at nominal concentrations of 0 (control), 0.01, 0.029, 0.098, 0.294, 0.980 and 2.939 mg a.s./L. The test was conducted in a water only test system. The test item was not maintained within ± 20 % of the nominal during the renewal periods of the study, in the overlying water; therefore geometric mean measured treatment concentrations were established at 0.008, 0.026, 0.086, 0.247, 0.813 and 2.64 mg a.s./L (based on measurements at test initiation, 'aged' and 'fresh' solutions- renewal occurred on day 7), the geometric mean measured treatment concentrations were used to establish the relevant endpoints for the study. The study met the relevant validity criteria according to the guideline (OECD, 239).

The following parameters were measured to investigate the effects of the test item, total length, wet weight and dry weight. At day 7 growth of algae was observed in all test concentrations and the control (the higher the concentration, the slighter the algae growth); however, this was deemed by the study author to be at levels not effecting performance of the study. From day 10 on, a total necrosis of the plant was observed in all replicates at the highest test concentration of 2.939 mg a.s./L (nominal). At day 14 in the test concentration of 0.980 mg a.s./L (nominal) in four replicates single blades show some necrosis and in one replicate a complete necrosis of the plant was observed, phytotoxicity was not reported in concentrations of 0.294 mg a.s./L (nominal) and below. Statistically significant inhibition of growth rate compared to control was observed at the three highest test item concentrations based on wet weight and dry weight (Welch t-test with Bonferroni-Holm adjustment for total length and wet weight and Dunnett's multiple t-test for dry weight, α = 0.05, one-sided smaller) and at the four highest test item concentrations based on total length (Welch t-test with Bonferroni-Holm adjustment, α = 0.05, one-sided smaller). The growth rate results are summarised in Table 45.

Table 45: **Inhibition of growth rate for each of the measured parameters**

Geometric mean test concentration (mg a.s./L)	% inhibition of growth rate at study termination (14 days)		
	Based on total length	Based on wet weight	Based on dry weight
Control	--	--	--
0.008	-5.0	-2.6	0.1
0.026	11.4	6.3	0.7
0.086	37.7*	26.0	15.7
0.247	65.3*	67.0*	40.8**
0.813	92.0*	95.9*	60.4**
2.64	93.3*	98.7*	65.9**

-- = not applicable. Negative values indicate an increase compared to control. * Statistically different compared to control (Welch t-test with Bonferroni-Holm adjustment, α = 0.05, one-sided smaller), ** Statistically different compared to control (Dunnet's multiple t-test, α = 0.05, one-sided smaller).

The E_rC_{50} values were calculated to be 0.137 mg a.s./L based on total length, 0.159 mg a.s./L based on wet weight and 0.621 mg a.s./L based on dry weight. The E_rC_{10} values are 0.023, 0.044 and 0.035 mg a.s./L based on total length, wet weight and dry weight respectively. The overall NOE_rC value is 0.026 mg a.s./L. The study was considered suitable for use in hazard assessment according to the GB CLP Regulation.

Cinmethylin water-sediment Myriophyllum spicatum toxicity test under static conditions (Rzodeczko H., 2017c and addendum (Kubitza, 2019c)).

The toxicity of cinmethylin to *M. spicatum* was assessed in a study performed to the guideline OECD 239 (2014) and according to GLP. Exposure to the test item was for 14 days under static conditions, at nominal concentrations of 0 (control), 0.0179, 0.0572, 0.183, 0.586, 1.88 and 6.0 mg a.s./L. The test was conducted in a water-sediment test system. The test item was not maintained within ± 20 % of the nominal. Furthermore, analytical samples were taken at all test concentrations at study initiation but not at the end of the study. The analytical data is shown in Tables 46 and 47 below for water and sediment concentrations respectively.

Table 46: Measured concentrations during study (in aqueous phase)

Nominal concentration (mg a.s./L)	Day 0		Day 7		Day 14	
	MC (mg a.s./L)	% of nominal	MC (mg a.s./L)	% of nominal	MC (mg a.s./L)	% of nominal
0	< LoD	n.a.	< LoD	n.a.	< LoD	n.a.
0.0179	0.0166	92.7	0.0096	53.6	0.0081	45.3
0.0572	0.0566	99.0	--	--	--	--
0.183	0.195	106.6	--	--	--	--
0.586	0.570	97.3	--	--	--	--
1.88	1.84	97.9	1.14	60.6	0.797	42.4

Nominal concentration (mg a.s./L)	Day 0		Day 7		Day 14	
	MC (mg a.s./L)	% of nominal	MC (mg a.s./L)	% of nominal	MC (mg a.s./L)	% of nominal
6	5.13	85.5	3.67	61.2	3.08	51.3

MC = measured concentration, n.a. = not applicable, LoD = 0.001 mg a.s./L, -- = not tested, NP = Not possible to determine.

Table 47: Measured concentrations during study (in sediment)

Nominal concentration (mg a.s./kg)	Day 7	Day 14
	MC (mg a.s./kg)	MC (mg a.s./kg)
0	< LoD	< LoD
0.0179	< LoD	< LoD
0.0572	--	--
0.183	--	--
0.586	--	--
1.88	0.44	0.38
6	1.62	0.95

MC = measured concentration, n.a. = not applicable, LoD = 0.02 mg a.s./kg, -- = not tested

Given not all test concentrations were analysed at study termination the applicant extrapolated values where appropriate using the following method:

Single First Order (SFO) kinetics (equation 1):

$c(t) = c(0) \times e^{kt}$

c(t): concentration at time t

c(0): initial concentration

k: rate constant

To simplify further analysis the SFO is linearized (equation 2):

$\tilde{c}(t) = \tilde{c}(0) + k \times t$

$\tilde{c}(t)$: natural logarithm of concentration at time t

$\tilde{c}(0)$: natural logarithm of initial concentration

k: rate constant

For each of the concentration levels at which the concentration was determined at all three-time steps, the linearized SFO was calibrated (using the “lm” function in R 3.5.2). From those calibration results the estimated rate constant (i.e. slope in a linear model) that indicates the strongest decline (equivalent to shortest DT_{50}) was selected as a ‘worst-case’ representative.

This worst-case rate constant was used to predict the concentrations of the concentration levels where only the initial concentration had been determined. For this the ‘worst-case’ rate constant was used in

Equation 1 as k, the initial concentration as $\tilde{c}(0)$, and 7 and 14 days as t to yield the predicted concentrations after 7 and 14 days.

Finally, the geometric mean is calculated from the determined and extrapolated (which is based on the 'worst-case' slope) concentrations. The concentrations calculated are shown in Table 48:

Table 22: Analytical measurements and extrapolated values calculated by applicant

Nominal concentration (mg a.s./L)	Day			Geometric mean (mg a.s./L)
	0	7	14	
0.0179	0.0166	0.0096	0.0081	**0.0109**
0.0572	0.0566	0.0373*	0.0245*	**0.0373***
0.183	0.195	0.1283*	0.0845*	**0.1283***
0.586	0.570	0.3751*	0.2469*	**0.3751***
1.88	1.84	1.14	0.797	**1.1868**
6	5.13	3.67	3.08	**3.8706**

* Extrapolated value based on method described above.

The study met the relevant validity criteria according to the guideline (OECD, 239).

The following parameters were measured to investigate the effects of the test item, total shoot length, fresh and dry weight. At exposure termination, no visible morphological changes were observed in the control plants and in test item concentrations 0.0179, 0.0572 and 0.183 mg a.s./L (nominal). The inhibition of growth rate for total shoot length ranged from 2.1 to 57.2 %, for fresh weight 1.3 to 68.3 % and for dry weight -4.2 to 75.0 %, compared to control. Statistically significant changes compared to control were observed for each endpoint parameter in the five highest test item concentrations (Williams Multiple Sequential t-test and Multiple Sequentially-rejective U-test after Bonferroni-Holm, one-sided smaller, α = 0.05) except for growth rate based on dry weight in which statistically significant differences compared to control were observed at the 3 highest test item concentrations (Williams Multiple Sequential t-test, p = 0.05). The growth rate results are summarised in Table 49.

Table 49: Inhibition of growth rate for each of the measured parameters

Geometric mean test concentration (mg a.s./L)	% inhibition of growth rate at study termination (14 days)		
	Based on total shoot length	Based on fresh weight	Based on dry weight
Control	--	--	--
0.0109	2.1	1.3	-4.2
0.0373[#]	8.7*	12.8*	-4.1
0.1283[#]	27.4*	35.6*	0.8
0.3751[#]	38.1*	50.7*	30.5*
1.1868	49.6*	64.6*	65.1*
3.8706	57.2*	68.3*	75.0*

-- = not applicable. Negative values indicate an increase compared to control. * Statistically different compared to control (Williams Multiple Sequential t-test, α = 0.05, one-sided smaller), # = extrapolated values as only initial concentration was analytically determined.

The E_rC_{50} values were calculated to be 1.219 mg a.s./L based on total shoot length, 0.414 mg a.s./L based on fresh weight and 0.810 mg a.s./L based on dry weight. The E_rC_{10} values are 0.010, 0.019 and 0.111 mg a.s./L based on total shoot length, fresh weight and dry weight respectively. The overall NOE_rC value is 0.0109 mg a.s./L. The study was considered suitable as supporting information for use in hazard assessment according to the GB CLP Regulation. All aquatic plant studies and available endpoints have been discussed further in Section 11.6.3.

Cinmethylin water-sediment Elodea canadensis toxicity test under static conditions (Rzodeczko H., 2018a).

The toxicity of cinmethylin to *E. canadensis* was assessed in a study performed to the guideline OECD 239 (2014) and according to GLP. Exposure to the test item was for 14 days under static conditions, at nominal concentrations of 0 (control), 0.0179, 0.0572, 0.183, 0.586, 1.88 and 6.0 mg a.s./L. The test was conducted in a water-sediment test system. The test item was not maintained within ± 20 % of the nominal. Furthermore, analytical samples were taken at all test concentrations at study initiation but not at the end of the study. The analytical data is shown in Tables 50 and 51 for water and sediment concentrations respectively.

Table 50: Measured concentrations during study (in aqueous phase)

Nominal concentration (mg a.s./L)	Day 0		Day 7		Day 14	
	MC (mg a.s./L)	% of nominal	MC (mg a.s./L)	% of nominal	MC (mg a.s./L)	% of nominal
0	< LoD	n.a.	< LoD	n.a.	< LoD	n.a.
0.0179	0.0203	113.4	--	--	--	--
0.0572	0.061	106.6	--	--	--	--
0.183	0.191	104.4	--	--	--	--
0.586	0.595	101.5	0.430	73.4	0.295	50.3
1.88	2.07	110.1	1.36	72.3	1.04	55.3
6	6.42	107.0	4.34	72.3	3.12	52.0

MC = measured concentration, n.a. = not applicable, LoD = 0.001 mg a.s./L, -- = not tested, NP = Not possible to determine

Table 51: Measured concentrations during study (in sediment)

Nominal concentration (mg a.s./kg)	Day 7	Day 14
	MC* (mg a.s./kg)	MC* (mg a.s./kg)
0	< LoD	< LoD
0.0179	--	--
0.0572	--	--

Nominal concentration (mg a.s./kg)	Day 7	Day 14
	MC* (mg a.s./kg)	MC* (mg a.s./kg)
0.183	--	--
0.586	0.230	0.286
1.88	1.065	1.180
6	2.720	3.090

MC = measured concentration, n.a. = not applicable, LoD = 0.02 mg a.s./kg, -- = not tested, * based on average of measurements taken by two different columns used for analysis.

Given not all test concentrations were analysed at study termination the applicant extrapolated values where appropriate using the following method:

Single First Order (SFO) kinetics (equation 1):

$c(t)= c(0) \times e^{k t}$

c(t): concentration at time t

c(0): initial concentration

k: rate constant

To simplify further analysis the SFO is linearized (equation 2):

$\tilde{c}(t) = \tilde{c}(0) + k \times t$

$\tilde{c}(t)$: natural logarithm of concentration at time t

$\tilde{c}(0)$: natural logarithm of initial concentration

k: rate constant

For each of the concentration levels at which the concentration was determined at all three-time steps, the linearized SFO was calibrated (using the "lm" function in R 3.5.2). From those calibration results the estimated rate constant (i.e. slope in a linear model) that indicates the strongest decline (equivalent to shortest DT_{50}) was selected as a 'worst-case' representative.

This worst-case rate constant was used to predict the concentrations of the concentration levels where only the initial concentration had been determined. For this the 'worst-case' rate constant was used in Equation 1 as k, the initial concentration as $\tilde{c}(0)$, and 7 and 14 days as t to yield the predicted concentrations after 7 and 14 days.

Finally, the geometric mean is calculated from the determined and extrapolated (which is based on the 'worst-case' slope) concentrations. The concentrations calculated are shown in Table 52.

The study met the relevant validity criteria according to the guideline (OECD, 239).

The following parameters were measured to investigate the effects of the test item, total shoot length, fresh and dry weight. At exposure termination, control plants were healthy, with green leaves and stems, without discolorations and good development of roots. In test item concentration 0.0179 mg a.s./L (nominal) no changes of the plant part above sediment and moderately developed roots for all plants were

Table 5223: Analytical measurements and extrapolated values calculated by applicant

Nominal concentration (mg a.s./L)	Day			Geometric mean (mg a.s./L)
	0	7	14	
0.0179	0.0203	0.0142*	0.0099*	0.0142*
0.0572	0.061	0.0425*	0.0296*	0.0425*
0.183	0.191	0.1332*	0.0928*	0.1332*
0.586	0.595	0.43	0.295	0.4226
1.88	2.07	1.36	1.04	1.4306
6	6.42	4.34	3.12	4.4299

* Extrapolated value based on method described above.

observed in comparison with plants in the control. In the tested range of test item concentrations, the inhibition of growth rate for total shoot length ranged from 0.1 to 81.2 %, for fresh weight from -5.1 to 97.1 % and for dry weight from 0.3 to 68.1 % in comparison with plants in the control. Statistically significant changes compared to control were observed for each endpoint parameter in the five highest test item concentrations (Williams Multiple Sequential t-test, one-sided smaller, α = 0.05) for growth rate based on total shoot length. For growth rate based on fresh weight and dry weight, the statistically significant differences compared to control were observed at the 4 highest test item concentrations (Williams Multiple Sequential t-test, one-sided smaller, α = 0.05). The growth rate results are summarised in Table 53.

Table 53: Inhibition of growth rate for each of the measured parameters

Geometric mean test concentration (mg a.s./L)	% inhibition of growth rate at study termination (14 days)		
	Based on total shoot length	Based on fresh weight	Based on dry weight
Control	--	--	--
0.0142#	0.1	-5.1	0.3
0.0425#	8.3*	7.2	4.9
0.1332#	14.1*	20.2*	18.6*
0.4226	43.5*	76.6*	26.6*
1.4306	59.1*	90.6*	49.0*
4.4299	81.2*	97.1*	68.1*

-- = not applicable. Negative values indicate an increase compared to control. * Statistically different compared to control (Williams Multiple Sequential t-test, α = 0.05, one-sided smaller), # = extrapolated values as only initial concentration was analytically determined.

The E_rC_{50} values were calculated to be 0.764 mg a.s./L based on total shoot length, 0.247 mg a.s./L based on fresh weight and 1.481 mg a.s./L based on dry weight. The E_rC_{10} values are 0.058, 0.076 and 0.067 mg a.s./L based on total shoot length, fresh weight and dry weight respectively. The overall NOE_rC value is 0.0142 mg a.s./L. The study was considered suitable as supporting information for use in hazard assessment according to the GB CLP Regulation. It should be noted all aquatic plant studies and available endpoints have been discussed further in section 11.6.3.

Cinmethylin- Water-sediment Egeria densa toxicity test under static conditions (Rzodeczko H., 2017d).

The toxicity of cinmethylin to *E. densa* was assessed in a study performed to the guideline OECD 239 (2014) and according to GLP. Exposure to the test item was for 14 days under static conditions, at nominal concentrations of 0 (control), 0.0179, 0.0572, 0.183, 0.586, 1.88 and 6.0 mg a.s./L. The test was conducted in a water-sediment test system. The test item was not maintained within ± 20 % of the nominal. Furthermore, analytical samples were taken at all test concentrations at study initiation but not at the end of the study. The analytical data is shown in Tables 54 and 55 below for water and sediment concentrations respectively.

Table 54: Measured concentrations during study (in aqueous phase)

Nominal concentration (mg a.s./L)	**Day 0**		**Day 7**		**Day 14**	
	MC (mg a.s./L)	**% of nominal**	**MC (mg a.s./L)**	**% of nominal**	**MC (mg a.s./L)**	**% of nominal**
0	< LoD	n.a.	< LoD	n.a.	< LoD	n.a.
0.0179	0.0172	96.1	--	--	--	--
0.0572	0.0573	100.2	--	--	--	--
0.183	0.178	97.3	--	--	--	--
0.586	0.557	95.1	0.347	59.2	0.234	39.9
1.88	1.72	91.5	1.17	62.2	0.928	49.4
6	5.58	93.0	3.66	61.0	3.20	53.3

MC = measured concentration, n.a. = not applicable, LoD = 0.001 mg a.s./L, -- = not tested, NP = Not possible to determine.

Table 55: Measured concentrations during study (in sediment)

Nominal concentration (mg a.s./kg)	**Day 7**	**Day 14**
	MC* (mg a.s./kg)	**MC* (mg a.s./kg)**
0	< LoD	< LoD
0.0179	--	--
0.0572	--	--
0.183	--	--
0.586	0.036	0.052
1.88	0.119	0.495
6	0.700	1.590

MC = measured concentration, n.a. = not applicable, LoD = 0.02 mg a.s./kg, -- = not tested, * based on average of measurements taken by two different columns used for analysis.

Given not all test concentrations were analysed at study termination the applicant extrapolated values where appropriate using the following method:

Single First Order (SFO) kinetics (equation 1):

$c(t) = c(0) \times e^{kt}$

c(t): concentration at time t

c(0): initial concentration

k: rate constant

To simplify further analysis the SFO is linearized (equation 2):

$\tilde{c}(t) = \tilde{c}(0) + k \times t$

$\tilde{c}(t)$: natural logarithm of concentration at time t

$\tilde{c}(0)$: natural logarithm of initial concentration

k: rate constant

For each of the concentration levels at which the concentration was determined at all three-time steps, the linearized SFO was calibrated (using the "lm" function in R 3.5.2). From those calibration results the estimated rate constant (i.e. slope in a linear model) that indicates the strongest decline (equivalent to shortest DT_{50}) was selected as a 'worst-case' representative.

This worst-case rate constant was used to predict the concentrations of the concentration levels where only the initial concentration had been determined. For this the 'worst-case' rate constant was used in Equation 1 as k, the initial concentration as $\tilde{c}(0)$, and 7 and 14 days as t to yield the predicted concentrations after 7 and 14 days.

Finally, the geometric mean is calculated from the determined and extrapolated (which is based on the 'worst-case' slope) concentrations. The concentrations calculated are shown in Table 56:

Table 56: Analytical measurements and extrapolated values calculated by applicant

Nominal concentration (mg a.s./L)	**Day**			**Geometric mean (mg a.s./L)**
	0	**7**	**14**	
0.0179	0.0172	0.0111*	0.0072*	0.0111*
0.0572	0.0573	0.0371*	0.0241*	0.0371*
0.183	0.178	0.1154*	0.0748*	0.1154*
0.586	0.557	0.347	0.234	0.3563
1.88	1.72	1.17	0.928	1.2315
6	5.58	3.66	3.2	4.0280

* Extrapolated value based on method described above.

The study met the relevant validity criteria according to the guideline (OECD, 239).

The following parameters were measured to investigate the effects of the test item, total shoot length, fresh and dry weight. The inhibition of growth rate for total shoot length ranged from 2.0 to 90.1 %, for fresh weight from -3.8 to 110.1 %, for dry weight from 1.4 to 74.5 % in comparison with plants in the control. Statistically significant changes compared to control were observed for each endpoint parameter in the four highest test item concentrations (Williams Multiple Sequential t-test, Multiple Sequentially-rejective U-test after Bonferroni-Holm and Multiple Sequentially Welch t-test after Bonferroni-Holm; p = 0.05, one-sided smaller) except for growth rate based on total shoot length in which statistically significant

differences compared to control were observed in the five highest test item concentrations (Williams Multiple Sequential t-test; p = 0.05, one-sided smaller). The growth rate results are summarised in Table 57.

Table 57: Inhibition of growth rate for each of the measured parameters

Geometric mean test concentration (mg a.s./L)	% inhibition of growth rate at study termination (14 days)		
	Based on total shoot length	Based on fresh weight	Based on dry weight
Control	--	--	--
0.0111#	2.0	-3.8	1.4
0.0371#	6.3*	-2.3	4.5
0.1154#	14.6*	58.2*	18.1*
0.36	33.2*	75.4*	41.4*
1.23	70.5*	80.9*	64.1*
4.03	90.1*	110.1*	74.5*

-- = not applicable. Negative values indicate an increase compared to control. * Statistically different compared to control (Williams Multiple Sequential t-test, α = 0.05, one-sided smaller), # = extrapolated values as only initial concentration was analytically determined.

The E_rC_{50} values were calculated to be 0.624 mg a.s./L based on total shoot length, 0.116 mg a.s./L based on fresh weight and 0.659 mg a.s./L based on dry weight. The E_rC_{10} values are 0.098, 0.037 and 0.040 mg a.s./L based on total shoot length, fresh weight and dry weight respectively. The overall NOE_rC value is 0.0111 mg a.s./L. The study was considered suitable as supporting information for use in hazard assessment according to the GB CLP Regulation. It should be noted all aquatic plant studies and available endpoints have been discussed further in section 11.6.3.

Overall conclusion

There are sufficient suitable studies to allow classification of the acute hazard to algae and other aquatic plants from exposure to cinmethylin. **The endpoint selected for use in hazard classification is the 7-day E_rC_{50} of 0.0888 mg a.s./L (Vlechev, 2017a)**, this is based on the most sensitive taxon (*L. gibba*) considered in the available valid studies. Aquatic plants are the group of organisms most acutely sensitive to exposure to cinmethylin based on the available information and will be used in the acute hazard classification.

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

No acute toxicity studies on other organisms were available for use in the hazard assessment.

11.6 Long-term aquatic hazard

Studies available during the evaluation of cinmethylin as an active substance, circa 2020, are summarised in Table 58. All the listed studies have been conducted according to GLP. The studies have been evaluated, considered reliable for quantitative use or as supporting information (see footer of table) and deemed suitable for hazard classification purposes. Only studies submitted testing the technical substance, cinmethylin, have been summarised below. Studies not deemed suitable for use in hazard classification have not been included or discussed further.

Table 58: Summary of relevant information on chronic aquatic toxicity

Method	Species	Test material	Results[1]	Reference
Fish				
Fish early-life stage toxicity **OECD 210** **GLP** **35-days, flow through**	***Pimephales promelas***	**Batch** **COD-002038** **Cinmethylin** **(purity 93.5 %)**	***NOEC (survival) 0.59 mg /L (m.m.)** ***EC_{10} (body weight) 0.92 mg /L (m.m.)** ***EC_{10} (body length) 1.47 mg /L (m.m)**	**Anon (2017g)**
Aquatic invertebrates				
Reproduction study **OECD 211** **GLP** **21-days, semi-static**	***Daphnia magna***	**Batch** **COD-002038** **Cinmethylin** **(purity 93.5 %)**	**NOEC 0.29 mg /L (g.m.)**** **EC_{10} > 0.29 mg /L (g.m.)****	**Rzodeczko (2017b)**
Algae				
Freshwater algal growth inhibition OECD 201 GLP 72-hours, static	*Pseudokirchneriella subcapitata*	Batch COD-002038 Cinmethylin (purity 93.5 %)	NOEC (g.r.) 0.651 mg /L (g.m) E_rC_{10} >1.765 mg /L (g.m.)[#]	Kauf (2017a)
Freshwater algal growth inhibition OECD 201, EPA 850.4500, EPA 850.4550 GLP 96-hours, static exposure	*Anabaena flos-aquae*	Batch COD-002038 Cinmethylin (purity 93.5 %)	NOEC (g.r.) 8.22 mg /L (g.m.) E_rC_{10} 24.55 mg /L (g.m.)	Kauf (2017b)

Other aquatic plants				
Growth inhibition test OECD 221 GLP 7-days, static, water only	*Lemna gibba*	Batch COD-002038 Cinmethylin (purity 93.5 %)	NOEC (g.r.) 0.006 mg /L (g.m.) E_rC_{10} 0.0285 mg /L (g.m.)	Vlechev (2017a)
Water-sediment toxicity test **OECD 239** **GLP** **14-days, static renewal of water**	***Glyceria maxima***	**Batch** **COD-002038** **Cinmethylin** **(purity 93.5 %)**	NOEC (g.r.) 0.026 mg /L (g.m.) **E_rC_{10} 0.023 mg /L (g.m.)**	**Vlechev (2017b)**
Water-sediment toxicity test OECD 239 GLP 14-days, static, water/sediment	*Myriophyllum spicatum*	Batch COD-002038 Cinmethylin (purity 93.5 %)	NOEC (g.r.) 0.0109 mg /L (g.m.)[##] E_rC_{10} 0.010 mg /L (g.m.)[##]	Rzodeczko (2017c) and addendum (Kubitza, 2019a)
Water-sediment toxicity test OECD 239 GLP 14-days, static, water/sediment	*Elodea canadensis*	Batch COD-002038 Cinmethylin (purity 93.5 %)	NOEC (g.r.) 0.0142 mg /L (g.m.)[##] E_rC_{10} 0.058 mg /L (g.m.)[##]	Rzodeczko (2018a) and addendum (Kubitza, 2019b)
Water-sediment toxicity test OECD 239	*Egeria densa*	Batch COD-002038 Cinmethylin (purity 93.5 %)	NOEC (g.r.) 0.0111 mg /L (g.m.)[##] E_rC_{10} 0.037 mg /L (g.m.)[##]	Rzodeczko (2017d) and addendum (Kubitza, 2019c)

GLP 14-days, static, water/sediment				

Bold entries are the endpoints considered most suitable to be considered for the hazard classification for the active substance for each group of organisms.
1 m.m. = mean measured concentration; g.m.= geometric mean measured concentration; g.r. = growth rate
* It should be noted the EC10 value calculated only considered body length and body weight. The NOEC is based on survival.
** Based on time weighted average concentration. Due to lack of analytical measurements during study for three test concentrations only the lowest and highest concentrations could be calculated. Therefore, this endpoint is considered conservative.
# Uncertainty regarding statistically derived value hence conservative approach has been taken and a greater than value reported.
Applicant 'extrapolated' missing analytical data based on linearized single first order kinetics using measured values for other test concentrations. Hence calculated endpoints are not considered suitable for quantitative use by the GB evaluator. It should be noted that all available aquatic plant studies/endpoints have been discussed in detail in section 11.6.3.

11.6.1 Chronic toxicity to fish

A long-term fish toxicity study was provided and considered suitable for use in the risk assessment of cinmethylin. The study is presented below.

Cinmethylin: Early-Life-Stage toxicity test on the fathead minnow (Pimephales promelas) in a flow through system (Anon, 2017g).

The chronic toxicity of cinmethylin to juvenile P. *promelas* was assessed in a study performed to OECD 210 (2013) and in accordance with GLP. Exposure to the test item was under flow-through conditions, at nominal concentrations of 0 (control), 0.27, 0.60, 1.33, 2.93 and 6.45 mg a.s./L.

The test item was not maintained within ± 20 % of the nominal concentration during the study; therefore arithmetic mean measured treatment concentrations were established by the study author as 0.25, 0.59, 1.19, 3.01 and 6.61 mg a.s./L, the mean measured treatment concentrations were used to establish the relevant endpoints for the study. It was noted the values calculated by the study author has excluded some samples from analysis as duplicate samples were taken. In order to investigate the difference the UK evaluator included all samples available and found the mean measured treatment concentrations were either identical or comparable to those calculated by the study author at; 0.25, 0.61, 1.19, 2.97 and 6.62 mg a.s./L. The validity criteria of OECD 210 (2013) were met.

Hatching started simultaneously in all test groups on day 2 and was complete by day 6. Hatching success ranged from 80 – 84 % for the replicates of the control group. Hatching success was statistically significantly decreased in comparison to the control group in the highest treatment group 5 (6.61 mg a.s./L mean measured and 6.00 mg a.s./L nominal). From the end of hatch to the end of exposure (day 6 – 35) survival was 95 – 100 % for the replicates of the control group. The survival from the end of hatch to the end of exposure (day 6 – 35) was statistically significantly decreased in the treatment groups ≥ 1.19 mg a.s./L (mean measured concentration) and ≥ 1.24 mg a.s./L (nominal concentration) in comparison to the control group.

A reduced growth was observed in the treatment group 4 (3.01 mg a.s./L mean measured and 2.73 mg a.s./L nominal) starting on day 28 to the end of exposure. There were no other observed morphological abnormalities or signs of toxicity.

In comparison to the control group the mean wet weights of the surviving fish at the end of the exposure period were statistically significantly decreased in the test groups ≥3 (1.19 mg a.s./L mean measured and 1.24 mg a.s./L nominal). The total body lengths of the surviving fish at the end of the exposure period were statistically significantly decreased in comparison to the control group in the test groups ≥2 (0.59 mg a.s./L mean measured and 0.56 mg a.s./L nominal). Overall weight was affected to a greater degree than length

as a percent of the control value. Consequently, the EC_{10} value for growth as weight is considered the most relevant effect metric for this endpoint. The results are summarised in Table 59.

Table 24: Chronic toxicity of cinmethylin to fathead minnow (*Pimephales promelas*) in a fish early life stage test (35 d)

Concentration corrected for purity (nominal) [mg a.s./L]	**Control**	**0.25**	**0.56**	**1.24**	**2.73**	**6.00**
Concentration corrected for purity (mean measured) [mg a.s./L]	**<LoQ**	**0.25**	**0.59**	**1.19**	**3.01**	**6.61**
Average % Hatch (range)	83 (80 – 84)	86 (84 – 88)	83 (76 – 88)	94 (84 – 100)	87 (80 – 100)	**77* [3]** **(76 – 80)**
Survival of young fish (day 6 - 35) [%]	99 (95 – 100)	98 (95 – 100)	97 (91 – 100)	**86** [2]** **(80 – 91)**	**91** [2]** **(85 – 100)**	**0** [2]** **(0 – 0)**
Survival from day 0 to test termination (35 d) [%] (range)	82 (80 – 84)	84 (83 – 84)	80 (76 – 84)	81 (76 – 84)	79 (68 – 88)	**0** [2]** **(0 – 0)**
Symptoms	none	none	none	none	3G	--
Mean weight (35 d) [mg] (SD)	173 (± 41.9)	177 (± 40.7)	168 (± 40.2)	**145** [1]** (± 36.2)	**80** [1]** (± 23.5)	--
Mean length (35 d) [cm] (SD)	2.7 (± 0.22)	2.7 (± 0.20)	**2.6* [1]** (± 0.20)	**2.5** [1]** (± 0.25)	**2.0** [1]** (± 0.20)	--
Parameters	**Endpoints [mg a.s./L]**					
	Mean measured					
$EC_{20\ body\ weight}$ (35 d)	1.34 (95 % CI 1.21 – 1.50)					
$EC_{20\ body\ length}$ (35 d)	2.57 (95 % CI 2.47 – 2.68)					
$EC_{10\ body\ weight}$ (35 d)	**0.92 (95 % CI 0.78 – 1.08)**					
$EC_{10\ body\ length}$ (35 d)	1.47 (95 % CI 1.37 – 1.56)					
$NOEC_{mortality}$ (35 d) proposed endpoint [4]	3.01					
Overall NOEC (35d) based on all parameters	**0.59**					

Values printed in **bold** show statistically significant differences compared to the control; LoQ: limit of quantification (LoQ < 2.5 ng a.s./ml); Symptoms: G: reduced growth; --: all fish dead, CI = confidence limits, SD = Standard Deviation- calculated by UK evaluator for all replicates, study report calculated values for replicates

[1] Statistically significant differences compared to the control (one-sided William´s test, * $p \leq 0.05$; ** $p \leq 0.01$).

[2] Statistically significant differences compared to the control (one-sided Jonkheere-Terpstra test, * $p \leq 0.05$; ** $p \leq 0.01$).

[3] Statistically significant differences compared to the control (one-sided Wilcoxon test, * $p \leq 0.05$; ** $p \leq 0.01$).

[4] NOEC value was based on the higher endpoint mortality by study author since juvenile survival does not follow a dose-response relationship.

In terms of survival for 'young juvenile fish' (day 6 to 35) statistically significant effects were observed at the three highest test concentrations (from 1.19 mg a.s./L upwards) reaching 100 % mortality at the highest test concentration of 6.61 mg a.s./L. However, the changes in survival were variable at 1.19 and 3.01 mg a.s./L and only at the highest test concentration was there a clear decrease. Nonetheless, there were statistically significant effects compared to control at 1.19 mg a.s./L with survival ranging from 80 to 91 % (maximum mortality of 20 % or 19 % corrected for control). Therefore, the next lowest test concentration of 0.59 mg a.s./L is considered an appropriate survival NOEC by the Agency.

Reduced growth was observed at a concentration of 3.01 mg a.s./L. Statistically significant effects on weight and length were noted from concentrations of 1.19 and 0.59 mg a.s./L respectively. The Agency considered an overall NOEC of 0.59 mg a.s./L appropriate despite the statistically significant effects on length recorded at 0.59 mg a.s./L. This is because the change was relatively low; means of 2.6 (± 0.22) and 2.7 (± 0.22) cm in the treatment and control respectively. This decision is further supported by the EC_{10} based on body length of 1.47 mg a.s./L. It was noted that at the higher test concentration of 3.01 mg a.s./L there was a relatively large decrease in length.

The overall NOEC for the study was concluded to be 0.59 mg a.s./L by the Agency and EC_{10} was 0.92 mg a.s./L. The study was considered suitable for use in hazard assessment according to the GB CLP Regulation.

Overall conclusion

There is sufficient toxicity data to allow classification of the chronic/long-term hazard to fish from exposure to cinmethylin and overall the key fish endpoint to use for hazard classification purposes is considered to be the **35-day NOEC of 0.59 mg a.s./L** for *Pimephales promelas*, from Anon (2017g). It is noted that ultimately fish are not the most sensitive taxa when considering chronic toxicity and therefore not critical for setting the hazard classification.

11.6.2 Chronic toxicity to aquatic invertebrates

A long-term invertebrate toxicity study was provided and considered suitable for use in the risk assessment of cinmethylin. The study is presented below.

Cinmethylin – Daphnia magna reproduction test under semi-static conditions (Rzodeczko H., 2017b).

The chronic toxicity of cinmethylin to D. *magna* (≤ 24 hours old) was assessed in a study performed to the OECD 211 (2012) guideline and in accordance with GLP. Exposure to the test item was for 21 days under semi-static renewal conditions (renewal three times a week), at nominal concentrations of 0 (control), 0.31, 0.63, 1.25, 2.5 and 5.0 mg a.s./L.

Analytical measurements of 'fresh' and 'aged' solutions were not determined for all test concentrations. Specifically for the three middle concentrations (0.63, 1.25 and 2.5 mg a.s./L) 'fresh' and 'aged' samples were taken on the following days; 0, 7, 14 and 2, 9, 16, 21 respectively. In this semi-static study test solutions were renewed on days 2, 4, 7, 9, 11, 14, 16 and 18. This means that exposure was confirmed ('fresh' and 'aged') on days 0 – 2, 7 – 9 and 14 – 16 but not known for the rest of the 21 day duration at these test concentrations. Essentially analytical confirmation was not conducted for 6 of 9 renewals. Therefore, the Agency does not consider it appropriate to determine endpoints based on the three middle test concentrations (nominal 0.63, 1.25 and 2.5 mg a.s./L). Instead, where possible time-weighted mean measured concentrations have been used i.e. for the lowest and highest test concentrations (TWA of 0.29 and 4.797 mg a.s./L respectively) where analytical samples of 'fresh' and 'aged' solutions were taken throughout the study during all renewals.

After 21 days of exposure, no parent mortality occurred in the control groups and at the test item concentrations of up to and including the highest concentration tested. Statistically significant differences in the number of offspring per parent were observed at the two highest test item concentrations (Multiple Sequentially-rejected Welsh test after Bonferroni-Holm; α = 0.05, one-sided smaller). The intrinsic rate of increase and day of first brood were significantly affected at the three highest test item concentrations (Williams Multiple Sequential t-test; α = 0.05, one-sided smaller). Body length of the parent animals was significantly affected at the three highest test item concentration (Williams Multiple Sequential t-test; α = 0.05, one-sided smaller). The results are summarised in Table 60.

Table 60: Effects of cinmethylin on Daphnia magna parent mortality, reproduction and growth after 21 days of exposure

Concentration [mg test item/L] (nominal test item)	Control	0.31	0.63	1.25	2.5	5
Concentration [mg a.s./L] (nominal content of a.s.) [2)]	--	0.288	0.586	1.163	2.325	4.65
Time weighted mean measured concentration [mg a.s./L]	**Control**	**0.29**	-	-	-	**4.797**
Parent Mortality [%]	0	0	0	0	0	0
Cumulative offspring / parent	212.6	213.3	209.7	199.1	185.2*	34.4*
% reproductive effects to control based on cumulative offspring/parent	n.a.	-0.3	+1.4	+6.3	+12.9	+83.8
Body dry weight / parent [mg]	0.72	0.71	0.69	0.66	0.64	0.61
Mean dry weight of parent as compared with control [%]	n.a.	+1.4	+4.2	+8.3	+11.1	+15.3
Body length [mm]	3.82	3.78	3.71	3.58**	3.53**	3.17**
Mean body length of parent as compared with control [%]	n.a.	+1.0	+2.9	+6.3	+7.6	+17.0
Mean Intrinsic rate of increase (population growth rate)	0.412	0.405	0.400	0.392**	0.386**	0.220**
Mean intrinsic rate as compared with control [%]	n.a.	+1.7	+2.9	+4.9	+6.3	+46.6

* Statistically significant effects compared to the control (Multiple Sequentially-rejected Welsh test after Bonferroni-Holm; α = 0.05, one-sided smaller).
** Statistically significant effects compared to the control (Williams Multiple Sequential t-test; α = 0.05, one-sided smaller).
n.a. = not applicable, negative value indicates increase compared to control and positive value is decrease.

The parameters measured in the study were parental mortality, % reproductive effects, parent weight/length and population growth rate.

Given only the lowest and highest concentrations were analytically determined throughout the study, the overall NOEC was set at 0.29 mg a.s./L (geometric mean measured concentration) i.e. the highest concentration sufficiently verified by chemical analysis where no effects were observed. Given only two concentrations (lowest and highest) were analytically verified throughout the study it was not possible to derive robust EC_{10} values. Hence the EC_{10} has been stated as > 0.29 mg a.s./L, given effects occurred at the highest test concentration (reduced offspring numbers, body length and population growth rate).

The study was considered suitable for use in hazard assessment according to the GB CLP Regulation.

Overall conclusion

There are sufficient suitable studies to allow classification of the chronic/long-term hazard to aquatic invertebrates from exposure to cinmethylin and overall the key invertebrate endpoint to use for hazard classification purposes is considered to be the 21-day NOEC/EC_{10} for Daphnia magna of 0.29 mg a.s./L from Rzodeczko (2017b).

11.6.3 Chronic toxicity to algae or other aquatic plants

The available studies related to algae and aquatic plants have been summarised in Section 11.5.3.

Overall conclusion

Aquatic plants are the most sensitive group based on the available data and are driving the chronic classification. Several endpoints are available however, only two studies were fully valid for quantitative use (Vlechev 2017a & 2017b). Whilst three other studies were available as supporting information. The supporting information studies (Rzodeczko, 2017c, 2018a, 2017d & addendums Kubitza 2019 a,b and c) were conducted to GLP and considered valid but not all test concentrations were analytically determined meaning extrapolated analytical values were used in the statistical analysis based on the decline observed in those where concentrations were measured at the start and end of the study. It should be noted they were static studies and all test concentrations were analytically determined at study initiation but three of the five were measured at the end of the study (see discussion at the end of this section for further details regarding the reliability and overall choice of endpoints). The results have been summarised in Table 61 for all studies and where there is uncertainty the rows have been shaded.

Table 61: Summary of growth inhibition effects for all available cinmethylin aquatic plant toxicity data.

Conc mg a.s./L	**Growth rate inhibition compared to control for aquatic plant species tested (both valid and supporting information studies)**													
	[1)]L. gibba **(F.N.)**	***[1)]L. gibba*** **(d.w.)**	***[2)]G. maxima*** **(t.l.)**	***[2)]G. maxima*** **(w.w.)**	***[2)]G. maxima*** **(d.w.)**	***[3)]M. spicatum*** **(t.s.l.)**	***[3)]M. spicatum*** **(f.w.)**	***[3)]M. spicatum*** **(d.w.)**	***[4)]E. canad-ensis*** **(t.s.l.)**	***[4)]E. canad-ensis*** **(t.s.l.)**	***[4)]E. canad-ensis*** **(t.s.l.)**	***[5)]E. densa*** **(t.s.l.)**	***[5)]E. densa*** **(f.w.)**	***[5)]E. densa*** **(d.w.)**
0.0009	-0.88	-0.53	-	-	-	-	-	-	-	-	-	-	-	-
0.0023	0.39	-1.07	-	-	-	-	-	-	-	-	-	-	-	-
0.006	0.46	0.95	-	-	-	-	-	-	-	-	-	-	-	-
0.008	-	-	-5.0	-2.6	0.1									
0.0109	-	-	-	-	-	2.1	1.3	-4.2						
0.0111	-	-	-	-	-	-	-	-				2.0	-3.8	1.4

Conc mg a.s./ L	Growth rate inhibition compared to control for aquatic plant species tested (both valid and supporting information studies)													
	[1)] *L. gibba* (F.N.)	[1)] *L. gibba* (d.w.)	[2)] *G. maxima* (t.l.)	[2)] *G. maxima* (w.w.)	[2)] *G. maxima* (d.w.)	[3)] *M. spicatum* (t.s.l.)	[3)] *M. spicatum* (f.w.)	[3)] *M. spicatum* (d.w.)	[4)] *E. canad-ensis* (t.s.l.)	[4)] *E. canad-ensis* (t.s.l.)	[4)] *E. canad-ensis* (t.s.l.)	[5)] *E. densa* (t.s.l.)	[5)] *E. densa* (f.w.)	[5)] *E. densa* (d.w.)
0.0142	2.56	3.66	-	-	-	-	-	-	0.1	-5.1	0.3			
0.026			**11.4**	6.3	0.7	-	-	-						
0.0371						-	-	-				6.3	-2.3	4.5
0.0373						8.7	**12.8**	-4.1						
0.038	**15.1**	**12.46**				-	-	-						
0.0425						-	-	-	8.3	7.2	4.9			
0.086			**37.7**	**26.0**	**15.7**	-	-	-						
0.099	**57.12**	**26.01**				-	-	-						
0.1154						-	-	-				**14.6**	**58.2**	**18.1**
0.1283						**27.4**	**35.6**	0.8						
0.1332						-	-	-	**14.1**	**20.2**	**18.6**			
0.247			**65.3**	**67.0**	**40.8**	-	-	-						
0.258	**86.33**	**40.13**				-	-	-						
0.36						-	-	-				**33.2**	**75.4**	**41.4**
0.3751						**38.1**	**50.7**	**30.5**						
0.4226						-	-	-	**43.5**	**76.6**	**26.6**			
0.813			**92.0**	**95.9**	**60.4**	-	-	-						
1.1868						**49.6**	**64.6**	**65.1**						
1.23						-	-	-				**70.5**	**80.9**	**64.1**
1.4306						-	-	-	**59.1**	**90.6**	**49.0**			
2.64			**93.3**	**98.7**	**65.9**	-	-	-						

Conc mg a.s./ L	Growth rate inhibition compared to control for aquatic plant species tested (both valid and supporting information studies)												
	[1)] *L. gibba* (F.N.)	[1)] *L. gibba* (d.w.)	[2)] *G. maxima* (t.l.)	[2)] *G. maxima* (w.w.)	[2)] *G. maxima* (d.w.)	[3)] *M. spicatum* (t.s.l.)	[3)] *M. spicatum* (f.w.)	[3)] *M. spicatum* (d.w.)	[4)] *E. canad-ensis* (t.s.l.)	[4)] *E. canad-ensis* (t.s.l.)	[4)] *E. canad-ensis* (t.s.l.)	[5)] *E. densa* (t.s.l.)	[5)] *E. densa* (f.w.)	[5)] *E. densa* (d.w.)
3.8706	-	-	-	-	-	**57.2**	**68.3**	**75.0**						
4.03	-	-	-	-	-	-	-	-				**90.1**	**110.1**	**74.5**
4.4299	-	-	-	-	-	-	-	-	**81.2**	**97.1**	**68.1**			

Negative value indicates increase compared to control, bold values indicate > 10 % effects, - = not tested. Parameter displayed has highest effects at lowest concentrations tested. F.N. = frond number, d.w. = dry weight, t.l. = total length, t.s.l. = total shoot length and w.w. = wet weight, Conc = Concentration, [1)] = Vlechev (2017a) [2)] = Vlechev (2017b), [3)] = Rzodeczko (2017c), [4)] = Rzodeczko (2018a), [5)] = Rzodeczko (2017d)

There are sufficient suitable studies to allow classification of the chronic/long-term hazard to algae and other aquatic plants from exposure to cinmethylin. Based on the 'Guidance on the Application of the CLP Criteria' (ECHA, 2017) the E_rC_{10} value has been used preferentially over the NOE_rC to determine the chronic hazard classification.

The lowest calculated E_rC_{10} value from the study reports was 0.010 mg a.s. /L, derived in one of the studies considered suitable as supporting information (Rzodeczko, 2017c testing *M. spicatum*) where not all test concentrations were confirmed analytically throughout the study. This value is not supported by the experimental data for this species i.e. maximum of 2.1 % growth rate inhibition compared to control at 0.0109 mg a.s. /L for *M. spicatum* (see Table 60). Given the test concentration of 0.0109 mg a.s. /L was measured analytically at both the beginning and end of the study this raises further uncertainty around the E_rC_{10} of 0.010 mg a.s. /L for *M. spicatum*. Furthermore, the 95 % confidence limits are wide (over an order of magnitude) ranging from 0.003 to 0.021 mg a.s./L suggesting the endpoint is not robust. Therefore, the Agency does not consider the E_rC_{10} of 0.010 mg a.s. /L based on supporting information for *M. spicatum* suitable for use in hazard classification.

When considering the experimental data shown in Table 60, the lowest concentration where there are > 10 % effects is 0.026 mg a.s./L based on total length for *G. maxima*, which is slightly above the lowest E_rC_{10} value based on fully valid studies of 0.023 mg a.s. /L (g.m.) also for *G. maxima*. Therefore, the Agency proposes the use of this E_rC_{10} endpoint (lowest based on valid data) in the chronic hazard classification. This endpoint is also protective of the E_rC_{10} for the most sensitive aquatic plant species considering the acute data (E_rC_{10} of 0.0285 mg a.s. /L for *L. gibba*).

In conclusion based on the available data the **overall key algal/plant endpoint to use for hazard classification purposes is considered to be the 14-day E_rC_{10} of 0.023 µg a.s./L for *G. maxima* from Vlechev (2017b)**.

11.6.4 Chronic toxicity to other aquatic organisms

No chronic toxicity studies on other organisms were available for use in the hazard assessment.

11.7 Comparison with the GB CLP criteria

11.7.1 Acute aquatic hazard

Acute aquatic toxicity data regarding technical cinmethylin are available for fish, invertebrates, algae and other aquatic plants (i.e. there is appropriate data for all three trophic levels that need to be assessed for CLP classification). The lowest LC_{50}/EC_{50} value is the measured 7-day E_rC_{50} of 0.0888 mg a.s./L for the aquatic plant *Lemna gibba* (Vlechev, 2017a). This is > 0.01 mg/L but ≤ 0.1 mg/L, therefore cinmethylin meets the criteria for classification with Aquatic Acute Category 1 with an acute M-factor of 10.

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

Cinmethylin is considered to be 'not rapidly degradable' according to the CLP criteria, this decision has been explained in full in the environmental fate section (Section 11.2.1).

Bioaccumulation

The measured Log K_{ow} for cinmethylin is 4.5 which is greater than the CLP trigger of ≥ 4 indicating a potential for bioaccumulation. Two high quality studies are available to establish measured BCF estimates testing at nominal concentrations of 0.5 and 5 µg a.s./L (Anon, 2017c) and subsequently determining the BCF values for parent cinmethylin from those for total radioactive residues (Anon, 2018m). According to the guidance on the application of the CLP criteria (ECHA, 2017), measured estimates should be used in preference when available to conclude on the bioaccumulation potential of a substance (BCF ≥ 500 indicates bioaccumulation potential). Therefore, these data have been used to conclude on the potential for bioaccumulation of cinmethylin. The relevant whole fish 5 % lipid normalised BCF estimates for cinmethylin, based on exposure at the two nominal exposure concentrations, were 170 L kg-1 and 59 L kg^{-1} respectively. Given that there are fewer than four estimates a geometric mean value has not been established for CLP. Both BCF estimates are less than 500 and as such it is concluded that cinmethylin does not meet the CLP criterion for potential bioaccumulation according to CLP.

Chronic toxicity

Long-term aquatic toxicity data regarding technical cinmethylin are available for fish, invertebrates, algae and other aquatic plants (i.e. there is appropriate data for all three trophic levels that need to be assessed for CLP classification). Based on the Guidance on the Application of the CLP Criteria (ECHA, 2017) the E_rC_{10} value has been used preferentially over the NOE_rC to determine the chronic hazard classification. The lowest E_rC_{10} value considered valid (see Section 11.6.3) is the geometric mean measured 7-day E_rC_{10} of 0.023 mg a.s./L for Glyceria maxima (derived from Vlechev 2017b). This is > 0.01 mg/L but ≤ 0.1 mg/L, and since cinmethylin is considered to be 'not rapidly degradable', it meets the criteria for classification with Aquatic Chronic Category 1 with a chronic M-factor of 1.

11.8 CONCLUSION ON CLASSIFICATION AND LABELLING FOR ENVIRONMENTAL HAZARDS

Based on the information evaluated above; cinmethylin does not meet the criteria for potential bioaccumulation according to the CLP classification criterion (measured BCF ≥ 500); it is considered to be 'not rapidly degradable'; and is sufficiently toxic to warrant the highest CLP classifications for both acute and chronic hazards to the aquatic environment.

Classification:

Aquatic Acute 1; H400: Very toxic to aquatic life. Acute M-Factor of 10

Aquatic Chronic 1; H410: Very toxic to aquatic life with long lasting effects. Chronic M-Factor of 1

12 EVALUATION OF ADDITIONAL HAZARDS

Not assessed in this dossier.

13 ADDITIONAL LABELLING

No additional labelling is proposed.

It should be noted the GB evaluation of cinmethylin as a pesticide is not finalised.

14 REFERENCES (NON-CONFIDENTIAL)

Nb. Confidential references are in a separate Annex (Annex II).

Author	Year	Reference
General		
ECHA	2017	Guidance on the application of the CLP criteria: Guidance to regulation (EC) No 1272/2008 on classification, labelling and packaging (CLP) of substances and mixtures. Version 5.0 Published Available at https://echa.europa.eu/
Physchem. references		
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ANNEXES

ANNEX I: AVAILABLE AQUATIC TOXICITY DATA ON DEGRADANTS OF CINMETHYLIN

The available acute toxicity data available for the degradants of cinmethylin (M684H001 and M684H003) considered relevant under Regulation 1107/2009 are summarised below in Table 62. This information is included for information only, none of the degradants exhibit equivalent acute toxicity to cinmethylin or, based on this information, would require hazard classification in isolation. Therefore they are not considered to impact the hazard classification of cinmethylin.

Table 62: Summary of relevant information on acute aquatic toxicity

Species	Test material	Results[1]	Reference
Fish			
Oncorhynchus mykiss (formerly known as *Salmo gairdneri*)	M684H003	LC_{50} > 1000 mg /L (nom.)#	Anon (1988d)
Aquatic invertebrates			
D. magna	M684H001	EC_{50} > 100 mg /L (nom.)	Turek (2018a)
	M684H003	EC_{50} > 840 mg /L (nom.)#	Anon (1988d)
		EC_{50} > 100 mg /L (nom.)	Turek (2018b)
Other aquatic plants			
L. gibba	M684H001	E_rC_{50} > 78.3 mg /L (g.m)	Rzodeczko (2017e)
	M684H003	E_rC_{50} > 100 mg /L (g.m)	Rzodeczko (2017f)

1 nom. = nominal concentration; g.m = geometric mean measured

Not considered suitable for quantitative use as insufficient information provided to confirm analytical method was validated.

ANNEX II – CONFIDENTIAL REFERENCES (separate document)