CLH report

Proposal for Harmonised Classification and Labelling

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

International Chemical Identification: Clofentezine (ISO); 3,6-bis(o-chlorophenyl)-1,2,4,5-tetrazine

EC Number: 277-728-2

CAS Number: 74115-24-5

Index Number: -

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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 in the IUPAC nomenclature or other international chemical name	3,6-bis(o-chlorophenyl)-1,2,4,5-tetrazine
ISO common name	Clofentezine
EC number	277-728-2
EC name	3,6-bis(o-chlorophenyl)-1,2,4,5-tetrazine
CAS number	74115-24-5
Molecular formula	$C_{14}H_8Cl_2N_4$
Structural formula	
SMILES notation	Clc1ccccc1c1nnc(nn1)c1ccccc1Cl
Molecular weight or molecular weight range	303.1 g/mol

1.2 Composition of the substance

Table 2: Constituents (non-confidential information)

Constituent (Name and numerical identifier)	Concentration range (% w/w minimum and maximum in multi-constituent substances)	Current CLH in Annex V I Table 3.1 (CLP)	Current self- classification and labelling (CLP)
3,6-bis(o-chlorophenyl)-1,2,4,5-tetrazine CAS 74115-24-5	≥ 980 g/kg	Not available	According to C&L inventory (10 June 2019):

Classification		Labelling		Number of Notifiers
Hazard Class and Category Code(s)	Hazard Statement Code(s)	Hazard Statement Code(s)	Pictograms, Signal Word Code(s)	
Acute Tox.4	H312	H312	GHS09 GHS07 Wng	**69**
Aquatic Acute 1	H400	H400		
Aquatic Chronic 3	H412	H412 (Aquatic Chronic...)		**19**
Aquatic Acute 1	H400		GHS09 Wng	**2**
Aquatic Chronic 1	H410	H410		
Aquatic Chronic 3	H412	H412		**1**

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

Impurity (Name and numerical identifier)	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3.1 (CLP)	The impurity contributes to the classification and labelling

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

Additive (Name and numerical identifier)	Function	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)	The additive contributes to the classification and labelling

2 PROPOSED HARMONISED CLASSIFICATION AND LABELLING

2.1 Proposed harmonised classification and labelling according to the CLP criteria

Table 5:

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	No current Annex VI entry										
Dossier submitters proposal	-	clofentezine (ISO); 3,6-bis(o-chlorophenyl)-1,2,4,5-tetrazine	277-728-2	74115-24-5	Carc. 2 Aquatic Chronic 1	H351 H410	GHS08 GHS09 Wng	H351 H410		M = 10	
Resulting Annex VI entry if agreed by RAC and COM	-		277-728-2	74115-24-5	Carc. 2 Aquatic Chronic 1	H351 H410	GHS08 GHS09 Wng	H351 H410		M = 10	

Table 6: Reason for not proposing harmonised classification and status under public consultation

Hazard class	**Reason for no classification**	**Within the scope of public consultation**
Explosives	Data conclusive but not sufficient for classification	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	Hazard class not applicable	No
Flammable solids	Data conclusive but not sufficient for classification	Yes
Self-reactive substances	Data conclusive but not sufficient for classification	Yes
Pyrophoric liquids	Hazard class not applicable	No
Pyrophoric solids	Data conclusive but not sufficient for classification	Yes
Self-heating substances	Data conclusive but not sufficient for classification	Yes
Substances which in contact with water emit flammable gases	Data conclusive but not sufficient for classification	Yes
Oxidising liquids	Hazard class not applicable	No
Oxidising solids	Data conclusive but not sufficient for classification	Yes
Organic peroxides	Hazard class not applicable	No
Corrosive to metals	Data conclusive but not sufficient for classification	Yes
Acute toxicity via oral route	Data conclusive but not sufficient for classification	Yes
Acute toxicity via dermal route	Data conclusive but not sufficient for classification	Yes
Acute toxicity via inhalation route	Data conclusive but not sufficient for classification	Yes
Skin corrosion/irritation	Data conclusive but not sufficient for classification	Yes
Serious eye damage/eye irritation	Data conclusive but not sufficient for classification	Yes
Respiratory sensitisation	Data lacking	No
Skin sensitisation	Data conclusive but not sufficient for classification	Yes
Germ cell mutagenicity	Data conclusive but not sufficient for classification	Yes
Carcinogenicity	**Carc.2; H351**	Yes
Reproductive toxicity	Data conclusive but not sufficient for classification	Yes
Specific target organ toxicity-single exposure	Data conclusive but not sufficient for classification	Yes
Specific target organ toxicity-repeated exposure	Data conclusive but not sufficient for classification	Yes
Aspiration hazard	Data conclusive but not sufficient for classification	Yes
Hazardous to the aquatic environment	**Aquatic Chronic 1; H410; M=10**	Yes
Hazardous to the ozone layer	Hazard class not applicable	-

3 HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING

Clofentezine is not currently listed in Annex VI of Regulation of Regulation (EC) 1272/2008.

Clofentezine is an acaricide used as an active substance in plant protection products (PPP). Clofentezine was included in Annex I to Directive 91/414/EEC by Commission Directive 2008/69/EC subsequently amended by Commission Directive 2010/39/EU and has been deemed to be approved under Regulation (EC) No 1107/2009, in accordance with Commission Implementing Regulation (EU) No 540/2011. EFSA previously finalised a conclusion on this active substance on 4 June 2009 (EFSA Scientific Report (2009) 269, 1-113). EFSA proposed classification for clofentezine as R53 according to Directive 67/548.

Regarding the renewal of coflentezine as an active substance in the context of PPP Regulation, a Renewal Assessment Report (RAR) in accordance with Commission Regulation (EC) No. 844/2012 has been developed by the Spanish CA. The content of this CLH Report is based in data included in the RAR. It has to be noted that the RAR was submitted to public consultation (deadline for comments: 29 December 2018)

At the time of submission of this CLH report, clofentezine is not registered under REACH (Regulation (EC) 1907/2006).

4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

In accordance with article 36(2) of Regulation (EC) 1272/2008 on classification, labelling and packaging of substances and mixtures, being clofetenzine an active substance in the meaning of PPP Regulation, it should now be considered for harmonised classification and labelling for all physico-chemical, human health and environmental endpoints. This Annex VI dossier presents a classification and labelling proposal based on the information provided for the assessment of clofentezine under Regulation (EC) 1107/2009.

5 IDENTIFIED USES

Clofentezine is an active substance used as acaricide in plant protection products (PPP).

6 DATA SOURCES

This evaluation is mainly based on the Renewal Assessment Report (RAR, 2018) developed in accordance with Commission Regulation (EC) No. 844/2012 by the Spanish CA. Information on data sources used in this CLH report are included in section 14 (References) and 15 (Annexes).

7 PHYSICOCHEMICAL PROPERTIES

Table 7: Summary of physicochemical properties

Property	Value	Comment (e.g. measured or estimated)	Reference
Physical state at 20°C and 101.3 kPa	Homogenous magenta powder Clofentezine technical (Batch No. LF-151025). Purity 98.7% Homogenous magenta crystalline powder Clofentezine purified (Batch No. 426-026-04). Purity 99.3%	Visual assessment	**Demangel, B., 2015 B.2.3/01 (AS)**
Melting/freezing point	Clofentezine technical (99.3%): 182.1°C Analytical reference (100%): 182.3°C	Method: Differential Scanning Calometry (DSC) GLP:No	**Ball, R.W., 1987 B.2.1/01 (AS)**
	Clofentezine; Purity 99.7% Mean melting point 183.04°C Range 180 – 195°C Followed by decomposition in the temperature range 190-250°C.	Method: EEC A.1 GLP:Yes	**Smeykal, H., 2000a B.2.1/03 (AS)**
	Clofentezine; Purity 99.7% The melting stage is directly followed by exothermal effect. The test substance decomposes before reaching the boiling point. No boiling.	Method: EEC A.2 GLP:Yes	
Boiling point	The boiling point cannot be determined because of decomposition. Exothermic effect is observed at 233.6ºC		
Relative density	Clofentezine (purity 99.7%) Density 1.52 g/cm^3 at 21.2°C. Relative Density (compared with water at 4ºC) D^R_4: 1.52	EEC A.3 GLP : Yes	**Smeykal, H., 2000b B.2.14 (AS)**
Vapour pressure	Clofentezine (purity 99.7%) Calculated values: p(20°C) = 6.0 x 10^{-7} Pa p(25°C) = 1.4 x 10^{-6} Pa p(50°C) = 6.1 x 10^{-5} Pa Since vapour pressure was very low, direct measurements were conducted in the range of 91.6 – 146°C.	EEC A.4 GLP : Yes	**Smeykal, H., 2000c B.2.2/01 (AS)**
Surface tension	Test not necessary since solubility is lower than 1 mg/L.	EEC A.5 GLP: No	**Martínez, J., Rexer, K., 2000a B.2.12/01 (AS)**
Water solubility	Clofentezine (purity 98.2%) 3.42 x 10^{-5} g/L, column elution method at 20°C.	OPPTS 830.7840 GLP: Yes	**Van Meter, D.S., 2010 B.2.5/01 (AS)**
	Clofentezine; radiochemical purity 98.2% At pH5, solubility = 2.52 µg/L At pH7, solubility < 2 µg/L At pH9, solubility < 2 µg/L (LOQ = 2 µg/L)	EEC A.6 and OECD 105 GLP: No	**Smith, S., Kelly, I.D., 1985a B.2.5/01 (AS)**
Partition coefficient n-octanol/water	Log Pow = 4.09 at 25°C pH 6 (Clofentezine purity 99.9%)	OECD 107 GLP: Yes	**Bright A.A.S. and Stalker A.M.., 1990 B.2.7/01 (AS)**
	Log Pow = 3.1 at 20°C (pH not presented) (Clofentezine recrystallized)	OECD 107 GLP: No	**Lowes P.R., Bright A.A.S., 1986 B.2.7/01 (AS)**
	Log Pow = 4.1 at 40°C at pH 2.0, 7.0 and 9.0 (Clofentezine purity 99.7%)	OECD 117 GLP: Yes	**Mühlberger, B., 2001c B.2.7/01 (AS)**
Flash point	Not required for clofentezine, melting point is >40°C		

Property	Value	Comment (e.g. measured or estimated)	Reference
Flammability	Not flammable Purity: 99.7% (Batch: LF-051014)	EEC A10 GLP: Yes	**Anding, C., 2007 B.2.9/01 (AS)**
Self-ignition temperature	No self-ignition temperature up to 423°C. Purity: 99.7% (Batch: LF-051014)	EEC A.16 GLP: Yes	**Anding, C., 2007 B.2.9/02 (AS)**
Explosive properties	Not explosive. Purity: 96% (Batch: CF101)	EEC A.14 GLP: Yes	**Franke, J., 2002 B.2.11/01 (AS)**
Oxidising properties	Structural formula shows the substance cannot react exothermically with combustible material, therefore test to determine oxidising properties is not required.	EEC A.17 GLP: No	**Martínez, J., Rexer, K., 2000b B.2.13/01 (AS)**
Solubility in organic solvents	Clofentezine (purity 99.7%) At 20°C: Ethyl acetate: 5.67 g/L n-Heptane: 111.41 mg/L	Saturation method GLP: Yes	**Mühlberger, B., 2001b B.2.6/01**
	Clofentezine, Primary Analytical Reference Standard (purity > 99.0%) At 25°C: Acetone: 9.3 g/L Dichloromethane: 37.4 g/L Ethanol: 0.49 g/L Xylene: 5.0 g/L	Saturation method GLP: No	**Bright, A.A.S., 1987c B.2.6/01**
	Clofentezine, Primary Analytical Reference Standard (purity > 99.0%) At 25°C: DMSO: 11.8 g/L	Saturation method GLP: Yes	**Bright, A.A.S., 1988 B.2.6/01**
Dissociation constant	Due to the low solubility of clofentezine in water, as well as the hydrolytical instability at the pH range where a dissociation of clofentezine might be expected (estimated pKa > 9.0), the dissociation constant for clofentezine cannot be determined experimentally.		**Heintze, A., 2003b B.2.8/01**
Viscosity	Substance is a solid		

8 EVALUATION OF PHYSICAL HAZARDS

8.1 Explosives

Table 8: Summary table of studies on explosive properties

Method	Results	Remarks	Reference
EEC A.14 GLP: Yes	Not explosive. Purity: 96% (Batch: CF101)		**Franke, J., 2002 B.2.11/01 (AS)**

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

Clofentezine is not explosive according to the results of the study (Franke, J., 2002).

8.1.2 Comparison with the CLP criteria

Clofentezine does not meet CLP criteria to classify according to its explosive properties.

8.1.3 Conclusion on classification and labelling for explosive properties

Clofentezine does not require classification for explosive properties.

8.2 Flammable gases (including chemically unstable gases)

Hazard class not applicable (solid).

8.3 Oxidising gases

Hazard class not applicable (solid).

8.4 Gases under pressure

Not applicable.

8.5 Flammable liquids

Hazard class not applicable (solid).

8.6 Flammable solids

Table 9: Summary table of studies on flammable solids

Method	Results	Remarks	Reference
EEC A10 GLP: Yes	Not flammable Purity: 99.7% (Batch: LF-051014)		**Anding, C., 2007** **B.2.9/01 (AS)**

8.6.1 Short summary and overall relevance of the provided information on flammable solids

Clofentezine is not a flammable solid according to the results of the study (Anding, C., 2007).

8.6.2 Comparison with the CLP criteria

Clofentezine does not meet CLP criteria to be classified as flammable solid.

8.6.3 Conclusion on classification and labelling for flammable solids

Clofentezine is not a flammable solid.

8.7 Self-reactive substances

8.7.1 Short summary and overall relevance of the provided information on flammable solids

No data provided.

8.7.2 Comparison with the CLP criteria

A self-reactive substance corresponds to a thermally unstable solid liable to undergo a strongly exothermic decomposition even without participation of oxygen (air). Data on clofentezine does not indicate a potential self-reaction.

8.7.3 Conclusion on classification and labelling for flammable solids

Clofentezine is not a self-reactive substance.

8.8 Pyrophoric liquids

Hazard class not applicable (solid).

8.9 Pyrophoric solids

8.9.1 Short summary and overall relevance of the provided information on pyrophoric solids

No data provided.

8.9.2 Comparison with the CLP criteria

Clofentezine is not expected to be a pyrophoric solid according to CLP criteria since it does not correspond to a material which, even in small quantities, is liable to ignite within five minutes after coming into contact with air.

8.9.3 Conclusion on classification and labelling for pyrophoric solids

Clofentezine is not a pyrophoric solid.

8.10 Self-heating substances

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

Method	Results	Remarks	Reference
EEC A16 GLP: Yes	No self-ignition temperature up to 423°C.Purity: 99.7% (Batch: LF-051014)		**Anding, C., 2007** **B.2.9/01 (AS)**

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

No self-ignition temperature up to 423°C was observed in the self-ignition study (Anding, C., 2007).

8.10.2 Comparison with the CLP criteria

Although test method A.16 is not deemed appropriate to evaluate self-heating of solids towards CLP classification according to the ECHA Guidance on the Application of the CLP Criteria (version 5.0 July 2017), data on clofentezine indicate that is not a self-heating substance.

8.10.3 Conclusion on classification and labelling for self-heating substances

Clofentezine is not a self-heating substance.

8.11 Substances which in contact with water emit flammable gases

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

No data provided.

8.11.2 Comparison with the CLP criteria

Data on clofentezine does not indicate thar emits flammable gases in contact with water.

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

Clofentezine does not emit flammable gases in contact with water.

8.12 Oxidising liquids

Hazard class not applicable (solid).

8.13 Oxidising solids

Table 11: Summary table of studies on oxidising solids

Method	Results	Remarks	Reference
EEC A17 GLP: Yes	Structural formula shows the substance cannot react exothermically with combustible material, therefore test to determine oxidising properties is not required.		**Martínez, J., Rexer, K., 2000b B.2.13/01 (AS)**

8.13.1 Short summary and overall relevance of the provided information on oxidising solids

Clofentezine is not an oxidising solid according to the results of the report statement (Martinez, J., Rexer, K., 2000b).

8.13.2 Comparison with the CLP criteria

Clofentezine does not meet CLP criteria to be classified as oxidising solid.

8.13.3 Conclusion on classification and labelling for oxidising solids

Clofentezine is not a oxidising solid.

8.14 Organic peroxides

Clofentezine is not an organic peroxide. It does not contain the bivalent -O-O- structure and it is not thermally unstable.

8.15 Corrosive to metals

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

No data available.

8.15.2 Comparison with the CLP criteria

The test method required according to CLP Regulation corresponds to the outlined in section 37.4 of the UN RTDG Manual of Tests and Criteria and it is used to assess the corrosive properties of ‘liquids and solids that may become liquids on transport’.

Although there are no data on the cited method, clofentezine is a solid that decomposes before boiling (exothermic effect is observed at 233.6ºC). Conseqently, this hazard class is not relevant for the active substance.

8.15.3 Conclusion on classification and labelling on the hazard class corrosive to metals

Clofentezine is not a oxidising solid.

9 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

Table 12: Summary table of toxicokinetic studies

Type of study/species/route/dose	Remarks	Reference
Single dose studies		
Excretion and distribution Comparable to OECD TG 417 (1984) GLP: No **Study acceptable** Sprague Dawley rat Oral dose (gavage): 0.1 mg/kg bw Vehicle: 0.5% gum tragacanth Group: 5 rats/sex Purity: not available Radiolabel: [^{14}C]-tetrazine ring	Radiolabel excretion was rapid and virtually complete within 48 h. Faecal excretion was ≈75% dose and urinary excretion 22% dose, 89 h post-dosing. Tissue radioactivity levels were close to or below the limit of detection (0.01 mg/kg). There was no significant sex difference in the rate or route of excretion.	**Anonymous 1 (1981a)** **B.6.1.1-01 (AS)**
Excretion and distribution Comparable to OECD TG 417 (1984) GLP: No **Study acceptable** Sprague Dawley rat Oral dose (gavage): 10 mg/kg bw Vehicle: 0.5% gum tragacanth Group: 5 rats/sex Purity: not available Radiolabel: [^{14}C]-tetrazine ring	Radiolabel excretion was rapid and virtually complete within 48 h. Faecal excretion was ≈75% dose and urinary excretion 20% dose, 96 h post-dosing. The highest levels of radioactivity were found in liver (0.22-0.46 mg/kg) and kidney (0.12-1.28 mg/kg). Plasma levels were 0.07-0.22 mg/L, and the remaining tissues contained 0.01-0.2 mg/kg. There was no significant sex difference in the rate or route of excretion.	**Anonymous 2 (1982a)** **B.6.1.1-02 (AS)**
Excretion and distribution Comparable to OECD TG 417 (1984) GLP: No **Study acceptable** Sprague Dawley rat Oral dose (gavage): 1000 mg/kg bw Vehicle: 0.5% gum tragacanth Group: 5 rats/sex Purity: 99% Radiolabel: [^{14}C]-tetrazine ring	Within 48 h of dosing, 85-90% dose had been excreted and by 72 h excretion was virtually complete. Faecal excretion was 99% dose (♂) and 96% dose (♀) and urinary excretion 2% dose (♂) and 4.5% dose (♀), 96 h post-dosing. The highest levels of radioactivity were found in plasma (11-16 mg/L), adrenals (7-12 mg/kg), liver (6-11 mg/kg) and fat (6-8.5 mg/kg). The remaining tissues contained <6 mg/kg. There was no significant sex difference in the rate or route of excretion.	**Anonymous 3 (1982b)** **B.6.1.1-03 (AS)**
Excretion and distribution Comparable to OECD TG 417 (1984) GLP: No **Study acceptable** Sprague Dawley rat Intravenous dose: 0.1 mg/kg bw Vehicle: propane-1,2-diol Group: 5 rats/sex Purity: not available Radiolabel: [^{14}C]-tetrazine ring	Radiolabel excretion was rapid and essentially complete within 48 h. Faecal excretion was >80% dose and urinary excretion 25-29% dose, 96 h post-dosing. Livers from 3 animals contained residues of 0.01 mg/kg but no other tissues in any animals contained residues at or above the limit of detection (0.01 mg/kg). There was no significant sex difference in the rate or route of excretion.	**Anonymous 4 (1983)** **B.6.1.1-04 (AS)**

Type of study/species/route/dose	Remarks	Reference
Biliary excretion and absoprtion Guideline: Commission Directive 88/302/EEC Part B Toxicokinetics according to Commission Directive 94/79/EC Annex I Part 5.1 GLP: Yes **Study acceptable** Sprague Dawley rat Oral single dose (gavage): 10 mg/kg bw Vehicle: aqueous 0.5% gum tragacanth Group: 5 rats/sex Purity: 99.7% Radiolabel: [^{14}C]-tetrazine ring	Within 24 h of dosing, more than 93% dose was excreted. Excretion of radioactivity in the bile was higher for ♂ (43.5% dose) than ♀ (32.5% dose) while excretion of radioactivity in the urine was higher for ♀ (17% dose) than ♂ (7% dose). There were no sex differences in the pattern of faecal excretion (48% dose for ♂, 49% dose for ♀). Thus, the total clofentezine absorbed was the same in both sexes (≈ 50% dose).	**Anonymous 5 (2007)** **B.6.1.1-05 (AS)**
Excretion and distribution Method: not stated but comparable to OECD TG 417 (1984) GLP: No **Study acceptable** Mice CD-1 Oral single dose (gavage): 10 mg/kg bw Vehicle: gum tragacanth Group: A (5♀), B (5♂) anb C (3/sex) Purity: not available Radiolabel: [^{14}C]-tetrazine ring	Radiolabel excretion was rapid and virtually complete within 48 h. Faecal excretion was 68% dose and urine excretion 26% dose, 96 h post-dosing. Residues were < 0.01 mg/kg in the majority of tissues. The highest residues were detected in liver (0.11-0.18 mg/kg). There was no significant sex difference in the rate or route of excretion.	**Anonymous 6 (1982a)** **B.6.1.1-18 (AS)**
Excretion and distribution Method: not stated but comparable to OECD TG 417 (1984) GLP: No **Study acceptable** New Zealand White rabbit Oral single dose (gavage): 10 mg/kg bw Vehicle: 0.5% gum tragacanth Group: 3/sex Purity: not available Radiolabel: [^{14}C]-tetrazine ring	In the first 48 h, ≈ 90% dose was excreted and by 96 h excretion was complete. Faecal excretion was 57% dose and urinary excretion 36% dose, 96 h post-dosing. Except for the tissues of the GIT, residues were highest in liver and kidney (0.24 and 0.09 mg/kg, respectively). The lowest residues were detected in muscle and bone (0.01 mg/kg and <0.01 mg/kg, respectively). Plasma contained very low residues (0.01 mg/kg) while bile contained higher residues (0.74 mg/kg) than any of the soft tissues There was no significant sex difference in the rate or route of excretion.	**Anonymous 7 (1982b)** **B.6.1.1-20 (AS)**
Excretion, distribution and plasma kinetics Method: not stated but comparable to OECD TG 417 (1984) GLP: No **Study acceptable** Beagle dog Oral single dose (gavage): 10 mg/kg bw Vehicle: gelatine capsule Group: 3/sex Purity: not stated Radiolabel: [^{14}C]-tetrazine ring	In the first 48 h, 96% dose was excreted and by 96 h excretion was complete. Faecal excretion was 95% dose and urinary excretion 2% dose, 96 h post-dosing.Tissue residues were highest in bile, liver and thyroid (1.56, 0.21 and 0.16 mg/kg, respectively) and lowest in bone, lens and aqueous humour (≤0.01 mg/kg). Peak plasma radioactivity (0.05 mg/L) occurred 4-8 h post-dosing. By 72 hours post-dosing plasma concentrations were ≤0.01 mg/L. There was no significant sex difference in the rate or route of excretion.	**Anonymous 8 (1982c)** **B.6.1.1-21 (AS)**

Type of study/species/route/dose	Remarks	Reference
Excretion and distribution Method: not stated but comparable to OECD TG 417 (1984) GLP: No **Study acceptable** Beagle dog Intravenous dose: 10 mg/kg bw Vehicle: propylene glycol in water Group: 2/sex Purity: not stated Radiolabel: [^{14}C]-tetrazine ring	Radiolabel excretion was rapid and complete within 48 h. Faecal excretion was ≈ 70% dose and urinary excretion 22% dose, 144 h post-dosing. With the exception of liver (0.01 mg/kg), bile (0.01 mg/kg) and pituitary (0.03 mg/kg) all tissues contains residues <0.01 mg/kg. Peak plasma radioactivity (0.05 mg/L) occurred 30 min post-dosing (the first time point taken). By 12 h post-dosing plasma concentrations were ≤0.01 mg/L. By 96 h post-dosing, the concentration was ≈ 0.001 mg/L which was the limit of reliable measurement under the analytical conditions used. There was no significant sex difference in the rate or route of excretion	Anonymous 9 (1981) B.6.1.1-22 (AS)
Excretion and distribution Method: not stated but comparable to OECD TG 417 (1984) GLP: No **Study acceptable** Baboon Single oral dose of [^{14}C]-10 mg/kg bw day 1 + 56 days unlabelled (escalating dose) + [^{14}C]-10 mg/kg bw day 52 Vehicle: 0.5% carboxymethyl cellulose Group: 1/sex Purity: 98.9% Radiolabel: [^{14}C]-tetrazine ring	Radiolabel excretion was rapid and complete within 48 h. Faecal excretion was 43-44% dose, with cage debris and cage wash together accounting for 8% dose, and urinary excretion 15-28.5% dose, 96 h post-dosing. There was no significant sex difference in the rate or route of excretion. However, due to poor recovery of radioactivity, the study is considered only as additional information. 96 h after the 2nd radiolabelled dose, no significant sex difference was apparent in the distribution of radioactivity in tissues. The highest concentrations were detected in fat, liver and kidney (0.23-0.06-% of administered radioactivity/kg) where tissue-to-plasma ratios were in the order 10-3. The lowest concentration was in muscle, brain and whole blood	Anonymous 10 (1983) B.6.1.1-23 (AS)
Comparative excretion and metabolism Summary document Method: none cited GLP: not applicable **Study acceptable** Rat, mouse, rabbit, calf, dog and baboon Single oral dosing: 10 mg/kg bw except calf (5 mg/kg bw). Besides one group of rats was also dosed with 20 mg/kg bw. Vehicle: 0.5% Gum tragacanth or CMC Group: 1/sex except for calf (1♂) Purity > 96% Radiolabel: [^{14}C]-tetrazine ring	In all species, excretion of clofentezine was rapid and complete. The majority of elimination taking place in the first 48 h post-dosing. The major route of excretion is via faeces. Urinary levels range from 2% in the dog to 36% in the rabbit. In rat and mouse, urinary levels are 20-26%; levels in mouse tending to be higher than in rat. Absolute levels in excreta of baboon were difficult to assess owing to poor overall recovery of radioactivity There was no significant sex difference in the route or rate of excretion. The metabolism of clofentezine in all species studied was qualitatively similar, with hydroxylation and replacement of chlorine by a methylthio group being the major pathways. Many minor metabolites were formed in all species. Quantitative interspecies differences were apparent, notably the fact that in the calf and the baboon, hydroxylation and subsequent conjugation were the most prominent pathways, with methythiolation being a minor route of metabolism. This latter pathway is more pronounced in rodents and the rabbit.	Anonymous 11 (1985) B.6.1.1-25 (AS)
Whole-body autoradiography Method: not stated but comparable to OECD TG 417 (1984) GLP: No **Study acceptable** Sprague Dawley rat Single oral dosing: 10 mg/kg bw Vehicle: 0.5% gum tragacanth Group: 5/sex Purity: 98.9% Radiolabel: [^{14}C]-tetrazine ring	At 4 h post-dosing the highest levels of radioactivity were detected in the intestine but also in the kidney and nasal turbinates. Activity was also seen in liver, bone marrow and systemic circulation. A similar pattern was seen at 8 h (radioactivity mostly in the lower gut) with some evidence of biliary excretion. At 24 h, with the exception of the lower intestine, the radioactivity was virtually eliminated from the body.	Anonymous 12 (1982) B.6.1.1-6 (AS)

Type of study/species/route/dose	Remarks	Reference
Tissue clearance Method: not stated but comparable to OECD TG 417 (1984) GLP: Yes **Study acceptable** Sprague Dawley rat Single oral dosing: 10 and 1000 mg/kg bw Vehicle: 1% gum tragacanth Group: 18/sex Purity: 100% Radiolabel: [^{14}C]-tetrazine ring	The concentration of clofentezine derived residues was determined in the tissues of rats 6, 24, 48, 72, 96 and 144 h post-dosing. At both dose levels, the initial clearance of the residues was rapid and extensive over the first 24 h post-dosing. After dosing of 10 mg/kg, the highest tissue residue was detected in fat (9.2 mg/kg) at 6 h. This residue fell to 0.16 mg/kg at 24 h. After dosing of 1000 mg/kg, tissue residues levels in ♀ were slightly higher than those of the corresponding ♂ at 6 h but there was no apparent sex difference at any later time points. Fat also contained the highest tissue residue (177 and 114 mg/kg, respectively for ♀ and ♂) 6 h post-dosing. This residue fell to 8.9 mg/kg at 24 h. The mean terminal half-lives of the total radioactive residues in plasma were determined to be 31.6 and 43.1 h at the low and high doses respectively.	**Anonymous 13 (1991)** **B.6.1.1-7 (AS)**
Plasma kinetics Method: not stated but comparable to OECD TG 417 (1984) GLP: No **Study acceptable** COB rat Single oral dosing (gavage): 10 mg/kg bw Vehicle: gum tragacanth Group: 5 rats/sex//time point Purity: not stated Radiolabel: [^{14}C]-tetrazine ring	Residues in plasma were measured 5 min to 18 h post-dosing. Total plasma radioactivity rose to a maximum of 1.3-1.6 mg/L at 4-6 h post-dosing, and declined thereafter to a value of 0.25-0.37 mg/L at 18 h. The concentration of unchanged clofentezine rose to a maximum of 0.6-0.7 mg/L (46-48% of total radioactivity) at 4-6 h post-dosing, and then declined to 0.02 mg/L at 18 h, with a half-life of 2.5 h. There was no apparent sex difference.	**Anonymous 14 (1982)** **B.6.1.1-8 (AS)**
Plasma kinetics Method: not stated but comparable to OECD TG 417 (1984) GLP: No **Study acceptable** Sprague Dawley rat Single oral dosing (gavage): 1000 mg/kg bw Vehicle: gum tragacanth Group: 3 rats/sex//time point Purity: 99% Radiolabel: [^{14}C]-tetrazine ring	Residues in plasma were measured 1-24 h post-dosing. Total radioactivity and unchanged clofentezine in plasma rose to a maximum of 14-16 mg/L and 7-8.5 mg/l, respectively, 6-8 h post-dosing. Unchanged clofentezine, which accounted for 47-54% of the total radioactivity, declined, with a half-life of 3.6 h. There was no apparent sex difference.	**Anonymous 15 (1985a)** **B.6.1.1-9 (AS)**
Metabolism Method: not stated but comparable to OECD TG 417 (1984) GLP: No **Study acceptable** Sprague Dawley rat Single oral dosing (gavage): 10 mg/kg bw Vehicle: gum tragacanth Group: 3 rats/sex//time point Purity: >98% Radiolabel: [^{14}C]-tetrazine ring	Urine and faeces for metabolite identification were collected 24 h post-dosing. No sex difference was apparent in the metabolic profile. Absorbed clofentezine was extensively metabolized, with only 3% of the radioactivity in the urine present as the parent compound. 15 major and 10 minor metabolites were present in urine. Two major urinary pathways have been identified, one leading to a monochloro-sulphur-containing derivative, 3-(2'-methylthio-3'-hydroxyphenyl)-6-(2'-chlorophenyl)-1,2,4,5-tetrazine (in free and conjugated forms this metabolite accounted for 35% of the urinary radioactivity), and the other involving hydroxylation of clofentezine at the 3,4, or 5 positions, (free and conjugated hydroxyclofentezine isomers accounted for 34% of the urinary radioactivity). The majority of radioactivity (at least 62%) in faeces was present as unchanged clofentezine, probably representing unabsorbed dose. The remaining faecal material was extensively metabolised to more than 30 minor metabolites. Unidentified metabolites in urine and faeces were attributed to multiple isomers of mono and di hydroxylated products and conjugates thereof.	**Anonymous 16 (1985)** **B.6.1.1-10 (AS)**

Type of study/species/route/dose	Remarks	Reference
Metabolism Method: not stated but comparable to OECD TG 417 (1984) GLP: No **Study acceptable** Baboon Oral dose: [^{14}C]-10 mg/kg bw day 1 + 56 days unlabelled (escalating dose) + [^{14}C]-10 mg/kg bw day 52 [^{14}C]-tetrazine ring Vehicle: gum tragacanth Group: 1/sex Purity: 98.9% Radiolabel: [^{14}C]-tetrazine ring	Urine samples for metabolite identification were collected over a 96 h period post-dosing. No sex difference was apparent in the metabolic profile. Clofentezine was extensively metabolized, with only 5% of the urinary radioactivity being due to unchanged parent compound. The major metabolic pathway involves hydroxylation to 4-hydroxy-clofentezine which is excreted in both free form and as its glucuronide conjugate and accounts for over 70% of the radioactivity extractable from the urine. While a number of minor metabolites were also present, each of these, in general accounted for <1% extractable radioactivity. One of these is thought to be 3-hydroxy-clofentezine. A comparison of urine collected from the 1st and 2nd radiolabelled dose showed the metabolic profile was qualitatively similar.	**Anonymous 17 (1983)** **B.6.1.1-24 (AS)**
Repeated dose studies		
Excretion and distribution Method: not stated but comparable to OECD TG 417 (1984) GLP: No **Study acceptable** Sprague Dawley rat Repeated oral dosing (gavage): 10 mg/kg bw (14 days) Vehicle: 0.5% gum tragacanth Group: 5/sex Purity: >98% Radiolabel: [^{14}C]-tetrazine ring	Radiolabel excretion was rapid and substantially complete within 48 h. Faecal excretion was 83% dose and urinary excretion ≈ 20% dose, 96 h post-dosing. Tissue residues were frequently below 0.3 mg/kg. The highest levels of radioactivity were found in liver (0.3-0.7 mg/kg) and kidney (0.2-0.5 mg/kg). Some tissue levels (heart, lung, spleen, muscle, brain) were 2-4 times higher in ♂ than ♀. Plasma levels were 0.03-0.08 mg/L. There was no significant sex difference in the rate or route of excretion and the pattern of excretion was similar to that a single dose. Residue levels were generally comparable to those obtained in the equivalent single dose study though certain tissues from ♂ contained slightly higher. In addition, plasma levels were lower than in the equivalent single dose study.	**Anonymous 18 (1982c)** **B.6.1.1-11 (AS)**
Distribution, kinetics and bioaccumulation Method: not stated but comparable to OECD TG 417 (1984) GLP: No **Study acceptable** Sprague Dawley rat Repeated oral dosing (gavage): 20 mg/kg bw (25 days) Vehicle: 0.5% gum tragacanth Group: 18/sex Purity: >98% Radiolabel: [^{14}C]-tetrazine ring	Residues in tissues were measured 1, 5, 10, 15, 20 and 25 days after the 1st dose. The highest residues were found in liver (3-4 mg/kg) 20-25 days after the 1st dose. After 5-15 days, a plateau was reached in liver, kidney, ♀ heart, skin and ovaries with residues being 2-4 times higher than after 1 day. There was an increase in radioactive residues in adrenals, ♂ heart, muscle, lung and fat but no definite plateaus could be discerned. However, residues in fat were 8 times higher after 25 days than after 1 day. No accumulation occurred in bone, brain, eyes, spleen, testes, blood and plasma.	**Anonymous 19 (1981b)** **B.6.1.1-12 (AS)**
Distribution in maternal and foetal tissue and bioaccumulation Method: not stated but comparable to OECD TG 417 (1984) GLP: No **Study acceptable** Sprague Dawley rat Oral dose: 3200 mg/kg bw/d from GD 7-13 + 320 mg/kg bw/d on GD 14 + untreated from GD 15-19 + single [^{14}C]-10 mg/kg bw on GD 20 Vehicle: 0.5% carboxymethyl cellulose Group: 5 pregnant rats Purity: Not stated Radiolabel: [^{14}C]-tetrazine ring	Clofentezine was rapidly absorbed and distributed with maternal plasma levels of 4-6 µg/mL 6 h post-dosing. The highest maternal residues were found in fat and ranged from 8-13 µg/g 6 h post-dosing and 2-7 µg /g 24 h post-dosing. The tissue-to-plasma ratio increased from 2 to 5.3 over this period, indicating that residues may accumulate in fat after repeated dosing. The lowest maternal residues were found in eye and brain both 6 and 24 h post-dosing, and with the exception of liver and kidney, residue levels in the remaining tissues 24 h post-dosing were equivalent to or less than those found in plasma. Clofentezine and/or its metabolites does not appear to readily cross the placenta since 6 h post-dosing residue levels in foetuses were lower (0.7-1.2 µg/g) than in most maternal tissues except eye and brain. The clearance rate from the foetus appeared to be slower than from the maternal tissues, indicating that accumulation could take place after repeated dosing. However, the sensitivity of the study was limited by the use of LSC to measure residue levels rather than co-chromatography.	**Anonymous 20 (1981)** **B.6.1.1-14 (AS)**

Type of study/species/route/dose	Remarks	Reference
Plasma kinetics Method: not stated but comparable to OECD TG 417 (1984) GLP: No **Study acceptable** CD Sprague Dawley rat Oral doses: unlabelled 10 mg/kg bw/d for 14 d+single [^{14}C]-10 mg/kg bw Vehicle: 0.5% gum tragacanth Group: 26/sex Purity: 98.9% Radiolabel: [^{14}C]-tetrazine ring	Residues in plasma were measured 1-24 h post-dosing. There was no apparent sex difference. Total plasma radioactivity rose to a maximum of 0.80 mg/L at 4 h post-dosing, and declined thereafter to a value of 0.17 mg/L at 24 h. The concentration of unchanged clofentezine rose to a maximum of 0.17 mg/L (10-28% of total radioactivity) at 4 h post-dosing, and then declined rapidly to 0.01 at 10 h, with a half-life of 1.6 h. Pre-dosing with clofentezine for 14 days resulted in an increase in the rate of metabolism when compared to administration of a single dose.	**Anonymous 21 (1985b)** **B.6.1.1-13 (AS)**
Enzyme induction Method: not specified GLP: No **Study acceptable** Charles River CD rat Dietary administration 5 groups of 6♂: 1) phenobarbitone for 4 days; 2) 20-methylcholanthrene 2 days; 3) clofentezine (40 ppm) for 8 weeks; 4) clofentezine (27000 ppm) for 8 weeks; 5) basal diet. Purity: 100.2% Unlabelled	Livers were examined for aniline hydroxylase activity, levels of cytochromes P-450 and b5, and microsomal protein concentration, 24 h after the final dose. At 27000 ppm, a 50% increase in the level of cytochrome P450 and 2-fold increases in the level of cytochrome b5 and in the activity of aniline hydroxylase were found. No induction was observed at 40 ppm. Clofentezine appears to be an enzyme inducer of the phenobarbitone type	**Anonymous 22 (1983a)** **B.6.1.1-15 (AS)**
Enzyme induction Method: not specified GLP: No **Study acceptable** Charles River CD rat Dietary administration Groups of 6♂: 1) phenobarbitone for 4 days (positive control); 2) basal diet 3) clofentezine (27000 ppm) for 10 weeks after 2 week of withdrawal period Purity: 100.2% Unlabelled	A 2 wk withdrawal period has been shown to virtually reverse the effects of the induction of microsomal mixed-function oxidases resulting from 10 wks administration of clofentezine at 27000 ppm to rats. At the end of the withdrawal period liver weights and the level of cytochrome P450 were not significantly different from those of control animals. Moreover, the levels of cytochrome b5 were also reduced to 1.5 times that of control.	**Anonymous 23 (1983b)** **B.6.1.1-16 (AS)**
Enzyme induction Method: not specified GLP: No **Study acceptable** Charles River CD rat Dietary administration 5 groups of 10/sex: basal diet; clofentezine 2 weeks (10, 40 and 400 ppm); phenobarbitone. Purity: 98.8% Unlabelled	At 400 ppm there was an increase in the level of microsomal protein (+12%), aldrin epoxidase (22-32%), ethoxycoumarin deethylase (20-49%), and cytochrome P-450 (24-36%) in both sexes. There was also an increase in the unit relative liver weight (+10%) and level of cytochrome b5 (+53%) b5 in ♂ only. At 40 ppm, ethoxycoumarin deethylase was slightly increased in ♂ only. The results suggest that the threshold for EI lies between 40 and 400 ppm. The pattern of EI and the absorption maxima of the carbon monoxide binding spectra confirm that clofentezine is a phenobarbitone type enzyme inducer	**Anonymous 24 (1986)** **B.6.1.1-17 (AS)**
Enzyme induction Method: not specified GLP: No **Study acceptable** CD mice Dietary administration Groups for 2 weeks: basal diet (10♂); clofentezine (9♂) 8 weeks (400 and 27000 ppm); phenobarbitone 4 days (5♂) Purity: 99.6% Unlabelled	At 27000 ppm the levels of cytochrome P450 and b5 were increased 3 fold, and the mean liver weight increased by 37%. There was a marginal effect on EI at 400 ppm.	**Anonymous 25 (1984)** **B.6.1.1-19 (AS)**

Type of study/species/route/dose	Remarks	Reference
Evaluation of existing K data from studies: – Anonymous 13 (1991) [B.6.1.1-7 (AS)]: single dosing/rat/oral/10, 1000 mg/kg bw. – Anonymous 14 (1982) [B.6.1.1-8 (AS)]: single dosing/rat/oral/10 mg/kg bw. – Anonymous 15 (1985a) [B.6.1.1-9 (AS)]: single dosing/rat/oral/ 1000 mg/kg bw. – Anonymous 18 (1982c) [B.6.1.1-11 (AS)]: repeated dosing/rat/oral/ 10 mg/kg bw. – Anonymous 8 (1982c) [B.6.1.1-21 (AS)]: single dosing/dog/oral/10 mg/kg bw – Anonymous 9(1981) [B.6.1.1-22 (AS)]: (single dosing/dog/i.v./0.1 mg/kg bw)	The plasma concentration *vs* time profiles for clofentezine and total radioactivity were consistent with the oral or intravenous dose routes in rats and dogs. Following a post-dose absorption phase after oral administration, clofentezine levels declined in a generally mono-phasic manner. Plasma total radioactivity levels declined in a generally bi-phasic manner after oral or i.v. administration. There was no apparent sex difference in any of the pharmacokinetic parameters calculated. In rats, systemic exposure to total radioactivity was consistently greater than exposure to clofentezine at each dose within each study. Tmax of clofentezine and total radioactivity were generally comparable. Systemic exposure to clofentezine, as well as to total radioactivity, increased with dose in a non-proportional manner in both sexes. Systemic exposure to clofentezine was greater following a single administration than following multiple administrations, which indicates increased metabolism of parent. No consistent differences in T_{max} or $T_{1/2}$ of clofentezine between doses were noted. However, $T_{1/2}$ of total radioactivity was longer at 10 and 1000 mg/kg in the study where total radioactivity was quantified over a longer period of time (terminal samples taken up to 144 h) and therefore this half-life value is considered to be the most reliable. In dogs, following i.v. administration, clearance of total radioactivity was low compared to hepatic and renal blood flow and the volume of distribution was high compared to total body water. Absolute oral bioavailability of total radioactivity was low in males and females (4-5%). Systemic exposure to total radioactivity was greater in rats than in dogs following oral administration of clofentezine at 10 mg/kg. No consistent differences in Tmax or $T_{1/2}$ between species were noted.	**Webster (2016)** **B.6.1.1-26 (AS)**

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

Following oral administration to the rat, mouse, rabbit, dog and baboon, excretion of clofentezine was rapid and complete. The majority of elimination occurred in the first 48 h post-dosing (80-90%) and was complete by 96 h. The major route of excretion is via faeces. Urinary levels range from 2% in the dog to 36% in the rabbit. In rat and mouse, urinary levels are 20-26%; levels in mouse tending to be higher than in rat. There was no significant sex difference in the route or rate of excretion.

A comparison of oral and i.v. dosing indicates that faeces are the major route of excretion in both cases. The amount excreted via urine and faeces did not seem to differ after oral or intravenous administration. Data on biliary excretion in rats (B.6.1.1-05) confirm EFSA's original estimation of oral absorption (EFSA Scientific Report (2009) 269, 1-113) established in 50%.

Radioactivity is widely distributed but low levels of residues are found in organs and tissues. The levels in plasma reached a maximum 4 to 6 hours after an oral dose. The highest residues were found in the liver. Although the repeated dosing study suggests that residues may accumulate in fat, the results were not consistent and bioaccumulation is not considered for clofentezine.

The plasma concentration *vs* time profiles for clofentezine and total radioactivity were consistent with the oral or intravenous dose routes in rats and dogs. Following a post-dose absorption phase after oral administration, clofentezine levels declined in a generally mono-phasic manner. Plasma total radioactivity levels declined in a generally bi-phasic manner after oral or i.v. administration. There was no apparent sex difference in any of the pharmacokinetic parameters calculated.

In rats, systemic exposure to total radioactivity was consistently greater than exposure to clofentezine at each dose within each study. T_{max} of clofentezine and total radioactivity were generally comparable. Systemic exposure to clofentezine, as well as to total radioactivity, increased with dose

in a non-proportional manner in both sexes. Systemic exposure to clofentezine was greater following a single administration than following multiple administrations, which indicates increased metabolism of parent. No consistent differences in Tmax or $T_{1/2}$ of clofentezine between doses were noted. However, $T_{1/2}$ of total radioactivity was longer at 10 and 1000 mg/kg in the study where total radioactivity was quantified over a longer period of time (terminal samples taken up to 144 h) and therefore this $T_{1/2}$ value is considered to be the most reliable (32 and 43 h at the low and high doses respectively).

In dogs, following i.v. administration, clearance of total radioactivity was low compared to hepatic and renal blood flow and the volume of distribution was high compared to total body water. Absolute oral bioavailability of total radioactivity was low in males and females (4-5%).

Systemic exposure to total radioactivity was greater in rats than in dogs following oral administration of clofentezine at 10 mg/kg. No consistent differences in T_{max} or $T_{1/2}$ between species were noted.

Metabolism data from rats show that after oral dosing unchanged clofentezine was the major component in faeces whereas the material excreted in rat urine consisted mainly of metabolites. In the faeces 50% was excreted unchanged; the rest was metabolized to more than 20 minor metabolites. The percentage of the administered dose in the faeces increases with dose suggesting saturation. It is noted that the high dose saturation evident in some of the studies would confound any clear conclusions on dose response relationships.

The metabolism of clofentezine was determined in the urine of the rat, mouse, rabbit, dog, calf and baboon being qualitatively similar, with hydroxylation and replacement of chlorine by a methylthio group being the major pathways. Many minor metabolites were formed in all species. Quantitative interspecies differences were apparent, notably the fact that in the calf and the baboon, hydroxylation and subsequent conjugation were the most prominent pathways, with methythiolation being a minor route of metabolism. This latter pathway is more pronounced in rodents and the rabbit (Figure 1 and Figure 2).

Figure 1: Proposed metabolic pathway of clofentezine in the rat

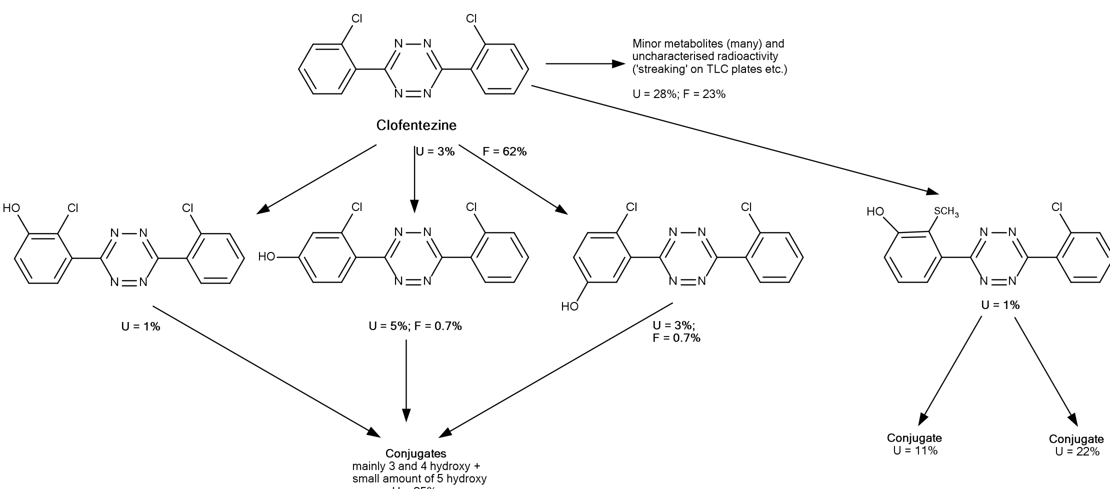


Figure 2: Proposed metabolic pathway of clofentezine in baboon

Clofentezine
(M = 3%; F = 5%)

4-hydroxy-clofentezine
(M = 25%; F = 12%)

3-hydroxy Clofentezine
(probable, <1%)

Possible

Conjugate
(M = 5%; F = 4%)

4-hydroxy-clofentezine-B-D-glucuronide
(M = 46%; F = 64%)

(Figures in brackets refer to percentages of the total radioactivity extractable from urine)

Finally, according to observations made in rats and mice clofentezine appears to be an enzyme inducer of the phenobarbitone type. In rats, at a dose level of 27000 ppm a 50% increase in the level of cytochrome P450 and 2-fold increases in the level of cytochrome b5 and in the activity of aniline hydroxylase were found, but the effect could be reversed after a 2-week withdrawal period; no induction was observed at 40 ppm.

10 EVALUATION OF HEALTH HAZARDS

10.1 Acute toxicity - oral route

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 study in rats Method comparable to OECD 401 GLP: No (prior to GLP enforcement) **Study acceptable as supporting information** Deviations: 3 animals/sex/dose instead of 5 animals/sex/dose	Purity: 99% Rat strain: Sprague-Dawley Oral (gavage) Vehicle: 0.5% gum tragacanth (aq) 3 rats/sex/dose Doses: 0, 800, 1131, 1600, 2261 and 3200 mg/kg bw 14-day observation period	**Mortality:** not occurred. **Clinical signs:** slight urinary incontinence in 1♂ and 1♀ at 3200 mg/kg bw and slight salivation in 1♂ at 1131 mg/kg bw. Pink coloration of faeces (attributed to the test chemical) between 20 and 22 hours was seen after dosing in females at all dose levels and in males at ≥ 2261 mg/kg bw. **Bodyweight**: no treatment related effects. **Necropsy**: no treatment related effects. **LD_{50}: > 3200** mg/kg bw for both sexes	**Anonymous 26 (1980)** **(AS)** **B.6.2.1.1-01**
Acute oral toxicity study in rats Method: OECD 401 GLP: No (prior to GLP enforcement) **Study acceptable**	Purity: 99.3% Rat strain: Sprague-Dawley Oral (gavage) Vehicle: 0.5% (w/v) carboxymethyl cellulose (aq) 5 rats/sex/dose Doses: 5200 mg/kg bw (limit test) and controls 14-day observation period	**Mortality:** not occurred. **Clinical signs:** not observed. **Bodyweight**: no effects. **Necropsy**: no treatment related effects. **LD_{50}: > 5200** mg/kg bw for both sexes	**Anonymous 27 (1986a)** **(AS)** **B.6.2.1.1-02**
Acute oral toxicity study in mice Method comparable to OECD 401 GLP: No (prior to GLP enforcement) **Study acceptable**	Purity: 99.1% Mice strain: CD-1 Oral (gavage) Vehicle: 0.5% gum tragacanth (aq) 6 mice/sex/dose Doses: 3200 mg/kg bw (limit test) and controls 14-day observation period	**Mortality:** not occurred. **Clinical signs:** not observed. **Bodyweight**: (↓) significant in ♀ (days 1-8). **Necropsy**: spleen with pale and pitted appearance or apparently small in 4/6♂ and 1/6♀ *vs.* 0/6♂ and 1/6♀ of controls. **LD_{50}: > 3200** mg/kg bw for both sexes	**Anonymous 28 (1980a)** **Snowdon, P.J. (1980a)[a]** **(AS)** **B.6.2.1.2-01**
Acute oral toxicity study in mice Method: OECD 401 GLP: No (prior to GLP enforcement) **Study acceptable**	Purity: 99.3% Mice strain: Swiss CR1:CD1 (ICR) BR Oral (gavage) Vehicle: 0.5% (w/v) carboxymethyl cellulose (aq) 5 mice/sex/dose Doses: 5200 mg/kg bw (limit test) and controls 14-day observation period	**Mortality:** not occurred. **Clinical signs:** not observed. **Bodyweight**: no effects. **Necropsy**: no treatment related effects. **LD_{50}: > 5200** mg/kg bw for both sexes	**Anonymous 29 (1986b)** **(AS)** **B.6.2.1.2-02**

Method, guideline, deviations if any	Species, strain, sex, no./group, test substance, dose levels, duration of exposure	Value LD_{50}	Reference
Acute oral toxicity study in hamster Method comparable to OECD 401 GLP: No (prior to GLP enforcement) **Study acceptable**	Purity: 99.1% Hamster strain: Syrian Oral (gavage) Vehicle: 0.5% gum tragacanth (aq) 6 hamsters/sex/dose Doses: 3200 mg/kg bw (limit test) and controls 14-day observation period	**Mortality:** not occurred. **Clinical signs:** not observed. **Bodyweight**: no effects. **Necropsy**: no treatment related effects. **LD_{50}: > 3200** mg/kg bw for both sexes	**Anonymous 30 (1980)** **Snowdon, P.J. (1980b)** [a] **(AS) B.6.2.1.3-01**
Acute oral toxicity study in dogs Method comparable to OECD 401 GLP: No (prior to GLP enforcement) **Study acceptable**	Purity: 98.8-99.6% Dog strain: Beagle Oral (gavage) Vehicle: 0.5% gum tragacanth (aq) 2 dogs/sex in controls and at 2000 mg/kg bw and 1♂ at 1000 mg/kg bw 14-day observation period	**Mortality:** not occurred. **Clinical signs:** not observed. **Bodyweight**: no effects. **Necropsy**: slight focal hyperplasia of the renal papillary epithelium was observed amongst treated male and female dogs. Although such changes were not evident in controls, this is a common, spontaneous lesion in laboratory dogs. **LD_{50}: > 2000** mg/kg bw for both sexes	**Anonymous 31 (1981)** **Snowdon, P.J. (1981a)** [a] **(AS) B.6.2.1.4-01**

[a] Study for the determination of concentrations in aqueous gum tragacanth suspension

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

In an acute oral toxicity study in rats (B.6.2.1.1-01) acceptable as additional information the LD_{50} (both sexes) was greater than 3200 mg/kg bw/day and in another acute oral toxicity study in rats (B.6.2.1.1-02) the LD_{50} (both sexes) was greater than 5200 mg/kg bw/day.

In acute oral toxicity study in mice (B.6.2.1.2-01) the LD_{50} (both sexes) was greater than 3200 mg/kg bw/day and in another oral toxicity study in mice (B.6.2.1.2-01) the LD_{50} (both sexes) was greater than 5200 mg/kg bw/day.

In an acute oral toxicity study in hamster (B.6.2.1.3-01) the LD_{50} (both sexes) was greater than 3200 mg/kg bw/day.

In an acute oral toxicity study in dogs (B.6.2.1.4-01) the LD_{50} (both sexes) was greater than 2000 mg/kg bw/day.

10.1.2 Comparison with the CLP criteria

The LD_{50} observed in the six studies performed in four species are clearly above the threshold value of 2000 mg/kg bw for triggering acute oral toxicity classification according to CLP Regulation.

10.1.3 Conclusion on classification and labelling for acute oral toxicity

Data available indicates that clofentezine does not require classification for acute oral toxicity.

10.2 Acute toxicity - dermal route

Table 14: 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 study in rats Method comparable to OECD 402 GLP: No (prior to GLP enforcement) Deviations: Limit dose was below 2000 mg/kg bw. Occlusive dressing for 21 h instead of semi-occlusive for 24 h **Study acceptable as supporting information**	Purity: 99.1% Rat strain: Sprague Dawley Vehicle: 0.5% gum tragacanth (aq) 6 rats /sex/dose Doses: 1332 mg/kg bw (limit test) and controls 21 h of exposure (occlusive dressing) 14-day observation period	**Mortality:** not occurred. **Clinical signs:** not observed. **Bodyweight**: no effects. **Necropsy**: no treatment related effects. **LD_{50}:** > **1332** mg/kg bw for both sexes	**Anonymous 32 (1980b)** **Snowdon, P.J. (1980c)[a]** **(AS) B.6.2.2-01**
Acute dermal toxicity study in rats Method comparable to OECD 402 GLP: No (prior to GLP enforcement) Deviations: Occlusive dressing instead of semi-occlusive **Study acceptable**	Purity: 99.3% Rat strain: Sprague Dawley Vehicle: 0.5% carboxymethyl cellulose (aq) 5 rats /sex/dose Doses: 2100 mg/kg bw (limit test) and controls 24 h of exposure (occlusive dressing) 14-day observation period	**Mortality:** not occurred. **Clinical signs:** not observed. **Bodyweight**: no effects. **Necropsy**: no treatment related effects. **LD_{50}:** > **2100** mg/kg bw for both sexes	**Anonymous 33 (1987)** **(AS) B.6.2.2-02**

[a] Study for the determination of concentrations in aqueous gum tragacanth suspension

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

In an acute dermal toxicity study in rats (B.6.2.2-01) acceptable as additional information the LD_{50} (both sexes) was greater than 1332 mg/kg bw/day.

In another acute dermal toxicity study in rats (B.6.2.2-02) the LD_{50} (both sexes) was greater than 2100 mg/kg bw/day.

10.2.2 Comparison with the CLP criteria

LD_{50} greater than 2100 mg/kg bw is above the threshold value of 2000 mg/kg bw for triggering acute dermal toxicity classification.

10.2.3 Conclusion on classification and labelling for acute dermal toxicity

Data available indicates that clofentezine does not require classification for acute dermal toxicity.

10.3 Acute toxicity - inhalation route

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

<table>
<tr><th>Method, guideline, deviations if any</th><th>Species, strain, sex, no./group, duration of exposure</th><th>Test substance, Dose levels, form and particle size (MMAD)</th><th>Value LC$_{50}$</th><th>Reference</th></tr>
<tr>
<td>Acute inhalation toxicity study in rats
Method comparable to OECD 403
GLP: No (prior to GLP enforcement)
Deviations: 6 h of exposure instead of 4 h
Study acceptable as supporting information</td>
<td>Rat strain: Sprague Dawley
Whole body exposure system for 6 hour and thereafter 14-day observation
5 animals/sex/dose</td>
<td>The study was performed with a preparation: wettable powder (WP) containing 77.6-82.4% w/w of clofentezine.
<table>
<tr><th rowspan="2">Parameter</th><th colspan="2">Value</th></tr>
<tr><th>Active ingredient</th><th>Preparation</th></tr>
<tr><td>Nominal concentration (mg/L)</td><td>9.08</td><td>11.35</td></tr>
<tr><td>Mean achieved atmosphere concentration (mg/L)</td><td>1.51</td><td>1.89</td></tr>
<tr><td>Particle size (MMAD ± GSD)</td><td colspan="2">2.7 ±0.2</td></tr>
<tr><td>% inspirable (< 4 µm)</td><td colspan="2">>79.6%</td></tr>
</table></td>
<td>Mortality: not seen.
Clinical signs: coolness to touch and pale eyes on removal of animals from the exposure chamber.
Bodyweight: no effects.
Necropsy: no treatment related effects.
LC$_{50}$ > 1.51 mg/L</td>
<td>Anonymous 34 (1982)
(AS) B.6.2.3-01</td>
</tr>
<tr>
<td>Acute inhalation toxicity study in rats
Method comparable to OECD 403
GLP: Yes
Study acceptable</td>
<td>Rat strain: HsdHanTM: WIST rats
Nose-only for 4 hour exposure and thereafter 14-day observation
5 animals/sex/dose</td>
<td>Purity: 98.2%.
<table>
<tr><th>Parameter</th><th>Value</th></tr>
<tr><td>Nominal concentration (mg/L)</td><td>6.17</td></tr>
<tr><td>Mean achieved atmosphere concentration (mg/L)</td><td>5.20</td></tr>
<tr><td>Chamber flow rate (L/min)</td><td>50</td></tr>
<tr><td>Particle size (MMAD ± GSD)</td><td>3.24 ±2.45</td></tr>
<tr><td>% inspirable (< 4 µm)</td><td>59.3%</td></tr>
</table></td>
<td>Mortality: not seen.
Clinical signs: not seen.
Bodyweight: no effects.
Necropsy: abnormally dark lungs in 2/5 ♀.
LC$_{50}$ > 5.20 mg/L</td>
<td>Anonymous 35 (2010)
(AS) B.6.2.3-02</td>
</tr>
</table>

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

In an acute inhalation toxicity study in rats (B.6.2.3-01), performed with a preparation consisting of a wettable powder (WP) of clofentezine and deemed acceptable only a supporting infomrmation, LC$_{50}$ was found to be greater than 1.51 mg/L after 6-hour exposure.

In another acute inhalation toxicity study in rats carried out with clofentezine LC$_{50}$ was found to be greater than 5.20 mg/L after 4-hour exposure.

10.3.2 Comparison with the CLP criteria

The 4-hour inhalation LC$_{50}$ of > 5.20 mg/L for rats is above the value for classification in the CLP Regulation (i.e. 5 mg/l dust/mist). No classification for acute inhalation toxicity is proposed.

10.3.3 Conclusion on classification and labelling for acute dermal toxicity

Data available indicates that clofentezine does not require classification for acute inhalation toxicity.

10.4 Skin corrosion/irritation

Table 16: Summary table of animal studies on skin corrosion/irritation

Method, guideline, deviations if any	Species, strain, sex, no./group Test substance, Dose levels duration of exposure	Results -Observations and time point of onset -Mean scores/animal -Reversibility	Reference
Skin irritation study in guinea pigs Method comparable to OECD 404 GLP: No (prior to GLP enforcement) Deviations: Guinea pigs used rather than rabbits; exposure time 24 hours rather than 4 hours; test chemical applied under an occlusive dressing rather than a semi-occlusive dressing. **Study acceptable as supporting information**	Purity: 99.1% Guinea pig strain: Dunkin-Hartley 6 animals (female) Vehicle: 0.5% gum tragacanth (aq) Test preparation: 333 mg/mL of the test chemical in the vehicle. 4 sites of application/animal: 0.2 mL of the test preparation in two sites (A and D), 1 with 0.2 mL of vehicle (B) and one for blank (C). 24 h of exposure and 7 days of observation.	Only slight oedema (not graded) was observed in 2/12 test application areas: – 1 until 2 ½ days after washing application area. – 1 from 2 ½ hours after washing application area until 2 ½ days. It is not known according to data available if both areas corresponded to 1 or 2 animals. Conclusion: **Negligible primary skin irritation**	**Anonymous 36 (1980c)** **Snowdon, P.J (1980d)[a]** **(AS)** **B.6.2.4-01**
***In vitro* skin irritation: human skin model test** Method OECD 439: EPISKIN-SMTM GLP: Yes **Study acceptable**	Purity: 98.7% Skin model: (non-cancerous), adult human-derived epidermal keratinocytes (NHEK) cultured to form a multilayered, highly differentiated model of the human epidermis Control negative (10 µL): Phospahte Buffered Saline (PBS) Control positive (10 µL) : 5% sodium dodecyl sulfate (SDS) Clofentezine: (10 mg + 10 µL distilled water)	<table><tr><td></td><td>Negative Control</td><td>Positive Control</td><td>Test Chemical</td></tr><tr><td>Mean relative tissue viability (%)±SD</td><td>100 ±4.8</td><td>18.5±6.1</td><td>**101.4 ±4.5**</td></tr></table>Evaluation criteria according to the method: Irritant: ≤ 50% mean tissue viability (% negative control). Non-Irritant: > 50% mean tissue viability (% negative control) Conclusion: **Non-irritant**	**Gehrke H., (2015)** **(AS)** **B.6.2.4-02**

[a] Study for the determination of concentrations in aqueous gum tragacanth suspension

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

In Crome S.J., Sanderson D.M. and Brooks P.N. (1980c) guinea pig skin irritation study slight oedema (not graded) was observed in 2/12 application sites of 6 animals (two sites/animal). It is not known if these two positive responses in two application sites occurred in one or two animals. The result indicated negligible primary irritation of clofentezine. The study is considered acceptable as additional information due to a large number of deficiencies (see Table 16). Besides, the test material used is not deemed appropriate to evaluate the irritation potential of clofentezine since consisted of a suspension of 333 mg/ml of the test chemical in the vehicle. According to the method when testing solids (which may be pulverised, if considered necessary), the test substance should be moistened with the smallest amount of water (or, where necessary, of another suitable vehicle) sufficient to ensure good skin contact.

In Gehrke H., (2015) i*n vitro* human skin irritation based on OECD 439 method the result of mean relative tissue viability > 50% indicated the non-irritative potential of clofentezine. It has to be noted that according to the ECHA Guidance on the application of the CLP criteria (July 2017) this method

can reliably distinguish non-classified from classified substances and it is considered valid in the evaluation of skin irritation potential of substances.

10.4.2 Comparison with the CLP criteria

The result of mean relative tissue viability obtained in the *in vitro* human skin irritation was 101.4%. The evaluation criteria included in the OECD 439 method establishes that a test material substance is considered non-irritant when the mean relative tissue viability is > 50%. Consequently, clofentezine is not irritant to the skin.

10.4.3 Conclusion on classification and labelling for skin corrosion/irritation

Data available indicates that clofentezine does not require classification as skin irritant.

10.5 Serious eye damage/eye irritation

Table 17: 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, test substance, 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>Eye irritation study in rabbits
Guideline: US EPA 81-4 comparable to OECD 405
GLP: No (prior to GLP enforcement)
Study acceptable</td><td>Purity: 99.3%
Rabbit strain: New Zealand albino
6 animals (female)
70 mg of undiluted test material equivalent to a volume of 0.1 ml instilled into one eye. The other one served as control.
Eyes remained unwashed after instillation.
Observations after 1, 24, 48 and 72 h and day 4 and 7 (end of the study)</td><td>Results of animals with unwashed eyes after instillation:
<table>
<tr><th rowspan="2"></th><th colspan="6" rowspan="2">Cornea</th><th colspan="6" rowspan="2">Iris</th><th colspan="12">Conjunctiva</th></tr>
<tr><th colspan="6">Redness</th><th colspan="6">Chemosis</th></tr>
<tr><td>After 24 hours</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>1</td><td>0</td><td>0</td><td>0</td><td>1</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td></tr>
<tr><td>After 48 hours</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td></tr>
<tr><td>After 72 hours</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td></tr>
</table>
2/6 animals showed slight erythema of 0.33 (mean score after 24, 48 and 72 h).
Conclusion: Not eye irritant.</td><td>Anonymous 37 (1986)
(AS) B.6.2.5-01</td></tr>
</table>

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

In Liggett M.P. & Parcell B.I. (1986) eye irritation study in rabbits the only lesion observed was conjunctival erythema of 0.33 (mean score after 24, 48 and 72 h) in 2/6 animals.

10.5.2 Comparison with the CLP criteria

According to the ECHA Guidance on the application of the CLP criteria (July 2017) when 6 rabbits are used in the eye irritation study the test material is considered irritant to the eye when conjunctival erythema is ≥ 2 in at least 4/6 animals. The erythema of 0.33 in 2/6 animals obtained in the study does not meet the criteria for classification as irritating to the eyes according to CLP.

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

Data available indicates that clofentezine does not require classification as eye irritant.

10.6 Respiratory sensitisation

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

No data available.

10.6.2 Comparison with the CLP criteria

No data available.

10.6.3 Conclusion on classification and labelling for respiratory sensitisation

Data lacking.

10.7 Skin sensitisation

Table 18: Summary table of animal studies on skin sensitisation

<table>
<tr><th>Type of study/data</th><th>Test substance, species, strain, sex, no./group</th><th>Dose levels
duration of exposure and results</th><th>Reference</th></tr>
<tr>
<td>Guinea pig maximisation test (GPMT)

Guideline: OECD 406.

Deviations: An additional topical application of the test chemical was applied immediately post intradermal injection on day 1.

GLP: No (prior to GLP enforcement)

Study acceptable</td>
<td>Purity: not stated

Female Dunkin Hartley guinea pigs (females)

20 animals for main tested group and 20 for control

Vehicle: ethanol</td>
<td>Preliminary test:

Intradermal injection (induction): no test performed. A saturated solution (80 g/l) of the test compound in ethanol equivalent to a concentration of 8% p/v was used.

Topical application (induction and challenge): 4 guinea pigs with 4 application sites/animal were treated (occlusive patch 24 h) with the following concentrations in ethanol:
– 0.5 g of the neat test chemical moistened with 0.5 ml of ethanol.
– 50% and 25% suspensions.
– A saturated solution (0.8 g/l) in ethanol.
Results: individual irritation scores after 24/48 h were 0 (no reaction) in the 4 guinea pigs.

Main test:

<table>
<tr><th>Induction intradermal injection Day 1</th><th>Test</th></tr>
<tr><td>1</td><td>FCA (Freund's Complete Adyuvant)</td></tr>
<tr><td>2</td><td>Saturated solution (8 % p/v)</td></tr>
<tr><td>3</td><td>Saturated solution (8 % p/v) mixed with FCA in proportion 1:1</td></tr>
<tr><td>Induction topical application Day 1 (occlusive patch 48 h)</td><td>0.5 g of the neat test chemical moistened with 0.5 ml of ethanol</td></tr>
<tr><td>Induction topical application Day 8 (occlusive patch 48 h)</td><td>0.5 g of the neat test chemical moistened with 0.5 ml of ethanol</td></tr>
<tr><td>Challenge topical application Day 22 (occlusive patch 24 h)</td><td>-0.5 g of the neat test chemical moistened with 0.5 ml of ethanol
-50% suspension of the test material in ethanol*</td></tr>
</table>
*Used to ensure that a non-irritant concentration was used and applied in other flank of the animal

Control group had the same treatment on day 1 and 8 using ethanol instead of active substance.

No concurrent positive control was conducted, but historical data presented (results of 16 studies using positive controls from 1979 to 1982) confirmed the laboratory's proficiency in detecting known skin sensitizers.</td>
<td>Anonymous 38 (1982)

(AS)
B.6.2.6-01</td>
</tr>
</table>

<table>
<tr><th>Type of study/data</th><th>Test substance, species, strain, sex, no./group</th><th>Dose levels
duration of exposure and results</th><th>Reference</th></tr>
<tr><td></td><td></td><td>
Results
2/20 (10%) animals showed a weak response (grade 1) 24 h after challenge with the neat test chemical but not at 48 hours. No response in the controls was observed (0/20→ 0%)
<table>
<tr><th colspan="2">Challenge phase</th><th colspan="2">Incidence of significant responses</th></tr>
<tr><th colspan="2">Group</th><th>24 hours</th><th>48 hours</th></tr>
<tr><td rowspan="2">0.5 g of neat test chemical moistened with 0.5 mL ethanol</td><td>Control</td><td>0/20</td><td>0/20</td></tr>
<tr><td>Test</td><td>2/20 [a]</td><td>0/20</td></tr>
<tr><td rowspan="2">50% suspension of the test chemical in ethanol</td><td>Control</td><td>0/20</td><td>0/20</td></tr>
<tr><td>Test</td><td>0/20</td><td>0/20</td></tr>
</table>
[a] : The two positive responses are of grade 1
Conclusion: Not sensitising.
</td><td></td></tr>
</table>

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

A guinea pig maximisation test (B.6.2.6-01) was performed with 20 animals for the tested and control groups. Concentration of intradermal injection was an 8% p/v saturated solution of the test chemical in ethanol. It has to be noted that after intradermal injection the area was immediately tested with a topical application of 0.5 g of the test material moistened with 0.5 ml of ethanol. One week later this same concentration was used for the topical induction application. After two weeks of the topical induction, two concentrations were tested in the challenge phase:

- After challenge with 0.5 g of the test material moistened with 0.5 ml of ethanol a positive response was observed in 2/20 animals (10%). The response in these two animals was observed 24 hours after challenge but not after 48 hours.
- A 50% suspension of the test material in ethanol was used in another flank of the animal to ensure that a non-irritant concentration was used. No response was observed in any of the 20 animals (0%).

No response was observed in the 20 animals of the control group (0%). According to the results of the study, clofentezine did not show skin sensitization potential.

10.7.2 Comparison with the CLP criteria

A response in 30% of the animals in the maximisation test is required for classification. 2/20 (10%) guinea pigs displayed skin reactions after challenge with clofentezine. Consequently, the criteria for classification are not met.

10.7.3 Conclusion on classification and labelling for skin sensitisation

Data available indicates that clofentezine does not require classification as skin sensitiser.

10.8 Germ cell mutagenicity

Table 19: Summary table of mutagenicity/genotoxicity tests *in vitro*

Method, guideline, deviations if any	Test system	Test substance and dosage	Results	Remarks	Reference
Bacterial gene mutation (Ames Test) Pre-OECD TG 471 (1983) Deviations: Only a positive control that requires metabolic activation (2-AA) used for all strains; results not confirmed in an independent experiment. GLP: No (prior to GLP enforcement) **Study not acceptable**, due to the inadequacy of the positive controls.	*Salmonella typhimurium:* TA1535, TA100, TA1538, TA98, TA1537. S9 from livers of rats induced with Aroclor 1254.	Clofentezine Purity: Not stated 10, 33, 100, 330, 1000, 3300 µg/plate (± S9) Solvent: DMSO	Negative	No cytotoxicity. Precipitation at 330 µg/plate and above.	**McConville (1980)** **B.6.4.1.1-01 (AS)**
Bacterial gene mutation (Ames Test) OECD TG 471 (1997) Deviations: None GLP: Yes **Study acceptable**	*Salmonella. typhimurium:* TA1535, TA100, TA1537, TA98, TA102 S9 from livers of rats induced with phenobarbitone and β-naphthoflavone	Clofentezine, Purity: 98.4% 50, 150, 500, 1500, 5000 µg/plate (± S9) Solvent: DMF	Negative	No cytotoxicity. Precipitation at 500 µg/plate and above	**Bowles (2005)** **B.6.4.1.1-02 (AS)**
Mammalian cell gene mutation test. Pre-OECD TG 476 (1984) Deviations: None GLP: No (prior to GLP enforcement) **Study not acceptable,** since when data are assessed against current guideline requirements (OECD TG 490, 20145) the methodology used is considered insufficient to evaluate gene mutation, for example there was not long-term treatment –S9.	Mouse lymphoma L5178Y $TK^{+/-}$ cells. S9 from livers of rats induced with Aroclor 1254	Clofentezine Purity: 98.4% 4 h (-S9): 15, 30, 70, 100, 128 µg/mL 4 h (+S9): 2, 10, 30, 80, 128 µg/mL Solvent: Acetone	Negative		**Bootman and Rees (1982)** **B.6.4.1.2-01 (AS)**
Mammalian cell gene mutation test. OECD TG 476 (1997) Deviations: None GLP: Yes **Study acceptable**	Chinese hamster V79 cells (*Hprt* locus) S9 from livers of rats induced with phenobarbital and β-naphthoflavone	Clofentezine, Purity: 98.7% 4 h (±S9): 0.30, 0.76, 1.52, 2.27, 3.03, 7.58, 15.15, 22.73 µg/mL 20 h (-S9): 0.30, 0.61, 0.91, 1.21, 3.03, 6.06, 15.15, 18.18 µg/mL 4h (+S9): 15.15, 16.67, 18.18, 19.70, 21.21, 22.73, 24.24, 27.27 µg/mL Solvent: DMSO	Negative	At the highest concentration tested, in each experiment, a reduction of relative total growth (RTG) below 70% was observed	**Wallner. (2015a)** **B.6.4.1.2-02 (AS)**

Method, guideline, deviations if any	Test system	Test substance and dosage	Results	Remarks	Reference
Mammalian cell chromosome aberrations test OECD TG 473 (1983) Deviations: None GLP: No (prior to GLP enforcement) **Study acceptable only as supporting information**, since when data are assessed against current guideline requirements (OECD TG 473, 2014) the methodology used is considered insufficient to evaluate chromosomal aberration.	Chinese hamster ovary cells (CHO - K1- BH_4) S9 from livers of rats of Sprague-Dawley origin induced with Aroclor 1254	Clofentezine Purity: 99.6% 20 h (-S9): 0.4, 2, 4 µg/mL 2 h (+S9): 0.4, 2, 4 µg/mL Solvent:DMSO	Negative	There was no toxicity at the maximum soluble dose level, 4 µg/mL.	**Allen, Brooker, Godfrey (1987)** **B.6.4.1.3** **(AS)**
Gene conversion and mitotic recombination test in yeast Pre-OECD TG 481 (1986) Deviations: None GLP: No (prior to GLP enforcement) **Study acceptable only as supplementary information**, since this study is not required and OECD TG 481 (1986) was deleted on 2 April 2014.	*Saccharomyces cerevisiae,* D7 strain S9 from livers of rats induced with Aroclor 1254	Clofentezine Purity: 98.4% 12.5, 25, 50, 100, 200 µg/mL Solvent: DMF:ethanol (1:9)	Negative	-	**Riach and McGregor (1983)** **B.6.4.1.4-01** **(AS)**
Rec-assay No test guideline available GLP: No (prior to GLP enforcement) **Study acceptable only as supplementary information**, since it is not required.	*Bacillus subtillis* H17 (Rec^+) and M45 (Rec^-) S9 from livers of rats induced with phenobarbital and β-naphthoflavone	Clofentezine Purity: Not stated 156, 313, 625, 1250, 2500 µg/disk (-S9) 78.1, 156, 313, 625, 1250 µg/disk (+S9) Solvent: DMSO	Negative	-	**Inoue and Nakajima (1986)** **B.6.4.1.4-02** **(AS)**

Table 20: Summary table of mutagenicity/genotoxicity tests in mammalian somatic or germ cells *in vivo*

Method, guideline, deviations if any	Test system	Test substance and dosage	Results	Remarks	Reference
Micronucleus test (somatic cells) Pre-OECD TG 474 (1983) Deviations: A single sex (male). A single sampling time. GLP: No (prior to GLP enforcement) **Study not acceptable,** because the bone marrow sampling used (6 h post the 2nd dose) is insufficient.	Mice, CD-1 strain	Clofentezine Purity: 99.6% 800, 1600, 3200 mg/kg bw/day (two oral administrations separated by 24 h). Vehicle: 0.5% aqueous gum tragacanth	Negative	-	**Anonymous 39 (1982)** **B.6.4.2.1-01** **(AS)**
Micronucleus test (somatic cells) OECD TG 474 (1983) Deviations: None GLP: No (prior to GLP enforcement) **Study acceptable**	Mice, CD-1 strain	Clofentezine Purity: 99.6% 8000 mg/kg bw (single oral administration) Vehicle: 0.5% sodium carboxymethyl cellulose	Negative	No toxicity. Exposure is assumed	**Anonymous 40 (1987)** **B.6.4.2.1-02** **(AS)**

Method, guideline, deviations if any	Test system	Test substance and dosage	Results	Remarks	Reference
Rodent dominant lethal test (germ cells). Pre-OECD TG 478 (1984) Deviations: No inclusion of a positive control. Exposure for 10 weeks exceeded the recommended one. GLP: No (prior to GLP enforcement) **Study acceptable only as supporting information** since this study is not required and by some deficiencies in methodology.	Rats, Sprague Dawley strain	Clofentezine Purity: ≥ 98.1% Diet containing 0.28, 2.81 and 27.8 mg/kg bw/day for 10 weeks following by pairing each treated male with two untreated females for up to14 days.	Negative	Liver weights and plasma cholesterol levels higher than controls at 27.8 mg/kg bw/day only, consistent with effects seen in short-term toxicity studies	**Anonymous 41 (1983)** **B.6.4.3-01** **(AS)**

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

The genotoxic potential of clofentezine has been investigated in a series of *in vitro* and *in vivo* studies.

The *in vitro* bacterial gene mutation study (Ames test) (Bowles, 2005) showed no evidence of mutagenicity following testing in five *Salmonella* strains when tested up to 5000 µg/plate (the maximum recommended concentration in accordance with current regulatory guidelines for *in vitro* bacterial genotoxicity assays) in the absence and presence of metabolic activation using the plate incorporation method.

In the *in vitro* mammalian cell gene mutation study (Wallner, 2015a) clofentezine did not induce forward mutation at the *Hprt* locus of V79 Chinese hamster cells. These conditions included treatment in both the absence (4 and 20 hours) and presence (4 hours) of a rat liver metabolic activation system which was limited by toxicity (a reduction of relative total growth below 70%).

Although both the Ames study (McConville, 1980) and the mammalian cell gene mutation assay with mouse lymphoma L5178Y cells (Bootman & Rees, 1982) were not considered acceptable for the assessment due to deficiencies noted, both gave negative results.

Regarding DNA damage studies, as supplementary information for the risk assessment, clofentezine was negative in a gene conversion and mitotic recombination test in yeast (*Saccharomyces cerevisiae* strain D-7) (Riach and McGregor, 1983) and in a Rec-assay with H17 (Rec+) and M45 (Rec-) strains of *Bacillus subtilis* (Inoue and Nakajima, 1986).

A photomutagenicity study was not provided. According to Commission Regulation (EU) No. 283/2013 *in vitro* photomutagenicity studies are indicated if the ultraviolet/visible (UV/VIS) molar extinction/absorption coefficient of the active substance and its major metabolites is more than 1000 L x mol^{-1} x cm^{-1}. In the case of clofentezine the molar extinction/absorption coefficient is >1000 L/mol cm at 290 nm, rapidly decreasing to 465 L/mol cm at a maximum absorbance of 534.5 nm and photomutagenicity testing should be triggered. However, as the *in vitro* 3T3 NRU phototoxicity assay returned a negative result (Roth, 2015) it is considered as unlikely that clofentezine induces photomutagenicity.

Three studies have been considered to assess the chromosomal aberration potential: the *in vitro* clastogenicity test in Chinese hamster ovary (CHO) cells (Allen, Brooker and Godfrey, 1987), the *in vivo* mouse bone marrow micronucleus test (B.6.4.2.1-02) and the *in vivo* rat lethal dominant mutation assay (B.6.4.3-01). Although it is true that these studies show some deficiencies in the methodology, it is considered that the available database is sufficient for overall assessment of this

endpoint. Clofentezine did not induce either micronuclei or bone marrow cell toxicity in the mouse (single oral dose at 8000 mg/kg bw) although is reasonable to assume the exposure of the target organ based on the available ADME data. Clofentezine was also negative in both *in vitro* clastogenicity test and *in vivo* lethal dominant mutation assay. Based on all data, it can be concluded that clofentezine showed no evidence for chromosomal aberration induction.

In the other *in vivo* mouse bone marrow micronucleus test (B.6.4.2.1-01), the results were negative, but it was not considered acceptable because the bone marrow sampling used was insufficient in order to evaluate chromosomal aberrations.

In conclusion, based on the weight of evidence clofentezine is considered to be non-genotoxic.

10.8.2 Comparison with the CLP criteria

Clofentezine was not mutagenic in a valid *in vivo* somatic cell mutagenicity test and so according to the guidance on the application of the CLP criteria no classification is warranted. The overall body of toxicological data from a number of *in vitro* and *in vivo* assays indicates that clofentezine is of no genotoxic concern. Therefore no classification for mutagenicity under the CLP regulation is required.

10.8.3 Conclusion on classification and labelling for germ cell mutagenicity

Not classified (conclusive but not sufficient for classification).

10.9 Carcinogenicity

Table 21: Summary table of animal studies on carcinogenicity

For more detailed information see RAR B6 (AS) chapter 6.5

<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
[Effects statistically significant and dose-related unless stated otherwise as not significant (n.s.) or not dose-related (ndr) or not clearly dose-related (ncdr)]</th><th>Reference</th></tr>
<tr>
<td>Long-term oral toxicity and carcinogenicity study in rats
(27 months)
FBC Limited
Method OECD 453
GLP: No (prior to GLP enforcement)
Study acceptable
Rat strain: Charles River Sprague Dawley
50 rats/sex/dose for carcinogenicity phase (sacrificied after 27 months of treatment)
20 rats/sex/dose for chronic toxicity phase (sacrificed after 12 months)
10 rats/sex/dose for an additional group for blood sampling</td>
<td>Purity: 98.7%
Oral (diet)
Doses: 0, 10, 40 and 400 ppm equivalent to:
Males: 0, 0.43, 1.72 and 17.3 mg/kg bw/day.
Females: 0, 0.55, 2.18, 22.1 mg/kg bw/day
Parameters observed: Mortality, clinical signs, bodyweights, food and water consumption, ophthalmology, haematology, urinalysis, clinical chemistry, gross pathology, organ weights and histopathology (neoplastic and non-neoplastic lesions)</td>
<td>Mortality: no evidence of any treatment related effect
<table>
<tr><th rowspan="3">Parameter</th><th colspan="8">Carcinogenicity groups (ppm)</th></tr>
<tr><th colspan="4">Males</th><th colspan="4">Females</th></tr>
<tr><th>0</th><th>10</th><th>40</th><th>400</th><th>0</th><th>10</th><th>40</th><th>400</th></tr>
<tr><td>No. of survivors at week 72</td><td>47</td><td>48</td><td>47</td><td>43</td><td>48</td><td>49</td><td>45</td><td>49</td></tr>
<tr><td>No. of survivors at week 96</td><td>38</td><td>37</td><td>35</td><td>36</td><td>32</td><td>38</td><td>32</td><td>34</td></tr>
<tr><td>No. of survivors at week 104</td><td>34</td><td>32</td><td>32</td><td>27</td><td>26</td><td>28</td><td>28</td><td>28</td></tr>
<tr><td>% survival at week 116</td><td>26</td><td>25</td><td>28</td><td>21</td><td>20</td><td>19</td><td>21</td><td>24</td></tr>
<tr><td>% mortality at week 116</td><td>74</td><td>75</td><td>72</td><td>79</td><td>80</td><td>81</td><td>79</td><td>76</td></tr>
</table>
400 ppm (17.3♂/22.1♀ mg/kg bw/day)

<u>Haematology:</u>
▪ (↓) Mean cell volume (MCV) in ♂ [month 6 (3%)].
▪ (↓) Hb in ♀ [month 18 (5% ncdr) and month 27 (7% ndr)].
▪ (↓) MCHC in ♀ [month 18 (3% ncdr) and month 27 (2%)].
▪ (↓) total WBC in ♀ [month 6 (21% ncdr) and month 12 (30% ncdr).
▪ (↓) total no. lymphocites in ♀ [month 12 (30% ncdr).
▪ (↓) total no. neutrophiles in ♂ [month 27 (45%)].

<u>Clinical chemistry:</u>
▪ (↓) albumin in ♂ [month 12 (5%)].
▪ (↓) globulin in ♀ [month 6 (8% ncdr)].
▪ (↑) albumin/globulin (A/G) ratio in ♀ [month 6 (8% ncdr)] and in ♀ [month 18 (8% ndr)].
▪ (↓) sodium in ♂ [month 6 (5% ndr) and month 12 (7%)].
▪ (↑) calcium in ♂ [month 6 (8% ndr)].
▪ (↓) phosphate in ♂ [month 6 (16% ndr) and month 12 (17% ndr)] and in ♀ [month 27 (12% ncdr)].
▪ (↑) glucose in ♂ [month 6 (9% ndr) and month 12 (17%).
▪ (↑) creatinine in ♂ [month 6 (10% ndr)] and in ♀ [month 6 (8% ndr)].
▪ (↓) AST in ♂/♀ [month 18 (45% ndr/32% ndr)] and ♀ [month 27(19% ndr)].
▪ (↓) ALT in ♂ [month 18 (55%)].
▪ (↓) BUN in ♀ [month 18 (18% ndr)].
▪ (↓) urate in ♂ [month 27 (19% ncdr)].
▪ (↑) free T4 in ♂ [month 27 (49%)].
▪ (↑) cholesterol in ♀ [month 27 (33% ncdr)].
▪ (↓) LDH in ♀ [month 27 (41%)].

<u>Organ weights:</u>
▪ Liver: (↑) abs wt in ♂/♀ (month 27 (24%/12% ncdr) and (↑) rel wt in ♂/♀ [month 12 (13% ncdr/6% ncdr) and month 27 (19% ncdr/9% ncdr)].
▪ Spleen: (↓) abs wt in ♀ [month 12 (8% ndr)].
▪ Brain: (↑) abs wt in ♂ [month 27 (3% ncdr)].
▪ Gonads: (↑) abs wt in ♂ [month 27 (17% ndr)].

<u>Histopathology:</u>
Interim sacrifice (I), interim deaths (D) and terminal deaths (T).
Total = I+D+T</td>
<td>Anonymous 42 (1985a)
Anonymous 43 (1985-1988) (report addendum)
Anonymous 44 (1988) (consideration of thyroid changes)
Seamons, M.C. & Crofts, M. (1985) (dietary concentrations)
(AS)
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 [Effects statistically significant and dose-related unless stated otherwise as not significant (n.s.) or not dose-related (ndr) or not clearly dose-related (ncdr)]	Reference

	Males							
	I		D		T		Total	
Dose (ppm)	0	400	0	400	0	400	0	400
Liver								
Centrilobular hepatocyte vacuolation	3/20†	**10/20***	0/26	0/29	0/24	1/21	3/70†	**11/70***
Centrilobular hepatocyte enlargement	0/20†	**18/20***	1/26	3/29	0/24†	**10/21***	1/70†	**31/70***
Focal hepatocyte necrosis	2/20†	5/20	3/26	3/29	0/24	0/21	5/70†	8/70
Focal cyst degeneration of hepatocytes	0/20	0/20	8/26	10/29	11/24	16/21	19/70†	26/70
Foci areas of telangiectasis	1/20	0/20	1/26	3/29	9/24	9/21	11/70	12/70
Fat deposits in non-specific distribution	0/20	0/20	4/26	8/29	7/24	11/21	11/70†	19/70
Thyroid								
Agglomeration of colloid	10/20†	14/20	17/26	13/29	12/24†	**18/21***	39/70†	45/70
Follicular cell hyperplasia	0/20	1/20	1/26	1/29	1/24	3/21	2/70	5/70
Harderian gland								
Interstitial mononuclear cell infiltration	3/20†	**10/20***	4/26	6/29	5/24	8/21	12/70†	**24/70***
	Females							
Kidney								
Glomerular tubular nephropathy	1/20	0/20	6/30†	**14/27***	6/20	8/23	13/70	22/70
Liver								
Foci areas of telangiectasis	0/20	0/20	2/30	6/27	10/20	16/23	12/70†	22/70

*indicates pairwise statistical significance. † Significative positive trend significant

Neoplastic changes

Thyroid

- Follicular cell tumor (n.s.) in ♂ considering total tumors in interim deaths during treatment (D) and terminal sacrifice animals (T): (8/50 (3 benign, 2 probably malignant and 3 malignant) *vs.* 2/50 of controls (1 benign and 1 malignant) with significative positive trend along dose levels ($p<0.01$). The incidence of thyroid follicular cell tumours in high dose ♂ treated with clofentezine was marginally higher than the control group from the other concurrently run study (8/50 *vs.* 6/49) performed at the same conductig laboratory with the same procedure, strain, diet and pathologist.

40 ppm (1.72♂/2.18♀ mg/kg bw/day)

<u>Clinical chemistry:</u>

- (↓) sodium in ♂ [month 6 (3% ndr)].
- (↑) calcium in ♂ [month 6 (11% ndr)].
- (↓) urate in ♂ [month 27 (24% ncdr)].

<u>Organ weights:</u>

- Liver: (↑) abs wt in ♂ in month 27 (12%).

<u>Histopathology:</u>

Non-neoplastic changes

No liver effects of centrilobular hepatocyte enlargement or centrilobular hepatocyte vacuolation in ♂ at this dose level.

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results [Effects statistically significant and dose-related unless stated otherwise as not significant (n.s.) or not dose-related (ndr) or not clearly dose-related (ncdr)]	Reference
		(see table below) *Neoplastic changes* Thyroid ▪ Follicular cell tumor (n.s.) in ♂ considering total tumors in interim deaths during treatment (D) and terminal sacrifice animals (T): 2/50 (2 malignant) *vs.* 2/50 (1 bening and 1 malignant) of controls regarded not relevant. **10 ppm** (0.43♂/0.55♀ mg/kg bw/day) Clinical chemistry: ▪ (↓) sodium in ♂ [month 6 (3% ndr)]. Organ weights: ▪ Liver: (↑) abs wt in ♂ in month 27 (11%). Histopathology: *Neoplastic changes* Thyroid ▪ Follicular cell tumor (n.s.) in ♂ considering total tumors in interim deaths during treatment (D) and terminal sacrifice animals (T): (2/50 (1 benign and 1 malignant) *vs.* 2/50 (1 benign and 1 malignant) of controls regarded not relevant. NOAEL$_{toxicity}$: **40 ppm** corresponding to **1.72/2.18 mg/kg bw**/day for ♂ and ♀ respectively. NOAEL$_{carcinogenicity}$: >**400 ppm** corresponding to **17.3/22.1 mg/kg bw/day** for ♂ and ♀ respectively.	
Long-term oral toxicity and carcinogenicity study in mice **(105 weeks)** *Huntingdon Research Centre* Method OECD 451 GLP: No (prior to GLP enforcement) **Study acceptable** Mice strain: Charles River CD-1 52 mice/sex/dose (main group)	Purity: 98.7% Oral (diet) Doses: 0, 50, 500 and 5000 ppm equivalent to: Males: 0, 5.0, 50.7 and 543.4 mg/kg bw/day Females: 0, 5.3, 56.9, 557.1 mg/kg bw/day Parameters observed: Mortality, clinical signs, bodyweights, food consumption, haematology, macroscopic	*Mortality:* At the end of the treatment period, the number of deaths in female mice at 5000 ppm was significantly higher than controls and other treated groups. (see mortality table below) *Clinical signs:* no treatment related clinical signs. **5000 ppm** (543.4♂/557.1♀ mg/kg bw/day) Bodyweight gain ▪ (↓) bw gain in ♂ on 0-26 weeks (22%) and 0-52 weeks (15%). Haematology: ▪ (↓) RBC in ♂ [week 52 (12%)] not observed at week 105. ▪ (↑) MCV in ♀ [week 52 (7% ndr)] not observed at week 105. ▪ (↓) WBC in ♂ [week 105 (51% ndr)] and lymph counts in ♂ [week 105 (53% ndr)]. Organ weights and gross pathology: ▪ (↑) Abs liver wt in ♀ (18% ncdr). ▪ (↑) Abs heart wt in ♀ (22% ncdr) not associated to histopathology. ▪ (↑) Abs testes wt in ♂ (21%). The weight of the testes (0.40 g) was above	**Anonymous 45 (1985)** **Cherry C.P., et al, (1985) (Addendum to histopathology)** **Crofts M., (1985) (Determination dietary concentration)** **ADAMA Makhteshim Ltd (Feb 2015) (Historical hepatocellular tumours data)** **Earl L., (2016) (Historical Histopathology Data Report)** **(AS) B.6.5.2**

	Males							
	I		**D**		**T**		**Total**	
Dose (ppm)	0	40	0	40	0	40	0	40
Thyroid								
Follicular cell hyperplasia	0/20	2/20	1/26	1/23	1/24	5/27	2/70	8/70
Harderian gland								
Interstitial mononuclear cell infiltration	3/20	5/20	4/26	3/23	5/24	7/27	12/70	15/70

Parameter	Dose Group (ppm)							
	Males				Females			
	0	**50**	**500**	**5000**	**0**	**50**	**500**	**5000**
No. of deaths[1]	38	35	35	40	27	24	25	**42****
No. of survivors at week 105	14	17	17	12	25	28	27	10
% survival at week 105	27	33	33	23	48	54	52	19
% mortality at week 105	73	67	67	77	52	46	48	81

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results [Effects statistically significant and dose-related unless stated otherwise as not significant (n.s.) or not dose-related (ndr) or not clearly dose-related (ncdr)]	Reference
10 mice/sex/dose for an additional group for blood sampling	and microscopic pathology	(see below)	

of historical controls provided conducted at Huntingdon Research Centre between March 1980 and July 1983 (range 0.323-0.39 g) and not associated to histopathology.

<u>Microscopic findings</u>

<u>Non-neoplastic findings (statistics not performed)</u>

- (↑) Amyloidosis (♀) in one or more organs: major contributory factor to death (19/42 *vs* 6/27 of controls). There was no evidence of this effect in ♂.

Amyloidosis		Females Dose level (ppm)	
		0	**5000**
Number of animals examined	**D**	**27**	**42**
	T	**25**	**10**
Amyloid present (in one or more organs)	D	6 (21%)	19 (44%)
	T	12 (48%)	3 (30%)

D: Animals dying or killed during study
T: Animals killed at termination

The amyloidosis in animals dying or killed during study (D) was dose-dependent but in animals killed at termination of study (T) was clearly not dose dependent.

- Liver: (↑) Foci/ areas of altered hepatocytes (eosinophilic) above of historical controls CD-1 mice in ♂ (D: 19.5% *vs.* 5.1% of controls and T: 18.2% *vs.* 7.7% of controls but ndr) and ♀ (D: 9.5% *vs.* 3.7% of controls and T: 50% *vs.* 8% of controls).

Non neoplastic findings liver		Males Dose level (ppm) 0	Males Dose level (ppm) 5000	HC	Females Dose level (ppm) 0	Females Dose level (ppm) 5000	HC
Number of animals examined	D	39	41	1775	27	42	1773
	T	13	11		25	10	
Eosinophilic hepatocytes	D	2 (5.1%)	8 (19.5%)	46 0.0-9.8%	1 (3.7%)	4 (9.5%)	35 0.0-9.1%
	T	1 (7.7%)	2 (18.2%)		2 (8%)	5 (50%)	

D: Animals dying or killed during study
T: Animals killed at termination
HC (Huntingdon Research Centre between March 1980 and July 1983).

In ♂, the incidence of focal eosinophilic hepatocytes in animals D was dose-dependent but not in T animals. Increases in ♀ (both D and T).

<u>Neoplastic findings</u>

Liver:

- (↑) Total beningn hepatic tumours [7/52 (13.5%)] in ♀ with respect to controls [4/52 (7.7%)] and above HCD (0-7.7%) from 26 studies with CD-1 mice of duration between 92 and 115 weeks conducted at Huntigdon Research Centre (1980-83). The incidence was not significant after pairwise comparison (p>0.05) but showed a positive trend after trend analysis (p<0.01). It has to be noted that the incidence in controls is in the upper HCD value of 7.7%.
- (↑) Total malignant hepatic tumours [1/52 (1.9%)] in ♀ with respect to controls [0/52 (0%)] below HCD (0-3.8%) and not statistically significant.
- The combined analysis of benign and/or malignant hepatic tumours in ♀ (8/52 *vs.* 4/52 of controls) was significant after pairwise comparison (p<0.05) and showed a positive trend after trend analysis (p<0.01).

500 ppm (50.7♂/56.9♀ mg/kg bw/day)<u>Haematology</u>:

- (↑) MCV in ♀ [week 52 (10% ndr)] not observed at week 105.
- (↓) Lymph counts in ♂ on [week 105 (40% ndr)].

<u>Microscopic findings (non-neoplastic findings):</u>

- Liver: (↑) Foci/ areas of altered hepatocytes (eosinophilic) above of historical controls in ♂ (D: 11.1% *vs.* 5.1% of controls and T: 25% *vs.* 7.7% of controls but ndr) and ♀ (D: 8% *vs.* 3.7% of controls and T: 18.5% *vs.* 8% of controls).

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results [Effects statistically significant and dose-related unless stated otherwise as not significant (n.s.) or not dose-related (ndr) or not clearly dose-related (ncdr)]	Reference

Non neoplastic findings liver		Males		HC	Females		HC
		Dose level (ppm)			Dose level (ppm)		
		0	500		0	500	
Number of animals examined	D	39	36	1775	27	25	1773
	T	13	16		25	27	
Eosinophilic hepatocytes	D	2 (5.1%)	4 (11.1%)	46 0.0-9.8%	1 (3.7%)	2 (8%)	35 0.0-9.1%
	T	1 (7.7%)	4 (25%)		2 (8%)	5 (18.5%)	

D: Animals dying or killed during study
T: Animals killed at termination
HC (Huntingdon Research Centre between March 1980 and July 1983).

Neoplastic findings

Liver:

- No increase in total beningn hepatic tumours [3/52 (5.8%)] in ♀ with respect to controls [4/52 (7.7%)] and within HCD (0-7.7%).
- No malignant hepatic tumours observed.

50 ppm (5♂/5.3♀ mg/kg bw/day)

Haematology:

- (↓) Lymph counts in ♂ on [week 105 (44% ndr)].

Non-neoplastic findings (statistics not performed)

- Liver: (↑) Foci/ areas of altered hepatocytes (eosinophilic) in ♂ above CD-1 mice historical controls for T animals (23.5% *vs.* 7.7% of controls but ndr) but not for D animals (8.6% *vs.* 5.1% of controls).

Non neoplastic findings liver		Males		HC	Females		HC
		Dose level (ppm)			Dose level (ppm)		
		0	50		0	50	
Number of animals examined	D	39	35	1775	27	24	1773
	T	13	17		25	28	
Eosinophilic hepatocytes	D	2 (5.1%)	3 (8.6%)	46 0.0-9.8%	1 (3.7%)	1 (4.1%)	35 0.0-9.1%
	T	1 (7.7%)	4 (23.5%)		2 (8%)	2 (7.1%)	

D: Animals dying or killed during study
T: Animals killed at termination
HC (Huntingdon Research Centre between March 1980 and July 1983).

Neoplastic findings

- No increase in total benign hepatic tumours [3/52 (5.8%)] in ♀ with respect to controls [4/52 (7.7%)] and within HCD (0-7.7%).
- No malignant hepatic tumours observed

$NOAEL_{toxicity}$: **50 ppm** corresponding to **5.0** and **5.3 mg/kg bw/day** for ♂and ♀ respectively

$NOAEL_{carcinogenicity}$: **500 ppm** corresponding to **50.7♂/56.9** ♀ mg/kg bw/day for ♂ and ♀ respectively.

Table 22: Summary table of other (mode of action) studies relevant for carcinogenicity

For more detailed information see RAR B6 (AS) chapter B.6.8.2

Type of study, laboratory, guideline, GLP, test substance(purity), route administration, strain, dose levels, no animals/group, acceptability	Results [Effects statistically significantly and dose-related unless stated otherwise as not significant (n.s.) or not dose-related (ndr) or not clearly dose-related (ncdr)]	Reference
See below	See below	**Anonymous 46 (1985)** Bright J.H.M. & Crofts M. (1985) (dietary concentrations) **B.6.8.2.1-01 (AS)**

Type of study, laboratory, guideline, GLP, test substance(purity), route administration, strain, dose levels, no animals/group, acceptability:

Oral studies (dietary) of 4 weeks in rats and mice.

Lab: Schering Agrochemicals Limited

Guideline: No test method available.

GLP: No (prior to GLP enforcement)

Test substance:

Radiolabelled:

- ✓ [^{14}C]-tetrazine ring labelled clofentezine, specific activity: 47.7 mCi/g, radiopurity: >99%.
- ✓ [125I]-Thyroxine specific activity: 200 µCi/g, radiopurity: --.
- ✓ [^{131}I]-Sodium iodide specific activity: 40 mCi/g, radiopurity: --.

Non radiolabelled:

- ✓ Clofentezine used for tissue residue accumulation study (purity: 98.8%; batch: CR 20099/12) and
- ✓ Clofentezine used for thyroid iodine uptake and thyroxine half-life studies (purity: 99.3%; batch: CR 20099/14).

Route administration: Oral (gavage)

Strain:

- ✓ Rat strain: Charles River Sprague Dawley
- ✓ Mice strain: Charles River CD-1

Dose levels/No animals:

Part 1: *Tissue residue accumulation study in male and female rats*

- ✓Dose and exposure: 20 mg/kg bw twice-daily for 10 days
- ✓No animals: 10 male and 10 female rats

Part 2: *Thyroxine half-life study in male rats*

- ✓Dose and exposure: 0, 30000 ppm (equivalent 3000 mg/kg bw/day) for 4 weeks
- ✓No animals: (10 male rats/group

Results:

Tissue residue accumulation in rat

Clofentezine accumulation in thyroid and pituitary is not significantly higher compared to other tissues.

Tissue	Accumulation ratios[a]	
	♂	♀
Adrenals	2.10	3.07
Thyroid	**2.99**	**3.60**
Pituitary	2.70	2.75
Testes/Ovaries	2.74	2.48
Eyes	2.06	2.72
Liver	3.57	3.39
Kidney	2.38	2.57
Heart	2.89	3.42
Lungs	2.82	3.45
Spleen	3.98	3.69
Bone	2.33	1.96
Brain	3.06	3.44
Muscle	2.35	2.35
Fat	0.94	1.89
Blood	3.25	4.42
Plasma	1.66	2.27
Mean	**2.61**	**2.97**

[a]Accumulation ratio = mean residue after 10 day dosing/mean residue after 1 day dosing

Thyroxine half-life in rat

↓Slight (6.8%) in T_4 half-life compared to control.

Half-life of [^{125}I]-thyroxine	Dose level (ppm)	
	0	**30000**
Before treatment (h)	16.70	17.05
After 1 month treatment (h)	17.61	16.42 (↓6.8%)

Thyroid iodine uptake

Rat:

↑ Rapid and significant in thyroid uptake of iodine by thyroid 6 hours after dosing.

Thyroid iodine uptake		Dose level (ppm)			
		0	**30000**	**0**	**30000**
		♂		♀	
Thyroid (cpm/ thyroid)	6 h	113400	**189600**** ↑67%	114000	**301700***** ↑164.6%
	24 h	163500	210200 ↑28.5%	144900	**277100**** ↑91%
Blood (cpm/mL)	6 h	13300	**10900**** ↓18%	15900	**12800**** ↓19.5%
	24 h	5500	**4600*** ↓16%	4800	**3900**** ↓18.8%

**= p<0.05, ** = p<0.01, *** = p<0.001 (Mann Whitney)*

<table>
<tr><th>Type of study, laboratory, guideline, GLP, test substance(purity), route administration, strain, dose levels, no animals/group, acceptability</th><th>Results
[Effects statistically significantly and dose-related unless stated otherwise as not significant (n.s.) or not dose-related (ndr) or not clearly dose-related (ncdr)]</th><th>Reference</th></tr>
<tr><td>Part 3: Thyroid iodine uptake study in rats and mice
✓Dose and exposure: 0, 30000 ppm (equivalent to 3000 and 4500 mg/kg bw/day for rats and mice respectively)[1] for 4 weeks
No animals: 20 of each sex and species, 10/group).
Study acceptable</td>
<td>Mice:
No ↑ in thyroid uptake of iodine by thyroid 6 hours after dosing. Levels of radioactivity 6 hours after dosing were similar in control and treated animals.
<table>
<tr><th rowspan="3" colspan="2">Thyroid iodine uptake</th><th colspan="4">Dose level (ppm)</th></tr>
<tr><th>0</th><th>30000</th><th>0</th><th>30000</th></tr>
<tr><th colspan="2">♂</th><th colspan="2">♀</th></tr>
<tr><td rowspan="2">Thyroid (cpm/thyroid)</td><td>6 h</td><td>77600</td><td>73000</td><td>67900</td><td>60100</td></tr>
<tr><td>24 h</td><td>85900</td><td>129600** ↑50.9%</td><td>60100</td><td>82400</td></tr>
<tr><td rowspan="2">Blood (cpm/mL)</td><td>6 h</td><td>1550</td><td>800*** ↓48%</td><td>1700</td><td>950** ↓44%</td></tr>
<tr><td>24 h</td><td>350</td><td>400</td><td>350</td><td>350</td></tr>
</table></td>
<td></td></tr>
<tr><td>Oral study (dietary) of 2-3 weeks in rats.
The effect of the clofentezine on the biliary excretion of thyroxine in the male rat (effect on thyroid hormone turnover)
Lab: Schering Agrochemicals Limited
Guideline: No test method available.
GLP: Yes
Test substance:
Radiolabelled:
✓ L- [125I]-Thyroxine specific activity: 200 µCi/ml radiopurity: no data available; assumed pure
✓ Route administration: intravenous (into a tail vein)
✓ Dose: 5µCi (approx 100 ng)
Non radiolabelled:
✓ Clofentezine technical (purity: 99.3 %/ batch no: CR 20099/15)
✓ Route administration: Oral dietary
✓ Dose: 0, 30000 ppm (equivalent to 0, 1500 mg/kg bw/day mg/kg bw/day)
Rat strain (male): Charles River (UK), Sprague Dawley CD (SD) rats.
No animals: 6 rats/dose
Study acceptable</td>
<td>Bile flow rate.
The bile flow rate was almost doubled in treated animals that of control animals over the entire four hour period (6.06 mL/hour/kg vs 3.1 mL/hour/kg in controls).
Bile flow rate (ml/hour/kg)
TIME (min)
o - Controls (bars represent S.D.) n=6
■ - Treated (bars represent S.D.) n=5

Excretion of 125I into bile
There was a marked increased from treated animals (59%) compared to control rats (10.2% of dose in four hours vs 6.4% control).
Cumulative biliary excretion of [125I] (%dose)
TIME (min)
o - Controls (bars represent S.D.) n=5
■ Treated (bars represent S.D.) n=5</td>
<td>Anonymous 47 (1988)

B.6.8.2.1-07 (AS)</td></tr>
</table>

[1] Test article consumption not calculated in the report. Based on recommendations from the JMPR, a conversion factor of 1 ppm equivalent to 0.1 mg/kg bw/day and 0.15 mg/kg bw/d for rats and mice, respectively was used (WHO/JMPR. Guidelines for the preparation of toxicological working papers for the WHO Core Assessment Group of the Joint Meeting on Pesticide Residues. Geneva, December 2000)

Type of study, laboratory, guideline, GLP, test substance(purity), route administration, strain, dose levels, no animals/group, acceptability	Results [Effects statistically significantly and dose-related unless stated otherwise as not significant (n.s.) or not dose-related (ndr) or not clearly dose-related (ncdr)]	Reference
	<u>Clearance of ^{125}I from bloom</u> In pretreated rats, the initial clearance rate (over the first 15 min) of an intravenous dose of [^{125}I]-thyroxine from blood was 52% of that in control animals (2.20 ml/hr *vs* 1.45 ml/hr control), but was the same at later time points (2.5 - 4 hours after dosing). <u>Concentration of ^{125}I in bile</u> The biliary concentration of ^{125}I was higher in treated animals only for the first 30 minutes when excretion was highest. Thereafter the concentration of ^{125}I in bile of treated rats was lower, owing to the increase in bile flow being greater than the increase in biliary excretion of ^{125}I. <u>The amount of ^{125}I present in bile as thyroxine and thyroxine glucuronide</u> ▪ The amount of **[^{125}I]-thyroxine** excreted in the bile was lower (approx. 60% expressed as percentage of total ^{125}I and approx. 50% expressed as pg thyroxine equivalents) in treated than of that in control rats, over a four hour period.	

Sampling time (minutes)	Dose (ppm)			
	0	30000	0	30000
	Percentage of total ^{125}I		pg thyroxine equivalents	
45 – 60	18.03	3.89 ↓78%	89.06	29.99 ↓66%
105 – 120	16.84	6.72 ↓60%	77.21	44.31 ↓43%
165 – 180	14.35	4.26 ↓70%	53.62	24.06 ↓55%
225 – 240	16.85	7.33 ↓56%	55.71	32.40 ↓42%

<table>
<tr><th>Type of study, laboratory, guideline, GLP, test substance(purity), route administration, strain, dose levels, no animals/group, acceptability</th><th>Results
[Effects statistically significantly and dose-related unless stated otherwise as not significant (n.s.) or not dose-related (ndr) or not clearly dose-related (ncdr)]</th><th>Reference</th></tr>
<tr><td></td><td>

- ↑ Excretion of **[125I]-thyroxine glucuronide** for the first two hours after dosing (approx. 50%) but fell afterwards to below that found in control rats. When the increased bile flow was considered the treated rats excreted more than double the amount of [125I]-thyroxine glucuronide in the first two hours, but over the second two hour time period the amount excreted was the same in both groups.

Sampling time (minutes)	Dose (ppm)			
	0	30000	0	30000
	Percentage of total ^{125}I		pg thyroxine equivalents	
45 – 60	7.06	11.02 ↑56%	34.87	84.97 ↑144%
105 – 120	9.80	14.93 ↑52%	44.93	98.46 ↑119%
165 – 180	15.64	10.47 ↓33%	58.44	59.14 ↑1%
225 – 240	13.71	11.56 ↓16%	45.33	51.09 ↑12%

The total excretion of ^{125}I into bile was higher at all time points in treated rats, but the amount excreted as thyroxine and its glucuronide conjugate was generally lower.

Sampling time (minutes)	Total ^{125}I		^{125}I-Thyroxine /thyroxine glucuronide	
	Dose (ppm)			
	0	30000	0	30000
	pg thyroxine equivalents			
45 – 60	493.93	771.01	123.93	114.96
105 – 120	458.49	659.43	122.14	142.77
165 – 180	373.65	564.85	112.06	83.20
225 – 240	330.64	441.97	101.04	83.49

These results indicate that the increased biliary excretion of ^{125}I from ^{125}I-thyroxine was not due solely to an increased glucuronidation, but it is likely that other metabolic routes for this compound are also increased (most probably oxidative deamination and decarboxylation).

Conclusion: The profile of metabolites in bile indicates that the action of clofentezine on the thyroid gland is caused by an increased turnover of thyroid hormones due to induction of the hepatic enzymes responsible for the catabolism of T_4 and resulting in an increased excretion of thyroxine metabolites. This mode of action closely mimics that of phenobarbitone.
</td><td></td></tr>
<tr><td>

Oral study (dietary) of 5 weeks in rats.

The effect of the clofentezine on the excretion of thyroxine in the male rat.

Lab: Schering Agrochemicals Limited

Guideline: No test method available.

GLP: Yes

Test substance:

Radiolabelled:

✓ L- [125I]-Thyroxine specific activity: 200 mCi/ml
</td><td>

- ↓ Urinary elimination of T_4 and/or its metabolites (15.31% of the dose vs 27.23% control)
- ↑ Faecal elimination of T_4 and/or its metabolites (40.4% of the dose vs 26.45 % control)
</td><td>

Anonymous 48 (1988)

B.6.8.2.1-08 (AS)
</td></tr>
</table>

<table>
<tr><th>Type of study, laboratory, guideline, GLP, test substance(purity), route administration, strain, dose levels, no animals/group, acceptability</th><th>Results
[Effects statistically significantly and dose-related unless stated otherwise as not significant (n.s.) or not dose-related (ndr) or not clearly dose-related (ncdr)]</th><th>Reference</th></tr>
<tr><td>radiopurity: no data available; assumed pure / batch no: 673BA
✓ Route administration: intravenous (into a tail vein)
✓ Dose: 5μCi (aprox 100 ng)
Non radiolabelled:
✓ Clofentezine technical (purity: 99.3 %/ batch no: CR 20099/15)
✓ Route administration: Oral dietary
✓ Dose: 0, 30000 ppm (equivalent to 0, 1500 mg/kg bw/day mg/kg bw/day)
Rat strain (male): Charles River (UK). Sprague Dawley CD.
No. animals: 5 rats/dose
Study acceptable</td><td>
<table>
<tr><th rowspan="3">Sampling time (minutes)</th><th colspan="4">Dose (ppm)</th></tr>
<tr><th>0</th><th>30000</th><th>0</th><th>30000</th></tr>
<tr><th colspan="2">% Urinary elimination</th><th colspan="2">% Faecal elimination</th></tr>
<tr><td>3 h</td><td>0.35</td><td>0.71</td><td>-</td><td>0.0</td></tr>
<tr><td>6 h</td><td>1.77</td><td>1.19</td><td>-</td><td>0.0</td></tr>
<tr><td>9 h</td><td>0.39</td><td>0.22</td><td>-</td><td>0.0</td></tr>
<tr><td>12 h</td><td>2.43</td><td>2.03</td><td>-</td><td>0.78</td></tr>
<tr><td>24 h</td><td>7.68</td><td>3.97</td><td>3.69</td><td>7.33</td></tr>
<tr><td>28 h</td><td>0.46</td><td>0.21</td><td>-</td><td>3.57</td></tr>
<tr><td>32 h</td><td>3.38</td><td>0.98</td><td>1.60</td><td>4.12</td></tr>
<tr><td>48 h</td><td>4.96</td><td>3.66</td><td>10.42</td><td>13.06</td></tr>
<tr><td>54 h</td><td>2.07</td><td>0.73</td><td>1.06</td><td>2.54</td></tr>
<tr><td>72 h</td><td>3.74</td><td>1.61</td><td>11.48</td><td>14.99</td></tr>
<tr><td>TOTAL</td><td>27.23</td><td>15.31</td><td>26.45</td><td>40.40</td></tr>
</table>
▪ Slight ↑ in the total excretion (58.3% vs 56.7% control)
<table>
<tr><th rowspan="2">Tissue</th><th colspan="2">Dose (ppm)</th></tr>
<tr><th>0</th><th>30000</th></tr>
<tr><td>Urine</td><td>27.23</td><td>15.31</td></tr>
<tr><td>Faeces</td><td>26.45</td><td>40.40</td></tr>
<tr><td>G.I. tract</td><td>3.03</td><td>2.57</td></tr>
<tr><td>TOTAL</td><td>56.71</td><td>58.27</td></tr>
</table>
CONCLUSION: the results show that clofentezine pretreatment produces a fundamental alteration in the route of elimination of thyroxine and/or metabolites increasing faecal elimination from 26.5% to 40.4% of the dose and decreasing urinary elimination from 27.2% to 15.3%. The overall changes are similar, both in magnitude and direction, to those previously documented for phenobarbitone.</td><td></td></tr>
<tr><td>Oral study (gavage) of 8 weeks in baboons.
Tolerance clofentezine in baboons
Lab: Schering Agrochemicals Limited
Guideline: No test method available
GLP: No (prior to GLP enforcement).
Test substance: Clofentezine technical NC 21314 (purity: 99.3%; batch no: CR 20099/14)
Route administration: Oral (gavage)
Baboon strain (male and female): Papio spp.
Dose: 4 doses daily at 0, 100, 200 or 400 mg/kg bw/day
No animals:1 baboon/sex
Study acceptable</td><td>Discolouration of the faeces from the first week of dosing in both animals.
Bouts of vomiting in the female baboon at 4 x 400 mg/kg bw/day, which continued when the dose level was reduced to 4 x 200 mg/kg bw/day. This ceased when a further reduction to 4 x 100 mg/kg bw/day and did not return when the level was increased to 4 x 200 mg/kg bw/day.
Serum triglyceride and cholesterol levels were unaffected.</td><td>Anonymous 49 (1983)

B.6.8.2.1-10
(AS)</td></tr>
</table>

<table>
<tr>
<th>Type of study, laboratory, guideline, GLP, test substance(purity), route administration, strain, dose levels, no animals/group, acceptability</th>
<th>Results
[Effects statistically significantly and dose-related unless stated otherwise as not significant (n.s.) or not dose-related (ndr) or not clearly dose-related (ncdr)]</th>
<th>Reference</th>
</tr>
<tr>
<td>
Oral study (dietary) of 9 weeks in rabbits.
The effect of the clofentezine on plasma cholesterol and triglyceride values in female rabbits.
<u>Lab</u>: Schering Agrochemicals Limited
<u>Guideline</u>: No test method available
<u>GLP</u>: No (prior to GLP enforcement).
<u>Test substance:</u> Clofentezine technical NC 21314 (purity: not stated ; batch no: CR 20099/8)
<u>Route administration</u>: Oral (fed diet)
<u>Rabbit strain</u> (female): New Zealand White
<u>Dose</u>: 0, 400, 4000/ 8000 ppm (equivalent to 0, 12, 120/240 mg/kg bw/day)
<u>No animals</u>: 6 rabbits/dose
Study acceptable
</td>
<td>
<u>8000 ppm</u>
<u>Plasma cholesterol and triglyceride levels</u>
▪ ↑ Plasma cholesterol (35%) and triglyceride (97%) values day 43. On day 57 the values were similar to the control group
<table>
<tr><th colspan="2">Parameter</th><th colspan="2">Dose levels (ppm)</th></tr>
<tr><th></th><th></th><th>0</th><th>8000</th></tr>
<tr><td rowspan="6">Cholesterol (mmol/l)</td><td>1st pre-test</td><td>3.06</td><td>-</td></tr>
<tr><td>2nd pre-test</td><td>3.09</td><td>-</td></tr>
<tr><td>Day 15</td><td>2.22</td><td>-</td></tr>
<tr><td>Day 29</td><td>2.27</td><td>-</td></tr>
<tr><td>Day 43</td><td>2.56</td><td>3.45*
↑35%</td></tr>
<tr><td>Day 57</td><td>2.74</td><td>3.28</td></tr>
<tr><td rowspan="6">Triglycerides (mmol/l)</td><td>1st pre-test</td><td>1.36</td><td>-</td></tr>
<tr><td>2nd pre-test</td><td>1.21</td><td>-</td></tr>
<tr><td>Day 15</td><td>1.46</td><td>-</td></tr>
<tr><td>Day 29</td><td>0.83</td><td>-</td></tr>
<tr><td>Day 43</td><td>0.73</td><td>1.44*
↑97%</td></tr>
<tr><td>Day 57</td><td>0.65</td><td>1.00</td></tr>
</table>
*p<0.05 (Mann Whitney U test)
<u>Macroscopic pathology</u>
▪ Slight to moderate excess abdominal fluid in 5/6 treatment animals.
▪ Retention of food in the stomach in 2/6 animals.
<u>Organ weight</u>
▪ ↑ Absolute and relative liver weight (30% absolute and 26% relative)
<table>
<tr><th>Parameter</th><th colspan="2">Dose levels (ppm)</th></tr>
<tr><th></th><th>0</th><th>8000</th></tr>
<tr><td>Terminal body weight (g)</td><td>2671</td><td>2770</td></tr>
<tr><td>Absolute liver weight (g)</td><td>54.79</td><td>71.30*
↑30%</td></tr>
<tr><td>Relative liver weight (%)</td><td>2.04</td><td>2.57*
↑26%</td></tr>
</table>
No histopathological abnormality was associated with the increases in liver weight
<u>4000 ppm</u>
<u>Plasma cholesterol and triglyceride levels</u>
Unaffected
<u>Organ weight</u>
▪ ↑ Relative liver weight (21%)
<table>
<tr><th>Parameter</th><th colspan="2">Dose levels (ppm)</th></tr>
<tr><th></th><th>0</th><th>4000</th></tr>
<tr><td>Terminal body weight (g)</td><td>2671</td><td>2792</td></tr>
<tr><td>Absolute liver weight (g)</td><td>54.79</td><td>68.15</td></tr>
<tr><td>Relative liver weight (%)</td><td>2.04</td><td>2.46*
↑21%</td></tr>
</table>
No histopathological abnormality was associated with the increases in liver weight
</td>
<td>
Anonymous 50 (1982)
B.6.8.2.1-09
(AS)
</td>
</tr>
</table>

Type of study, laboratory, guideline, GLP, test substance(purity), route administration, strain, dose levels, no animals/group, acceptability	Results [Effects statistically significantly and dose-related unless stated otherwise as not significant (n.s.) or not dose-related (ndr) or not clearly dose-related (ncdr)]	Reference

Oral studies (dietary) of 6 weeks in rats.

Lab: Schering Agrochemicals Limited

Guideline: No test method available.

GLP: No (prior to GLP enforcement)

Test substance: Clofentezine (purity: 99.3%; batch: CR 20099/14).

Route administration: Oral (dietary)

Rat strain: Charles River CR1: COBS CD (SD) BR Sprague Dawley

Dose levels/No animals:

***Part 1**: Investigation on thyroid function*

- ✓Dose: 0, 400, 30000 ppm (equivalent to 0, 40 and 3000 mg/kg bw/day)
- ✓No animals: 10 rats/sex/dose

***Part 2**: Electron microscopy of anterior pituitary and Thyroid*

- ✓Dose: 0, 400, 30000 ppm (equivalent to 0, 40 and 3000 mg/kg bw/day)
- ✓No animals: 5 rats/sex/group in pituitary gland and 5 rats/sex/group in thyroid gland

***Part 3**: Morphometric study on the thyroid glands in rats*

- ✓Dose: 0, 30000 ppm (equivalent to 0 and 3000 mg/kg bw/day)
- ✓No animals: 5 male rats /group

Study acceptable

30000 ppm (3000 mg/kg bw/day)

Hormone analysis:

- ↑ T_3 (♂22%)
- ↑ T_4 (28% ♂ and 31%♀)
- ↓T4-binding capacity (♀16%)
- ↑ Free T_4 index (♂ 38% and ♀ 54%),
- ↑ TSH (♂ 67% and ♀109%)
- ↑ Progesterone (♂ 170% and ♀51%) and DHEAS (♂ 171% and ♀405%).

Parameter	Dose (ppm)			
	0	30000	0	30000
	Males		Females	
Total T_3 (nmol/L)	0.9	**1.1*** ↑22.2%	1.1	1.1
T4: Thyroxine (nmol/L)	54	**69***** ↑28%	51	**67***** ↑31 %
T_4-binding capacity	0.77	0.71	0.77	**0.65*** ↓16%
Free T_4 index	71	**98***** ↑38%	68	**105***** ↑54%
TSH: Thyrothrophin (ng/mL)	5.4	**9*** ↑67%	3.5	**7.3***** ↑109%
Progesterone (nmol/L)	14.8	**40**** ↑170%	89.1	**134.7*** ↑51%
DHEAS: Dehydroepian-drosterone (µmol/L)	0.52	**1.41***** ↑171%	0.44	**2.22***** ↑405%

Organ weights:

- ↑ Liver weight (Abs: ♂ 62% and ♀ 54% and Rel ♂ 66% and ♀ 74%).

Parameter		Dose (ppm)			
		0	30000	0	30000
		Males		Females	
Terminal body weight (g)		350	340	224	**197***** ↓12%
Liver weight	Abs (g)	12.2	**19.8***** ↑62%	8.4	**12.9***** ↑54%
	Rel (%)	3.5	**5.8***** ↑66%	3.8	**6.6***** ↑74%

Histopathology:

Anterior pituitary

- Minimal hypertrophy and dilatation of rough endoplasmic reticulum in some of the cells that produce TSH (thyrotrophs) from 5/5 males.
- Occasional secretory granules within the cisternae of the rough endoplasmic reticulum in some thyrotrophs (4/5 male rats). Secondary lysosomes were seen in the cells of two of these rats.

Anterior pituitary analysis confirmed ↑ TSH synthesis.

Thyroid

- ↑Total thyroid area (58%): Enlargement
- ↑Total number of thyroid follicular cells (47%): Hyperplasia

Reference:

Anonymous 51 (1986)

(Investigation on thyroid function)

Anonymous 52 (1988)

(Addendum to histopathology)

Anonymous 53 (1986)

(Electron microscopy of anterior pituitary and Thyroid)

Anonymous 54 (1989)

Morphometric study on the thyroid glands in rats

B.6.8.2.1-02a

B.6.8.2.1-02b

B.6.8.2.1-02c

(AS)

Type of study, laboratory, guideline, GLP, test substance(purity), route administration, strain, dose levels, no animals/group, acceptability	Results [Effects statistically significantly and dose-related unless stated otherwise as not significant (n.s.) or not dose-related (ndr) or not clearly dose-related (ncdr)]	Reference
	(see below)	

Parameter	Dose (ppm)	
	0	30000
	Males	
Area of thyroid (mm)	2523	**3978*** (↑58%)
No. of follicles	730.3	909.7
No. of thyroid follicular cells	15781	**23132**** (↑47%)

*= p<0.05, ** = p<0.01 (one-sided significance test)

- Colloid depletion and follicular cell hypertrophy

400 ppm (40 mg/kg bw/day)

Hormone analysis:

- ↑ Serum T4 (15%) in ♂

Parameter	Dose (ppm)			
	0	400	0	400
	Males		Females	
T4: Thyroxine (nmol/L)	54	**62*** ↑15%	51	54

Organ weights:

- ↑ Liver weight in males only (Abs 24% and Rel 14%).

Parameter		Dose (ppm)			
		0	400	0	400
		Males		Females	
Terminal body weight (g)		350	380*	224	221
Liver weight	Abs (g)	12.2	**15.1***** ↑24%	8.4	8.8
	Rel (%)	3.5	**4.0***** ↑14%	3.8	4.0

Histopathology:

Anterior pituitary

- Minimal hypertrophy and dilatation of rough endoplasmic reticulum in thyrotrophs (1/4 males).

CONCLUSION

At 30000 ppm (3000 mg/kg bw/day) clofentezine altered thyroid metabolism in the male and females rat as evidenced by increases in serum thyroxine, free thyroxine index, thyrotropin (TSH) and (in males only) tri-iodothyronine.

Treatment at 400 ppm (40 mg/kg bw/day) affected thyroid metabolism only in males, causing a serum thyroxine increase.

Type of study, laboratory, guideline, GLP, test substance(purity), route administration, strain, dose levels, no animals/group, acceptability	Results [Effects statistically significantly and dose-related unless stated otherwise as not significant (n.s.) or not dose-related (ndr) or not clearly dose-related (ncdr)]	Reference
	Thyroids hormone analysis	

Parameter	Dose (ppm)					
	0	400	30000	0	400	30000
	Males			Females		
Total T_3 (nmol/L)	0.9	1	**1.1*** ↑22%	1.1	1.1	1.1
Thyroxine (nmol/L)	54	**62*** ↑15%	**69***** ↑28%	51	54	**67***** ↑31.4%
T_4-binding capacity	0.77	0.79	0.71	0.77	0.8	**0.65* ↓16%**
Free T_4 index	71	84	**98***** ↑38%	68	71	**105***** ↑54%
TSH (ng/mL)	5.4	5.7	**9*** ↑67%	3.5	3.7	**7.3***** ↑109%

*= p<0.05, ** = p<0.01, *** = p<0.001 (Bartlett´s test)

Progesterone and dehydroepiandrosterone were significantly and dose-dependent increased in both sexes at 30000 ppm (Progesterone increase 170% in males and 51% in females and DHEAS 171% in males and 405% in females). At 400 ppm dehydroepiandrosterone was increased in females only (50%)

Other hormone analysis

Parameter	Dose (ppm)					
	0	400	30000	0	400	30000
	Males			Females		
Testosterone (nmol/L)	12.3	12.6	21.4	0.8	0.7	0.7
Oestradiol (nmol/L)	0.5	0.3*	0.4	0.7	0.6	0.6
Progesterone (nmol/L)	14.8	17.7	**40**** ↑170%	89	90.7	**134.7*** ↑51%
DHEAS: Dehydroe-piandrosterone (µmol/L)	0.52	0.57	**1.41***** ↑171%	0.4	**0.66*** ↑50%	**2.22***** ↑405%

*= p<0.05, ** = p<0.01, *** = p<0.001 (Bartlett´s test)

Absolute and relative liver weight was significantly increased in both sexes at 30000 ppm and in males only at 400 ppm.

Liver weight

Parameter		Dose (ppm)					
		0	400	30000	0	400	30000
		Males			Females		
Terminal body weight (g)		350	380*	340	224	221	**197***** ↓12%
Liver weight	Abs (g)	12	**15.1***** ↑24%	**19.8***** ↑62%	8.4	8.8	**12.9***** ↑54%
	Rel (%)	3.5	**4.0***** ↑14%	**5.8***** ↑66%	3.8	4.0	**6.6***** ↑74%

*= p<0.05, ** = p<0.01, *** = p<0.001 (Students t test)

The morphological features of anterior pituitary at 30000 ppm included the presence of intracisternal secretory granules[2] and minimal hypertrophy and dilatation[3] of the rough endoplasmic reticulum (RER)

[2] *These ultrastructural changes are similar to those seen in thyroidectomized rats by Farquhar (1969), who suggests that enhanced hormone in the cisternae of the rough endoplasmic reticulum, resulting in the formation of intracisternal secretory granules.*

[3] *The dilated cisternae contained an amorphous material of medium electron density. Ghandially (1975) suggested this may represent an accumulation of secretory product, similar to that in plasma cells.*

Type of study, laboratory, guideline, GLP, test substance(purity), route administration, strain, dose levels, no animals/group, acceptability	Results [Effects statistically significantly and dose-related unless stated otherwise as not significant (n.s.) or not dose-related (ndr) or not clearly dose-related (ncdr)]	Reference
	in some thyrotrophs in males. This 7 an increase of TSH synthesis.	

Anterior pituitary histopathology

Parameter	Dose (ppm)					
	0	400	30000	0	400	30000
	Males			Females		
ANTERIOR PITUITARY						
Hypertrophy/dila-tation of RER	0/5	1/5	**5/5**	0/5	0/5	0/5
Secretory granules within the cisternae of RER	0/5	0/5	**4/5**	0/5	0/5	0/5
Secondary lysosomes	0/5	0/5	**2/5**	0/5	0/5	0/5

In thyroids, there was evidence of follicular cell hypertrophy (enlargement) associated with follicular cell hyperplasia (Increase of no. of thyroid follicular cells) at 30000 ppm in males. The usual mechanism of these responses by the follicular cell is mediated by increased TSH levels due to decrease in the levels of thyroid hormones.

Thyroid histopathology

Parameter	Dose (ppm)	
	0	30000
	Males	
THYROID		
Area of thyroid (mm): Enlargement	2523	**3978*** (↑58%)
No. of thyroid follicular cells	15781	**23132**** (↑47%)

*= p<0.05, ** = p<0.01 (Students t test)

Oral study (dietary) of 13 weeks in rats.

Lab: Schering Agrochemicals Limited

Guideline: No test method available.

GLP: Yes

Test substance: Clofentezine NC 21314 (batch: CR 20099/15; purity: ≥99.3%).

Route administration: Oral (fed diet)

Rat strain: Charles River Crl: COBS CD (SD) BR Sprague Dawley

Dose: 0, 10, 40, 400 or 30000 ppm (equivalent to 0, 0.71, 2.88, 28.9, or 2250 mg/kg bw/day) for 4, 8 or 13 weeks.

No. animals: 60 male rats/dose

Study acceptable

30000 ppm (2250 mg/kg bw/day)

Body weight:

- ↓Body weight (~10%) throughout the treatment period.

Serum biochemistry:

- ↑Total protein ncdr (11% at 4, 8 and 13 weeks).
- ↑Globulin ncdr (18, 19 and 16% at 4, 8 and 13 weeks respectively).
- ↓A:G ratio (10% throughout the treatment period.)
- ↑Cholesterol ncdr (48, 55 and 68% at 4, 8 and 13 weeks respectively).
-

Parameter		Dose level (ppm)	
		0	30000
		Males	
Total protein (g/L)	4 weeks	56.4	**62.4***** ↑11%
	8 weeks	57	**63***** ↑11%
	13 weeks	57.9	**64***** ↑11%
Total Globulin (g/L)	4 weeks	28.2	**33.2***** ↑18%
	8 weeks	27.9	**33.2***** ↑19%
	13 weeks	29.1	**33.9***** ↑16%

Reference: **Anonymous 55 (1990)**

B.6.8.2.1-03

(AS)

Type of study, laboratory, guideline, GLP, test substance(purity), route administration, strain, dose levels, no animals/group, acceptability	Results [Effects statistically significantly and dose-related unless stated otherwise as not significant (n.s.) or not dose-related (ndr) or not clearly dose-related (ncdr)]	Reference
	(see below)	

A/G ratio (1:1)	4 weeks	1	**0.9****	↓10%
	8 weeks	1	**0.9*****	
	13 weeks	1	**0.9***	
Cholesterol (mM)	4 weeks	1.79	**2.65***** ↑48%	
	8 weeks	2.1	**3.26***** ↑55%	
	13 weeks	2.36	**3.96***** ↑68%	

Hormone and enzyme analysis:

- ↑ TSH ncdr (107, 239 and 145 % at weeks 4, 8 and 13, respectively).
- ↑ Total T_4 ncdr at week 8 (27%)
- ↑ Free T_4 ndr at week 8 (19%)
- ↓ Free T_3 ncdr (19% at weeks 4)
- ↑ Liver microsomal UDPGT levels (321, 280 and 253% at weeks 4, 8 and 13, respectively)

Parameters		**Dose level (ppm)**	
		0	**30000**
		Males	
Thyrotrophin (TSH) (ng/mL)	4 wk	6	**12.4***** ↑107%
	8 wk	5.1	**17.3***** ↑239%
	13 wk	6.4	**15.7***** ↑145%
Total T_4(nM)	4 wk	79	84
	8 wk	62	**79**** ↑27%
	13 wk	64	77
Free T_4(pM)	4 wk	12.9	13.3
	8 wk	8.6	**10*** ↑19%
	13 wk	8.1	9
Total T_3(nM)	4 wk	2	2
	8 wk	2	1.9
	13 wk	1.7	1.9
Free T_3(pM)	4 wk	2.1	**1.7*** ↓19%
	8 wk	2	1.7
	13 wk	2	1.7
Microsomal UDPGT (U/L/mg)	4 wk	46	**194***** ↑321%
	8 wk	41	**156***** ↑280%
	13 wk	52	**184***** ↑253%

**= p<0.05, ** = p<0.01, *** = p<0.001*

Organ weight**:**

- ↑ Absolute and relative liver weight (Abs: 53% and 63% at weeks 4 and 13, respectively; Rel: 69% at weeks 4 and 13).
- ↑ Absolute and relative thyroid weight at 13 weeks (Abs 25% and Rel 50%)

Type of study, laboratory, guideline, GLP, test substance(purity), route administration, strain, dose levels, no animals/group, acceptability	Results [Effects statistically significantly and dose-related unless stated otherwise as not significant (n.s.) or not dose-related (ndr) or not clearly dose-related (ncdr)]	Reference
	(see below)	

Organ weights		Dose level (ppm) 0	30000
		♂	
LIVER			
Absolute (g)	4 weeks	11.86	**18.11***** ↑53%
	13 weeks	13.97	**22.81***** ↑63%
Relative (%)	4 weeks	3.79	**6.42***** ↑69%
	13 weeks	3.2	**5.4***** ↑69%
THYROID			
Absolute (g)	13 weeks	0.020	**0.025*** ↑25%
Relative (%)	13 weeks	0.004	**0.006***** ↑50%

*= $p<0.05$, ** = $p<0.01$, *** = $p<0.001$

Histopathology:

Thyroid gland

- ↑ Severity colloid depletion 10/10 [(1 slight/6 moderate/3 severe)] *vs* 6/10 control [(4 minimal/2 slight)] at week 4
- ↑ Severity follicular cell size [10/10 (8 moderate /2 severe)] *vs* [9/10 (3 minimal/6 slight)] control at week 4.

Pituitary:

- ↑ Incidence focal hypertrophy of thyrotrophs in 7/10 males *vs* 1/10 males in control at week 4

400 ppm (28.9 mg/kg bw/day)

Serum biochemistry:

- ↑Globulin at 13 week (8%)
- ↑Cholesterol at 13 weeks ncdr (22%)

Hormone and enzyme analysis:

- ↑ Free T_4 ndr at week 4 and 8 (22 and 19% respectively).
- ↑ Liver microsomal UDPGT levels (87, 63 and 73% at weeks 4, 8 and 13, respectively).

Parameters		Dose level (ppm) 0	400
		Males	
Microsomal UDPGT (U/L/mg)	4 weeks	46	**83***** ↑87%
	8 weeks	41	**67***** ↑63%
	13 weeks	52	**90***** ↑73%

*= $p<0.05$, ** = $p<0.01$, *** = $p<0.001$

Organ weight:

- ↑ Absolute and relative liver weight (Abs: 13% at week 13; Rel: 10 and 12.5 % at week 4 and 13 respectively).

Type of study, laboratory, guideline, GLP, test substance(purity), route administration, strain, dose levels, no animals/group, acceptability	Results [Effects statistically significantly and dose-related unless stated otherwise as not significant (n.s.) or not dose-related (ndr) or not clearly dose-related (ncdr)]	Reference
	(see below)	

Organ weights		Dose level (ppm) 0	400
		♂	
LIVER			
Absolute (g)	4 weeks	11.86	12.45
	13 weeks	13.97	**15.72*** ↑13%
Relative (%)	4 weeks	3.79	**4.17***** ↑10%
	13 weeks	3.2	**3.6**** ↑12.5%

*= $p<0.05$, ** = $p<0.01$, *** = $p<0.001$

CONCLUSION

At 30000 ppm (2250 mg/kg bw/day), body weight gain was reduced by 10% throughout the treatment period. Clinical chemistry investigations at 4, 8 and 13 weeks showed significant increases in total protein, total globulin, and cholesterol whilst there was a slight reduction in the A:G ratio.

Clinical chemistry values

Parameter		0	10	40	400	30000
		Dose level (ppm) Males				
Total protein (g/L)	4 weeks	56.4	56.5	59	57	**62.4***** ↑11%
	8 weeks	57	57.4	58.6	58.8	**63***** ↑11%
	13 weeks	57.9	58.9	**61****	60.1	**64***** ↑11%
Total Globulin (g/L)	4 weeks	28.2	28.3	29	28.3	**33.2***** ↑18%
	8 weeks	27.9	28.4	29.3	28.8	**33.2***** ↑19%
	13 weeks	29.1	30.3	**30.9***	**31.3***	**33.9***** ↑16%
A/G ratio (:1)	4 weeks	1	1	1	1	**0.9**** ↓10%
	8 weeks	1	1	1	1	**0.9***** ↓10%
	13 weeks	1	1	1	0.9	**0.9*** ↓10%
Cholesterol (mM)	4 weeks	1.79	1.94	1.97	1.92	**2.65***** ↑48%
	8 weeks	2.1	1.86	2.21	2.23	**3.26***** ↑55%
	13 weeks	2.36	2.35	2.31	**2.87*** ↑22%	**3.96***** ↑68%

At 30000 ppm hormone measurements showed an increase in thyrotrophin at each time point. A very slight but statistically significant decrease in free T_3 was observed at 4 weeks only and total T_4 was marginally elevated at 8 and 13 weeks.

Type of study, laboratory, guideline, GLP, test substance(purity), route administration, strain, dose levels, no animals/group, acceptability	Results [Effects statistically significantly and dose-related unless stated otherwise as not significant (n.s.) or not dose-related (ndr) or not clearly dose-related (ncdr)]	Reference
	Hormone analysis	

Parameters		Dose level (ppm) 0	10	40	400	30000
		Males				
Thyrotrophin (TSH) (ng/mL)	4 wk	6	5.3	6.9	7.1	**12.4***** ↑107%
	8 wk	5.1	6.5	6.5	5.9	**17.3***** ↑239%
	13 wk	6.4	5.7	6.7	6.7	**15.7***** ↑145%
Total T_4(nM)	4 wk	79	77	87	87	84
	8 wk	62	66	66	68	**79**** ↑27%
	13 wk	64	64	64	71	**77 ↑20%**
Free T_4(pM)	4 wk	12.9	14	14.3	**15.7**** ↑22%	13.3
	8 wk	8.6	9.9	8.5	**10.2*** ↑19%	**10*** ↑19%
	13 wk	8.1	8.8	9	8.5	9
Total T_3 (nM)	4 wk	2	2.1	2.1	2	2
	8 wk	2	1.9	1.9	1.9	1.9
	13 wk	1.7	1.8	1.7	1.8	1.9
Free T_3 (pM)	4 wk	2.1	2	2.1	1.8	**1.7*** ↓19%
	8 wk	2	2	1.9	1.8	1.7
	13 wk	2	1.9	1.9	1.9	1.7

At doses ≥ 400 ppm (≥28.9 mg/kg bw/day) there was an increase in liver microsomal UDPGT at 4, 8 and 13 weeks.

Enzyme analysis

Parameters		Dose level (ppm) 0	10	40	400	30000
		Males				
Microsomal UDPGT (U/L/mg)	4 wk	46	52	49	**83***** ↑87%	**194***** ↑321%
	8 wk	41	44	48	**67***** ↑63%	**156***** ↑280%
	13 wk	52	49	52	**90***** ↑73%	**184***** ↑253%

At doses ≥ 400 ppm (≥28.9 mg/kg bw/day) the absolute and relative liver weight was significantly increased (at 400 ppm, Abs: 13 % at 13 weeks and Rel: 10% at 4 and 13% at 13 weeks. At 30000 ppm, Abs: 53% and 63% at 4 and 13 weeks respectively and Rel: 69% at 4 and 13 weeks).

At dose 30000 ppm absolute and relative thyroid weight was significantly increased at 13 weeks (25% and 50% absolute and relative respectively).

Liver and Thyroid weight

Organ weights		Dose level (ppm) 0	10	40	400	30000
		♂				
LIVER						
Abs (g)	4 weeks	11.86	11.43	11.50	12.45	**18.11***** ↑53%
	13 weeks	13.97	14.29	14.78	**15.72*** ↑13%	**22.81***** ↑63%

<table>
<tr><th>Type of study, laboratory, guideline, GLP, test substance(purity), route administration, strain, dose levels, no animals/group, acceptability</th><th>Results
[Effects statistically significantly and dose-related unless stated otherwise as not significant (n.s.) or not dose-related (ndr) or not clearly dose-related (ncdr)]</th><th>Reference</th></tr>
<tr><td></td><td>
<table>
<tr><td rowspan="2">Rel (%)</td><td>4 weeks</td><td>3.79</td><td>3.82</td><td>3.88</td><td>4.17*** ↑10%</td><td>6.42*** ↑69%</td></tr>
<tr><td>13 weeks</td><td>3.2</td><td>3.1</td><td>3.2</td><td>3.6** ↑13%</td><td>5.4*** ↑69%</td></tr>
<tr><td colspan="7">THYROID</td></tr>
<tr><td>Abs (g)</td><td>13 weeks</td><td>0.020</td><td>0.020</td><td>0.018</td><td>0.018</td><td>0.025* ↑25%</td></tr>
<tr><td>Rel (%)</td><td>13 weeks</td><td>0.004</td><td>0.004</td><td>0.004</td><td>0.004</td><td>0.006*** ↑50%</td></tr>
</table>
At 30000 ppm, in the thyroid, there was a statistically significant increase in colloid depletion and follicular cell size after 4 weeks. Additionally, focal hypertrophy of pituitary thyrotrophs was seen in 7/10 animals.
Thyroid and Pituitary histopathology
<table>
<tr><th colspan="2" rowspan="3">Parameters</th><th colspan="5">Dose level (ppm)</th></tr>
<tr><th>0</th><th>10</th><th>400</th><th>3000</th><th>30000</th></tr>
<tr><th colspan="5">Males</th></tr>
<tr><td colspan="7">THYROID</td></tr>
<tr><td>Colloid depletion</td><td>Week 4</td><td>6/10</td><td>5/10</td><td>3/10</td><td>5/10</td><td>10/10**</td></tr>
<tr><td>Follicular cell size (hypertrophy)</td><td>Week 4</td><td>9/10</td><td>9/10</td><td>8/10</td><td>10/10</td><td>10/10**</td></tr>
<tr><td colspan="7">PITUITARY</td></tr>
<tr><td>Focal hypertrophy</td><td>Week 4</td><td>1/10</td><td>0/10</td><td>0/10</td><td>1/10</td><td>7/10</td></tr>
</table>
**p<0.01
Note: No tissues were examined from animals killed at subsequent sampling points.
No treatment-related effects were seen at 10 or 40 ppm.
</td><td></td></tr>
<tr><td>Oral study (dietary) of 13 weeks in rabbits.
Investigation of thyroid function in the male and female rabbits.
Lab: Schering Agrochemicals Limited
Guideline: No test method available.
GLP: No (prior to GLP enforcement)
Test substance: Clofentezine (batch: 20099/14; Purity: 99.3%)
Route administration: Oral (diet)
Rabbit strain: New Zealand White
Dose: 0 and 8000 ppm (equivalent to 0, 441♂/409♀ mg/kg bw/day)
No. animals: 5 rabbits/sex/dose
Study acceptable</td>
<td>8000 ppm (441♂/409♀ mg/kg bw/day)
No effect on thyroid hormone levels or thyroid morphology.
↑ Absolute liver weight (25%) only in ♀.</td>
<td>Anonymous 56
B.6.8.2.1-04
(AS)</td></tr>
<tr><td>Single oral dose study in several species
Influence of clofentezine on biofuntions
Lab: Schering Agrochemicals Limited
Guideline: No test method</td>
<td>At oral doses of 100, 300 and 1000 mg/kg bw/day or at concentrations of 10^{-9}, 10^{-8} and 10^{-7} (isolates tissues) no influence of clofentezine on biological functions:
Blood coagulation (plasma prothrombin time and activated partial thromboplastin) and skeletal muscle (twitch response induced by phrenic nerve stimulation in the diaphragm) in rats</td>
<td>Anonymous 57 (1987)
B.6.8.2.1-11
(AS)</td></tr>
</table>

Type of study, laboratory, guideline, GLP, test substance(purity), route administration, strain, dose levels, no animals/group, acceptability	Results [Effects statistically significantly and dose-related unless stated otherwise as not significant (n.s.) or not dose-related (ndr) or not clearly dose-related (ncdr)]	Reference
available GLP: No. Test substance: Clofentezine technical NC 21314 (purity: ≥99.3%; batch no: CR 20099/15) Animals: ✓Male Wistar **rats** a. Evaluation: blood coagulation Dose: 0, 100, 300, 1000 mg/kg bw/day. No animals: 6/dose Route administration: Oral (dietary) b. Evaluation: Skeletal muscle Dose: 0, 10^{-9}, 10^{-8}, 10^{-7} g/ml No animals: 5/dose Route administration: *in vitro* ✓Male ddY **mice** Evaluation: Central nervous system (CNS), behavioural observations, bleed time and digestive tract Dose: 0, 100, 300, 1000 mg/kg bw/day. No animals: 10/dose Route administration: Oral (dietary) ✓Male **cats** Evaluation: Respiratory and circulatory systems Dose: 0, 100, 300, 1000 mg/kg bw/day. No animals: 1/dose Route administration: oral (dietary) ✓Male Hartley **guinea pig** Evaluation: contraction movement ileum with agonists (Ach, His and $BaCl_2$) Dose: 0, 10^{-9}, 10^{-8}, 10^{-7} g/ml. No animals: 5/dose Route administration: *in vitro* ✓Male albino Japanesse **rabbit:** a. Evaluation: spontaneous movement ileum Dose: 0, 10^{-9}, 10^{-8},10^{-7} g/ml No animals: 3/dose Route administration: *in vitro* b. Evaluation: haemolysis test Dose: 0, 10^{-9}, 10^{-8}, 10^{-7} g/ml No animals: 3/dose Route administration: *in vitro* **Study acceptable**	▪ Central nervous system (CNS), behavioural observations, bleeding time and digestive tract (charcoal transportation in intestinal canal) in **mice** ▪ Respiratory (respiration) and circulatory systems (blood pressure, heart rate and EEG) in **cat**. ▪ Contraction movement ileum in **guinea pig** ▪ Spontaneous movement ileum and haemolysis test (haemolytic index) in **rabbits**	

Type of study, laboratory, guideline, GLP, test substance(purity), route administration, strain, dose levels, no animals/group, acceptability	Results [Effects statistically significantly and dose-related unless stated otherwise as not significant (n.s.) or not dose-related (ndr) or not clearly dose-related (ncdr)]	Reference
Oral study (dietary) of 2 weeks in rats. *Indirect effect of clofentezine on the thyroid of the male rat* Lab: Schering Agrochemicals Limited Guideline: No test method available. GLP: Yes Test substance: Clofentezine (batch: 20099/15; Purity: >99.3%) Route administration: Oral (diet) Rat strain (male): Charles River CR1: COBS CD (SD) BR Sprague Dawley Dose: 0, 30000 ppm (equivalent to 1915 mg/kg bw/day) No. animals: 50 rats/dose **Study acceptable**	See results below	**Anonymous 58 (1988)** **B.6.8.2.1-05** **(AS)**

30000 ppm (♂1915 mg/kg bw/day)

Hormone analysis:

- ↓ T_3 (20%) following 2 days of treatment and after 7 days (25%). After 14 days, T_3 levels had returned to control
- ↑ TSH after 4 days of treatment (42%). After 14 days, TSH levels remained elevated (37%).

Parameters		Dose level (ppm) 0	30000
		Males	
Total T_4 (nM)	24 h	83	92
	2 days	85	92
	4 days	70	73
	7 days	77	70
	14 days	80	79
Total T_3 (nM)	24 h	1.5	1.4
	2 days	2.0 [a]	**1.6*** ↓20%
	4 days	1.6	**1.4*** ↓12.5%
	7 days	1.6	**1.2**** ↓25%
	14 days	1.6	1.6
TSH: Thyrotrophin (ng/mL)	24 h	5.8	5.9
	2 days	8.4	7.2
	4 days	5.3	**7.5**** ↑42%
	7 days	5.2	**7.6***** ↑46%
	14 days	4.9	**6.7*** ↑37%

**= p<0.05, ** = p<0.01, *** = p<0.001*

Organ weights:

- ↑ Absolute and relative liver weight (abs 16% and rel 17%) following 2 days of treatment rising to 60% after 4 days and remaining elevated throughout the treatment period.

Parameters		Dose level (ppm) 0	30000
		Males	
Absolute (g)	2 days	13.9	12.38*
	3 days	11.40	**13.19**** ↑16%
	5 days	13.25	**20.59**** ↑55%
	8 days	14.25	**20.12**** ↑41%
	15 days	14.41	**22.47**** ↑56%
Relative (%)	2 days	3.59	3.347
	3 days	3.179	**3.705**** ↑17%
	5 days	3.362	**5.378**** ↑60%
	8 days	3.536	**5.019**** ↑42%
	15 days	3.390	**5.440**** ↑60%

Type of study, laboratory, guideline, GLP, test substance(purity), route administration, strain, dose levels, no animals/group, acceptability	Results [Effects statistically significantly and dose-related unless stated otherwise as not significant (n.s.) or not dose-related (ndr) or not clearly dose-related (ncdr)]	Reference
	▪ ↑ Absolute and relative thyroid weight (abs 16% and rel 19%) following 2 days of treatment. After 4 days the thyroid weight was similar to controls.	

Parameters		Dose level (ppm) 0	30000
		Males	
Absolute (g)	2 days	0.020	0.022
	3 days	0.019	**0.022*** ↑16%
	5 days	0.020	0.022
	8 days	0.020	0.020
	15 days	0.022	0.022
Relative (%)	2 days	0.0051	0.0058
	3 days	0.0052	**0.0062*** ↑19%
	5 days	0.0050	0.0057
	8 days	0.0050	0.0051
	15 days	0.0053	0.0057

*= p<0.05, ** = p<0.01, *** = p<0.001

Histopathology:

Morphological changes in the thyroid were not apparent after 1 or 2 days.

After 4 days proliferative stimulation of the follicular lining cells was evidenced by an increased incidence of mitotic activity. This cellular division was maximal at 7 days and some activity was still evident at 14 days. (4 days: 5/10 rats *vs* 1/10 control; 7 days: 7/10 *vs* 0/10 control; 14 days: 5/10 *vs* 0/10 control).

Colloid depletion, follicular cell hyperplasia and hypertrophy were seen at 7 days and were marked at 14 days.

Parameters		Dose level (ppm) 0	30000
		Males	
Colloid depletion	2 days	4/10	6/10
	4 days	7/10	8/10
	7 days	6/10	**9/10**
	14 days	4/10	**10/10**
Follicular cell size (hypertrophy)	2 days	7/10	7/10
	4 days	8/10	9/10
	7 days	8/10	**10/10**
	14 days	7/10	**10/10**
Follicular cell hyperplasia	2 days	0/10	1/10
	4 days	1/10	2/10
	7 days	0/10	**4/10**
	14 days	0/10	**8/10**
Increased mitotic activity follicular cells (lining)	2 days	0/10	1/10
	4 days	1/10	**5/10**
	7 days	0/10	**7/10**
	14 days	0/10	**5/10**
Central resting follicles	2 days	4/10	6/10
	4 days	0/10	**6/10**
	7 days	4/10	**7/10**
	14 days	7/10	7/10

<table>
<tr><th>Type of study, laboratory, guideline, GLP, test substance(purity), route administration, strain, dose levels, no animals/group, acceptability</th><th>Results
[Effects statistically significantly and dose-related unless stated otherwise as not significant (n.s.) or not dose-related (ndr) or not clearly dose-related (ncdr)]</th><th>Reference</th></tr>
<tr><td></td><td><u>CONCLUSION</u>
The study showed that in rats, clofentezine resulted in a very early (after 2 days) and marked increase in liver weight associated with reduced levels of the active thyroid hormone (T_3). After 4 days, feedback mechanisms compensations results in a restoration hormone balance as, after 14 days, T3 was no longer significantly reduced. As a result of the feedback mechanism a significant increase in TSH (which was maximal after 7 days) and the stimulation and proliferation of the thyroid glands (hypertrophy and hyperplasia) were observed. The increased thyroid stimulation was maintained by the levels of TSH that remained elevated up to and including the last sampling point after 14 days.

The Notifier concludes that over the longer term it is possible that this effect of clofentezine on the liver, leading to altered thyroid homeostasis could cause the small increase in thyroid follicular cell tumours observed in the high dose level male rats in the combined chronic and oncogenicity study. However it should be noted that the dose levels used in this study are much higher than those in the carcinogenicity study and the results are not nearly as marked as for compounds known to cause tumours via this feedback mechanism.</td><td></td></tr>
<tr><td>Oral study (dietary) of 4 weeks in rats.
Indirect effect on thyroid of clofentezine in the male rat
<u>Lab</u>: Schering Agrochemicals Limited
<u>Guideline</u>: No test method available.
<u>GLP</u>: No
<u>Test substance</u>: Clofentezine (batch: 20099/15; <u>Purity</u>: ≥99.3%)
<u>Purity</u>: Not stated
<u>Route administration</u>: Oral (fed diet)
<u>Rat strain</u> (male): Sprague Dawley
<u>Dose</u>[(*)]: 0, 10, 400, 3000 or 30000 ppm (equivalent to 0, 0.58, 22.69, 169.4 and 1635 mg/kg bw/day).
<u>No. animals</u>: 80 male rats/dose

Study acceptable

[(*)]<u>Note</u>: dose levels were selected on the following basis:
<u>10 ppm</u>: the same as the low dose level used in the rat chronic study
<u>400 ppm</u>: the same as the high dose level used in the rat chronic study
<u>3000 ppm</u>: to provide a broader dose range
<u>30000 ppm</u>: the highest dose known to be tolerated by the rat over</td><td><u>30000 ppm</u> (1635 mg/kg bw/day)
<u>Body weight and food consumption</u>:
▪ ↓Body weight gain (~14%) throughout the treatment period (5-29 days).
▪ ↓Food consumption (18%) during the first 4 days of treatment. At the end of the treatment period (28 days), food consumption was reduced by approximately 6%.
<table>
<tr><th colspan="2" rowspan="3">Parameters</th><th colspan="2">Dose level (ppm)</th></tr>
<tr><th>0</th><th>30000</th></tr>
<tr><th colspan="2">Males</th></tr>
<tr><td rowspan="4">Body weight (g)</td><td>5 days</td><td>386.7</td><td>380.2</td></tr>
<tr><td>8 days</td><td>392.2</td><td>390.4</td></tr>
<tr><td>15 days</td><td>404.6</td><td>408.7</td></tr>
<tr><td>29 days</td><td>442.9</td><td>428.3</td></tr>
<tr><td>Body weight gain (g)</td><td>(5-29 days)</td><td>56.2</td><td>48.1
(↓14%)</td></tr>
<tr><td rowspan="4">Food consumption (g/animal/day)</td><td>4 days</td><td>23.03</td><td>19.02*
↓17%</td></tr>
<tr><td>7 days</td><td>23.72</td><td>22.01*
↓7%</td></tr>
<tr><td>14 days</td><td>24.03</td><td>23.98</td></tr>
<tr><td>28 days</td><td>24.07</td><td>22.67*
↓6%</td></tr>
</table>
*= p<0.05 (Dunnett´s test of significance)</td><td>Anonymous 59 (1989)

B.6.8.2.1-06
(AS)</td></tr>
</table>

Type of study, laboratory, guideline, GLP, test substance(purity), route administration, strain, dose levels, no animals/group, acceptability	Results [Effects statistically significantly and dose-related unless stated otherwise as not significant (n.s.) or not dose-related (ndr) or not clearly dose-related (ncdr)]	Reference
subchronic period for demonstrating the mode of action on the liver and thyroid.	Clinical chemistry: ▪ ↑Liver microsomal UDPGT five times *vs* control from 5 days	

Parameters		Dose level (ppm) 0	Dose level (ppm) 30000
		Males	
Microsomal protein (mg/g)	5 days	21.76	**28.54*** ↑31%
	8 days	14.97	**25.00***** ↑67%
	15 days	19.00	**31.80*** ↑67%
	29 days	27.43	**35.99***** ↑31%
Microsomal UDPGT (DAminmg)	5 days	0.019	**0.098***** ↑416%
	8 days	0.034	**0.125***** ↑268%
	15 days	0.019	**0.101***** ↑432%
	29 days	0.018	**0.098***** ↑444%

*= $p<0.05$, ** = $p<0.01$, ***=$p<0.001$

Organ weight

▪ ↑Absolute and relative liver weight approximately 60%. This effect was throughout the treatment period.

Parameters		Dose level (ppm) 0	Dose level (ppm) 30000
		Males	
Absolute liver weight (g)	5 days	13.59	**18.44**** ↑36%
	8 days	13.46	**19.69**** ↑46%
	15 days	13.05	**21.90**** ↑68%
	29 days	14.46	**22.40**** ↑55%
Relative liver weight (%)	5 days	3.508	**4.852**** ↑38%
	8 days	3.350	**5.091**** ↑52%
	15 days	3.259	**5.265**** ↑62%
	29 days	3.262	**5.228**** ↑60%

*= $p<0.05$, ** = $p<0.01$

Type of study, laboratory, guideline, GLP, test substance(purity), route administration, strain, dose levels, no animals/group, acceptability	Results [Effects statistically significantly and dose-related unless stated otherwise as not significant (n.s.) or not dose-related (ndr) or not clearly dose-related (ncdr)]	Reference
	▪ ↑Absolute and relative thyroid weight after 7 days until termination (absolute 17-41% and relative 25-36%)	

Parameters		Dose level (ppm) 0	30000
		Males	
THYROID			
Absolute thyroid weight (g)	5 days	0.017	0.018
	8 days	0.016	**0.020**** ↑25%
	15 days	0.017	**0.024**** ↑41%
	29 days	0.024	**0.028**** ↑17%
Relative thyroid weight (%)	5 days	0.0044	0.0047
	8 days	0.0040	**0.0051**** ↑28%
	15 days	0.0042	**0.0057**** ↑36%
	29 days	0.0053	**0.0066**** ↑25%

*= $p<0.05$, ** = $p<0.01$

Histopathology:

- Marked depletion of colloid, with 20/20 animals showing at least a slight response compared with only 4/20 controls on 28 days.
- ↑ Incidence and severity of distinct follicular cell hypertrophy and hyperplasia after 4 and 7 days respectively until the end of treatment period.
- ↑ Mitotic activity in the follicular cells was observed from 4 days until the end of treatment period.

Parameters		Dose level (ppm) 0	30000
		Males	
Colloid depletion	4 days	9/20	15/20
	7 days	7/20	19/20
	14 days	5/20	18/20
	28 days	4/20	**20/20**
Follicular cell size	4 days	12/20	**16/20**
	7days	9/20	**19/20**
	14 days	7/20	**18/20**
	28 days	10/20	**20/20**
Follicular cell hyperplasia	4 days	0/20	0/20
	7 days	0/20	**6/20**
	14 days	2/20	**5/20**
	28 days	0/20	**8/20**
Increased mitotic activity follicular cells	4 days	1/20	**9/20**
	7days	0/20	**15/20**
	14 days	1/20	**12/20**
	28 days	0/20	**12/20**

3000 ppm (169.4 mg/kg bw/day)

Clinical chemistry:

- Not measured liver microsomal UDPGT

Type of study, laboratory, guideline, GLP, test substance(purity), route administration, strain, dose levels, no animals/group, acceptability	Results [Effects statistically significantly and dose-related unless stated otherwise as not significant (n.s.) or not dose-related (ndr) or not clearly dose-related (ncdr)]	Reference
	Organ weight ▪ ↑Absolute and relative liver weight approximately 30% throughout the treatment period.	

Parameters		Dose level (ppm)	
		0	3000
		Males	
Absolute liver weight (g)	5 days	13.59	**17.48**** ↑29%
	8 days	13.46	**17.04**** ↑27%
	15 days	13.05	**19.34**** ↑48%
	29 days	14.46	**20.51**** ↑42%
Relative liver weight (%)	5 days	3.508	**4.555**** ↑30%
	8 days	3.350	**4.284**** ↑28%
	15 days	3.259	**4.466**** ↑37%
	29 days	3.262	**4.569**** ↑40%

**= p<0.05, ** = p<0.01*

▪ ↑Absolute and relative thyroid weight approximately 30% after 7 and 15 days.

Parameters		Dose level (ppm)	
		0	3000
		Males	
THYROID			
Absolute thyroid weight (g)	5 days	0.017	0.019
	8 days	0.016	**0.021**** ↑31%
	15 days	0.017	**0.022**** ↑29%
	29 days	0.024	0.026
Relative thyroid weight (%)	5 days	0.0044	0.0050
	8 days	0.0040	**0.0052**** ↑30%
	15 days	0.0042	**0.0051**** ↑21%
	29 days	0.0053	0.0058 ↑9%

**= p<0.05, ** = p<0.01*

Histopatology:

- Marked depletion of colloid, with 19/20 animals showing at least a slight response compared with only 4/20 controls on 28 days.
- ↑ Incidence of distinct follicular cell hypertrophy after 4 days until the end of treatment period.
- ↑ Mitotic activity in the follicular cells was observed after 4 days, thereafter declining although still detectable after 28 days.

Type of study, laboratory, guideline, GLP, test substance(purity), route administration, strain, dose levels, no animals/group, acceptability	Results [Effects statistically significantly and dose-related unless stated otherwise as not significant (n.s.) or not dose-related (ndr) or not clearly dose-related (ncdr)]	Reference
	(see below)	

Parameters		Dose level (ppm)	
		0	3000
		Males	
Colloid depletion	4 days	9/20	17/20
	7 days	7/20	12/20
	14 days	5/20	13/20
	28 days	4/20	**19/20**
Follicular cell size	4 days	12/20	**18/20**
	7 days	9/20	**14/20**
	14 days	7/20	**18/20**
	28 days	10/20	**19/20**
Follicular cell hyperplasia	4 days	0/20	3/20
	7 days	0/20	3/20
	14 days	2/20	3/20
	28 days	0/20	4/20
Increased mitotic activity follicular cells	4 days	1/20	**13/20**
	7 days	0/20	**7/20**
	14 days	1/20	**7/20**
	28 days	0/20	**5/20**

400 ppm (22.69 mg/kg bw/day)

Clinical chemistry:

- ↑Liver microsomal UDPGT two times *vs* control from day 5.

Parameters		Dose level (ppm)	
		0	400
		Males	
Microsomal protein (mg/g)	5 days	21.76	20.23
	8 days	14.97	**21.00*** ↑40%
	15 days	19.00	25.17
	29 days	27.43	28.77
Microsomal UDPGT (DAminmg)	5 days	0.019	**0.032*** ↑68%
	8 days	0.034	**0.065***** ↑91%
	15 days	0.019	**0.045***** ↑137%
	29 days	0.018	**0.041***** ↑128%

*p<0.05, ** p<0.01 and *** p<0.001*

Organ weight

- ↑Absolute and relative liver weight approximately 10%. This effect was from 15 days to the end of the treatment period.

Type of study, laboratory, guideline, GLP, test substance(purity), route administration, strain, dose levels, no animals/group, acceptability	Results [Effects statistically significantly and dose-related unless stated otherwise as not significant (n.s.) or not dose-related (ndr) or not clearly dose-related (ncdr)]	Reference

Parameters		Dose level (ppm)	
		0	400
		Males	
Absolute liver weight (g)	5 days	13.59	14.87
	8 days	13.46	14.06
	15 days	13.05	**15.40**** ↑18%
	29 days	14.46	15.90 ↑10%
Relative liver weight (%)	5 days	3.508	3.692
	8 days	3.350	3.466
	15 days	3.259	**3.763**** ↑15%
	29 days	3.262	**3.578**** ↑10%

*= $p<0.05$, ** = $p<0.01$

- No clear effect was seen on thyroid weight

Parameters		Dose level (ppm)	
		0	400
		Males	
THYROID			
Absolute thyroid weight (g)	5 days	0.017	**0.020***
	8 days	0.016	0.016
	15 days	0.017	**0.019****
	29 days	0.024	0.022
Relative thyroid weight (%)	5 days	0.0044	0.0049
	8 days	0.0040	0.0041
	15 days	0.0042	**0.0047***
	29 days	0.0053	0.0049

*= $p<0.05$, ** = $p<0.01$

Histopatology:

Thyroid gland

- Marked depletion of colloid, with 12/20 animals showing at least a slight response compared with only 4/20 controls (28 days).
- ↑Mitotic activity in the follicular cells was observed only after 4 days.

Parameters		Dose level (ppm)	
		0	400
		Males	
Colloid depletion	4 days	9/20	**11/20**
	7 days	7/20	**13/20**
	14 days	5/20	**9/20**
	28 days	4/20	**12/20**
Follicular cell size	4 days	12/20	13/20
	7 days	9/20	**16/20**
	14 days	7/20	**10/20**
	28 days	10/20	**14/20**
Follicular cell hyperplasia	4 days	0/20	0/20
	7 days	0/20	0/20
	14 days	2/20	**3/20**
	28 days	0/20	**1/20**
Increased mitotic activity follicular cells	4 days	1/20	**6/20**
	7days	0/20	2/20
	14 days	1/20	0/20
	28 days	0/20	0/20

Type of study, laboratory, guideline, GLP, test substance(purity), route administration, strain, dose levels, no animals/group, acceptability	Results [Effects statistically significantly and dose-related unless stated otherwise as not significant (n.s.) or not dose-related (ndr) or not clearly dose-related (ncdr)]	Reference
	CONCLUSION: Dose of ≥ 400 ppm (≥22.69 mg/kg bw/day) resulted in early histological changes in the thyroid gland with concurrent effects in liver. Effects in **thyroid** were an increase in thyroid weight, an increase in mitotic activity of the follicular cells observed at maximum level after 4 days of treatment (at 400 and 3000 ppm) and after 7 day of treatment (at 30000 ppm), thereafter declining although still detectable after 28 days, colloid depletion (from day 4 until the end of treatment period at doses ≥400 ppm with higher incidence after 28 days), hypertrophy from day 7 until the end of treatment period at 400 ppm and from day 4 until the end of treatment period at doses ≥3000 ppm and hyperplasia of the follicular lining cells from day 7 until the end of treatment period at 30000 ppm with higher incidence and severity after 28 days. In addition, there was, in general terms, a clear dose response relationship for all conditions at each time point.	

CHANGE THYROID

✓ Organ weight

Parameters		Dose level (ppm) 0	10	400	3000	30000
		Males				
THYROID						
Absolute weight (g)	5 days	0.017	0.018	**0.020***	0.019	0.018
	8 days	0.016	0.016	0.016	**0.021**** ↑31%	**0.020**** ↑25%
	15 days	0.017	0.017	**0.019**** ↑12%	**0.022**** ↑29%	**0.024**** ↑41%
	29 days	0.024	0.021	0.022	0.026	**0.028**** ↑17%
Relative weight (%)	5 days	0.0044	0.0044	0.0049	0.0050	0.0047
	8 days	0.0040	0.0040	0.0041	**0.0052**** ↑30%	**0.0051**** ↑28%
	15 days	0.0042	0.0042	**0.0047*** ↑12%	**0.0051**** ↑21%	**0.0057**** ↑36%
	29 days	0.0053	0.0047	0.0049	0.0058	**0.0066**** ↑25%

**= p<0.05, ** = p<0.01*

✓ Histopathology

Parameters		Dose level (ppm) 0	10	400	3000	30000
		Males				
Colloid depletion	4 days	9/20	10/20	**11/20**	**17/20**	**15/20**
	7 days	7/20	5/20	**13/20**	**12/20**	**19/20**
	14 days	5/20	7/20	**9/20**	**13/20**	**18/20**
	28 days	4/20	3/20	**12/20**	**19/20**	**20/20**
Follicular cell size (hypertrophy)	4 days	12/20	11/20	13/20	**18/20**	**16/20**
	7 days	9/20	7/13	**16/20**	**14/20**	**19/20**
	14 days	7/20	13/20	**10/20**	**18/20**	**18/20**
	28 days	10/20	4/20	**14/20**	**19/20**	**20/20**
Follicular cell hyperplasia	4 days	0/20	0/20	0/20	3/20	0/20
	7 days	0/20	0/20	0/20	3/20	**6/20**
	14 days	2/20	0/20	3/20	3/20	**5/20**
	28 days	0/20	0/20	1/20	4/20	**8/20**
Increased mitotic activity follicular cells	4 days	1/20	0/20	**6/20**	**13/20**	**9/20**
	7 days	0/20	0/20	**2/20**	**7/20**	**15/20**
	14 days	1/20	0/20	0/20	**7/20**	**12/20**
	28 days	0/20	0/20	0/20	**5/20**	**12/20**

Type of study, laboratory, guideline, GLP, test substance(purity), route administration, strain, dose levels, no animals/group, acceptability	Results [Effects statistically significantly and dose-related unless stated otherwise as not significant (n.s.) or not dose-related (ndr) or not clearly dose-related (ncdr)]	Reference
	Concurrent effects on the **liver** were an increase in liver weight (up to 60%) at 30000 ppm (1635 mg/kg bw/day) and increase in microsomal UDPGT (2 and 5 fold after 4 days until the end of treatment period at 400 equivalent to 22.69 mg/kg bw/day and 30000 ppm equivalent to 1635 mg/kg bw/day respectively). Not measured at 3000 ppm	

CHANGE LIVER

- ✓ Hepatic enzymes

Parameters		Dose level (ppm)				
		0	10	400	3000	30000
		Males				
Microsomal protein (mg/g)	5 days	21.76	**13.89****	20.23		**28.54*** ↑31%
	8 days	14.97	17.84	**21.00*** ↑40%		**25.00***** ↑67%
	15 days	19.00	16.26	25.17		**31.80*** ↑67%
	29 days	27.43	25.21	20.23		**35.99***** ↑31%
Microsomal UDPGT (DAminmg)	5 days	0.019	**0.031*** ↑63%	**0.032*** ↑68%		**0.098***** ↑416%
	8 days	0.034	0.039	**0.065***** ↑91%		**0.125***** ↑268%
	15 days	0.019	**0.025*** ↑32%	**0.045***** ↑137%		**0.101***** ↑432%
	29 days	0.018	0.028	**0.041***** ↑128%		**0.098***** ↑444%

*= p<0.05, ** = p<0.01, *** = p<0.001

- ✓ Organ weight

Parameters		Dose level (ppm)				
		0	10	400	3000	30000
		Males				
LIVER						
Abs weight (g)	5 days	13.59	14.00	14.87	**17.48**** ↑29%	**18.44**** ↑36%
	8 days	13.46	13.60	14.06	**17.04**** ↑27%	**19.69**** ↑46%
	15 days	13.05	13.73	**15.40**** ↑18%	**19.34**** ↑48%	**21.90**** ↑68%
	29 days	14.46	14.69	15.90	**20.51**** ↑42%	**22.40**** ↑55%
Rel weight (%)	5 days	3.508	3.562	3.692	**4.555**** ↑30%	**4.852**** ↑38%
	8 days	3.350	3.409	3.466	**4.284**** ↑28%	**5.091**** ↑52%
	15 days	3.259	3.365	**3.763**** ↑15%	**4.466**** ↑37%	**5.265**** ↑62%
	29 days	3.262	3.286	**3.578**** ↑10%	**4.569**** ↑40%	**5.228**** ↑60%

*= p<0.05, ** = p<0.01, *** = p<0.001

Type of study, laboratory, guideline, GLP, test substance(purity), route administration, strain, dose levels, no animals/group, acceptability	Results [Effects statistically significantly and dose-related unless stated otherwise as not significant (n.s.) or not dose-related (ndr) or not clearly dose-related (ncdr)]	Reference
Oral study (dietary) of 4 weeks in rats. *Effect on thyroid and liver of clofentezine in the male rat* Lab: BSL BIOSERVICE. Scientific Laboratories Munich GmbH. Guideline: No test method available. GLP: Yes Test substance: Clofentezine technical (batch no: LF-140496, purity: 98.7%) Route administration: Oral (fed diet) Rat strain (male): Charles River, Sprague Dawley rats, CD (SD) Dose: 0, 600, 3000 and 9000 ppm (equivalent to 0, 36.4, 205.7 and 509.4 mg/kg bw/day) No animals: 15 rats/dose **Study acceptable**	(see below)	**Anonymous 60 (2016)** **B.6.8.2.1-13** **(AS)**

9000 ppm (509.4 mg/kg bw/day)

Body weight, body weight gain and food consumption

- Reduced overall body weight gain during the whole treatment period, when compared to controls (↓31% during 1-15 period and ↓22% during 1-29 period).
- ↓ Food consumption mainly in the first treatment week (23%).

Parameters		Dose level (ppm)	
		0	9000
		Males	
Body weight (g)	1 days	212.33	211.93
	8 days	256.87	293.60
	15 days	307.70	274.50
	29 days	359.00	323.80
Body weight gain (g)	(1-15 days)	95.20	**65.20*** ↓31%
	(1-29 days)	149.80	**116.40**** ↓22%
Food consumption (g/animal/day)	Week 1	25.75	**19.93***** ↓23%
	Week 2	24.95	27.60
	Week 3	27.50	25.60
	Week 4	28.24	26.52

*p<0.05, ** p<0.01 and *** p<0.001*

Serum TSH, T3* and T4

- There were no biologically relevant differences in serum T4 and TSH levels with controls. In week 3 slightly higher values of T4 (24%) and TSH (62%) were observed (n.s)

(*) As most serum T_3 values were below the lower limit of quantification, an evaluation of this parameter was not possible.

Parameters		Dose level (ppm)	
		0	9000
		♂	
Total T_4 (nmol/ L)	Pre-treatment	85.17	83.45
	Week 1	67.21	72.74
	Week 2	70.68	84.31
	Week 3	67.40	**83.72 ↑24%**
	Week 4	63.02	69.64
TSH: Thyrotrophin (ng/ ml)	Pre-treatment	1.06	1.14
	Week 1	1.25	1.39
	Week 2	1.64	1.62
	Week 3	1.26	**2.05 ↑62%**
	Week 4	1.32	1.34

PROD, BROD, 7-BQ and UGT activity in S9 Fraction

- ↑ Activity cytochrome P450 in the liver:
  - ✓ CYP2B: ↑PROD (approx. 13 and 18-fold for day 8 and day 15 respectively *vs* control) and ↑BROD (approx.. 21 and 32-fold for day 8 and day 15 respectively *vs* control)
  - ✓CYP3A: ↑7-BQ (approx. 2-fold for day 8 and day 15 *vs* control).

Type of study, laboratory, guideline, GLP, test substance(purity), route administration, strain, dose levels, no animals/group, acceptability	Results [Effects statistically significantly and dose-related unless stated otherwise as not significant (n.s.) or not dose-related (ndr) or not clearly dose-related (ncdr)]	Reference
	▪ ↑ Activity UDP glucuronosyltransferase enzymes in the liver: ↑UGT multienzime sustrato approx. 2 and 3-fold for day 8 and day 15 respectively *vs* control	

Parameters		Marker	Day necropsy	Dose level (ppm) 0	9000	Fold Induction
				Activity (pmol/mg/min)		
Cyto-chrome P450	CPY2B	PROD	Day 8	1.7	22.8	**13.2**
			Day 15	1.5	26.3	**17.8**
	CPY2B CPY3A	BROD	Day 8	6.6	136.2	**20.6**
			Day 15	5.9	187.5	**31.8**
	CPY3A	7-BQ	Day 8	922.0	1517.7	**1.6**
			Day 15	649.6	1582.7	**2.4**
UDPGT		UGT multienzime sustrato	Day 8	20.1	47.0	**2.3**
			Day 15	11.3	37.4	**3.3**

PROD: Pentoxyresorufin-O-dealkylase activity
BROD: 7-Benzyloxyresorufin O-dealkylase activity
7BQ: 7-benzyloxyquinoline dealkylase activity

Organ weight

- Moderately increased of absolute liver weight after one week (58%), after 2 weeks (40%) and after 4 weeks (50%).
- No effect on thyroid/parathyroid gland weight

Parameters		Dose level (ppm) 0	9000
		♂	
LIVER			
Absolute (g)	Day 8	7.37	**11.65***** ↑58%
	Day 15	9.30	**13.00**** ↑40%
	Day 29	10.02	**15.02***** ↑50%
THYROID / PARATHYROID			
Absolute (g)	Day 8	0.0161	0.0163
	Day 15	0.0198	0.0162
	Day 29	0.0225	0.0219

**p<0.05, ** p<0.01 and *** p<0.001.*

Macroscopic findings

- Enlarged liver: 4/5 (1 week) and 5/5 (2 weeks) *vs* 0/5 control. No enlarged livers were noted at necropsy after 4 weeks of treatment (day 29).

Histopathology

Liver:

- ↑ Incidence (5/5 on days 8, 15 and 29 *vs* 0/5 control) and severity (3.4, 3.6 and 3.8 on days 8, 15 and 29 respectively *vs* 0 control) of hepatocellular hypertrophy centrilobular.

Thyroid:

- ↑ Incidence (3/5, 4/5 and 5/5 on days 8, 15 and 29 respectively *vs* 0/5 control) and severity (1.3, 2.0 and 2.4 on days 8, 15 and 29 respectively *vs* 0 control) of follicular cell hypertrophy.
- ↑ Incidence (2/5 and 3/5 on days 15 and 29 respectively *vs* 0/5 control) and severity (1.0 and 1.0 on days 15 and 29 respectively *vs* 0 control) follicular colloid depletion.

Type of study, laboratory, guideline, GLP, test substance(purity), route administration, strain, dose levels, no animals/group, acceptability	Results [Effects statistically significantly and dose-related unless stated otherwise as not significant (n.s.) or not dose-related (ndr) or not clearly dose-related (ncdr)]	Reference
	(see tables and text below)	

Parameters		♂ Dose level (ppm) 0		9000	
		Incidence	Mean severity	Incidence	Mean severity
LIVER					
Hepatocellular hypertrophy centrilobular (pale eosinophilic or granular cytoplasmic appearance)[4]	Day 8	0/5	-	**5/5**	**3.4**
	Day 15	0/5	-	**5/5**	**3.6**
	Day 29	0/5	-	**5/5**	**3.8**
THYROID					
Follicular cell hypertrophy	Day 8	0/5	-	**3/5**	**1.3**
	Day 15	0/5	-	**4/5**	**2.0**
	Day 29	0/5	-	**5/5**	**2.4**
Follicular colloid depletion	Day 8	0/5	-	0/5	-
	Day 15	0/5	-	**2/5**	**1.0**
	Day 29	0/5	-	**3/5**	**1.0**

3000 ppm (205.7 mg/kg bw/day)

Serum TSH, T3 and T4

- There were no biologically relevant differences in serum T_4 and TSH levels with controls.

PROD, BROD, 7-BQ and UGT activity in S9 Fraction

- ↑ Activity cytochrome P450 in the liver:
  - ✓ CYP2B: ↑PROD (approx. 15 and 7-fold for day 8 and 15 respectively *vs* control) and ↑BROD (approx.. 18 and 12-fold for day 8 and 15 respectively *vs* control)
  - ✓CYP3A: ↑7-BQ (approx. 3-fold on day 15 only)
- ↑ Activity UDP glucuronosyltransferase enzymes in the liver: ↑UGT multienzime sustrato approx. 2 and 4-fold for day 8 and 15 respectively *vs* control.

Parameters		Marker	Day necropsy	Dose level (ppm) 0	3000	Fold Induction
				Activity (pmol/mg/min)		
Cyto-chrome P450	CPY2B	PROD	Day 8	1.7	25.2	**14.6**
			Day 15	1.5	10.6	**7.1**
	CPY2B CPY3A	BROD	Day 8	6.6	116.9	**17.7**
			Day 15	5.9	68.4	**11.6**
	CPY3A	7-BQ	Day 8	922.0	1211.6	1.3
			Day 15	649.6	1931.6	**3.0**
UDPGT		UGT multienzime sustrato	Day 8	20.1	44.9	**2.2**
			Day 15	11.3	41.5	**3.7**

PROD: Pentoxyresorufin-O-dealkylase activity
BROD: 7-Benzyloxyresorufin O-dealkylase activity
7BQ: 7-benzyloxyquinoline dealkylase activity

[4] *Hypertrophied hepatocytes were characterized by pale eosinophilic or granular cytoplasmic appearance, which is typically found in liver of rats treated with phenobarbital-type agents (Gopinath C. and Mowat V., 2014; Greaves P., 2012)*

Type of study, laboratory, guideline, GLP, test substance(purity), route administration, strain, dose levels, no animals/group, acceptability	Results [Effects statistically significantly and dose-related unless stated otherwise as not significant (n.s.) or not dose-related (ndr) or not clearly dose-related (ncdr)]	Reference
	Organ weight ▪ Moderately increased ncdr of absolute liver weight after one week (54%), after 2 weeks (55%) and after 4 weeks (56%). ▪ No effect on thyroid/parathyroid gland weight.	

Parameters		Dose level (ppm) 0 ♂	3000 ♂
LIVER			
Absolute (g)	Day 8	7.37	**11.34*** ↑54%**
	Day 15	9.30	**14.40*** ↑55%**
	Day 29	10.02	**15.65*** ↑56%**
THYROID / PARATHYROID			
Absolute (g)	Day 8	0.0161	0.0193
	Day 15	0.0198	0.0183
	Day 29	0.0225	0.0262

*p<0.05, ** p<0.01 and *** p<0.001.*

Macroscopic findings

▪ Enlarged liver: 3/5 after 2 weeks *vs* 0/5 control. No enlarged livers were noted at necropsy after 4 weeks of treatment (day 29).

Histopathology

Liver:

▪ ↑ Incidence (5/5, 4/4, 5/5 on days 8, 15 and 29 respectively *vs* 0/5 control) and severity (3.0, 3.5 and 3.0 on days 8, 15 and 29 respectively *vs* 0 control) of hepatocellular hypertrophy centrilobular.

Parameters		♂ Dose level (ppm) 0 Incidence	0 Mean severity	3000 Incidence	3000 Mean severity
LIVER					
Hepatocellular hypertrophy centrilobular (pale eosinophilic or granular cytoplasmic appearance)	Day 8	0/5	-	**5/5**	**3.0**
	Day 15	0/5	-	**4/4**	**3.5**
	Day 29	0/5	-	**5/5**	**3.0**

Thyroid:

▪ ↑ Incidence (2/5, 3/4 and 5/5 on days 8, 15 and 29 respectively *vs* 0/5 control) and severity (1, 1.3 and 2.4 on days 8, 15 and 29 respectively *vs* 0 control) of follicular cell hypertrophy.

▪ ↑ Incidence (1/4 and 3/5 on days 15 and 29 respectively *vs* 0/5 control) and severity (1.0 on days 15 and 29 *vs* 0 control) of follicular colloid depletion.

Type of study, laboratory, guideline, GLP, test substance(purity), route administration, strain, dose levels, no animals/group, acceptability	Results [Effects statistically significantly and dose-related unless stated otherwise as not significant (n.s.) or not dose-related (ndr) or not clearly dose-related (ncdr)]	Reference

Parameters		♂ Dose level (ppm)			
		0		3000	
		Incidence	Mean severity	Incidence	Mean severity
THYROID					
Follicular cell hypertrophy	Day 8	0/5	-	**2/5**	**1**
	Day 15	0/5	-	**3/4**	**1.3**
	Day 29	0/5	-	**5/5**	**2.4**
Follicular colloid depletion	Day 8	0/5	-	0/5	**-**
	Day 15	0/5	-	**1/4**	**1.0**
	Day 29	0/5	-	**3/5**	**1.0**

600 ppm (36.4 mg/kg bw/day)

Serum TSH, T3 and T4

- ↑ T_4 in week 3 (40%) and week 4 (36%). ↑TSH ns (54%) in week 3
- As most serum T_3 values were below the lower limit of quantification, a comprehensive evaluation of this parameter was not possible.

Parameters		Dose level (ppm)	
		0	600
		Males	
Total T_3 (ng/ ml)	Pre-treatment	0.32	0.34
	Week 1	-	-
	Week 2	-	-
	Week 3	0.21	0.26
	Week 4	0.88	0.66
Total T_4 (nmol/ L)	Pre-treatment	85.17	85.86
	Week 1	67.21	77.46 ↑15%
	Week 2	70.68	81.87 ↑16%
	Week 3	67.40	**94.38**** ↑40%
	Week 4	63.02	**85.92**** ↑36%
TSH: Thyrotrophin (ng/ ml)	Pre-treatment	1.06	1.04
	Week 1	1.25	1.32
	Week 2	1.64	1.65
	Week 3	1.26	**1.94 ↑54%**
	Week 4	1.32	1.41 ↑7%

*p<0.05, ** p<0.01 and *** p<0.001*

PROD, BROD, 7-BQ and UGT activity in S9 Fraction

- No induction of liver enzymes.

Organ weight

- At the end of the 4-weeks treatment period slightly but statistically significantly higher but ncdr liver weight was observed (21%).

Type of study, laboratory, guideline, GLP, test substance(purity), route administration, strain, dose levels, no animals/group, acceptability	Results [Effects statistically significantly and dose-related unless stated otherwise as not significant (n.s.) or not dose-related (ndr) or not clearly dose-related (ncdr)]	Reference
	(see tables and text below)	

Parameters		Dose level (ppm) 0	600
		♂	
LIVER			
Absolute (g)	Day 8	7.37	8.83
	Day 15	9.30	9.20
	Day 29	10.02	**12.08**** ↑21%
THYROID / PARATHYROID			
Absolute (g)	Day 8	0.0161	0.0164
	Day 15	0.0198	0.0173
	Day 29	0.0225	0.0255

**p<0.05, ** p<0.01 and *** p<0.001.*

Histopathology

Liver:

- ↑ Incidence (5/5, 5/5, 5/5 on days 8, 15 and 29 respectively *vs* 0/5 control) and severity (1.4, 1.6 and 1.2 on days 8, 15 and 29 respectively *vs* 0 control) of hepatocellular hypertrophy centrilobular.

Thyroid:

- ↑ Incidence (3/5 *vs* 0/5 control) and severity (1.0 *vs* 0 control) on day 29 of follicular cell hypertrophy.

Parameters		♂ Dose level (ppm) 0 Inciden-ce	0 Mean severity	600 Inciden-ce	600 Mean severity
LIVER					
Hepatocellular hypertrophy centrilobular (pale eosinophilic or granular cytoplasmic appearance)	Day 8	0/5	-	**5/5**	**1.4**
	Day 15	0/5	-	**5/5**	**1.6**
	Day 29	0/5	-	**5/5**	**1.2**
THYROID					
Follicular cell hypertrophy	Day 8	0/5	-	0/5	**-**
	Day 15	0/5	-	0/5	**-**
	Day 29	0/5	-	**3/5**	**1.0**

CONCLUSION: After 1 or 2 weeks of treatment at a dose level of 9000 ppm livers were enlarged in 4/5 or 5/5 animals respectively. Moreover, enlarged liver were also noted in 3/5 animals after 2 weeks of treatment with 3000 ppm. No enlarged livers were noted grossly at necropsy at any dose after 4 weeks of treatment. The macroscopic finding of enlarged liver was also reflected in markedly higher liver weights.

A significant increase in absolute liver weight after 1, 2 or 4 weeks at doses ≥ 3000 ppm (~50%). At the end of the 4 week treatment period slightly higher liver weight was also found at 600 ppm (21%). These increases were associated with centrilobular hepatocellular hypertrophy in a dose-dependent manner at all dose levels and at all time of sacrifice (day 8, 15 and 29). Hypertrophied hepatocytes were characterized by pale eosinophilic or granular cytoplasmic appearance,

Type of study, laboratory, guideline, GLP, test substance(purity), route administration, strain, dose levels, no animals/group, acceptability	Results [Effects statistically significantly and dose-related unless stated otherwise as not significant (n.s.) or not dose-related (ndr) or not clearly dose-related (ncdr)]	Reference
	which is typically found in liver of rats treated with phenobarbital-type agents[5]. At 9000 ppm the severity of this finding increased in a time dose-dependent manner. At 600 or 3000 ppm an increase in severity was observed between week 1 and 2 but severity had decreased again in week 4.	

Absolute liver weight

Parameters		Dose level (ppm)			
		0	600	3000	9000
		♂			
LIVER WEIGHT					
Absolute (g)	Day 8 (week 1)	7.37	8.83	**11.34***** ↑54%	**11.65***** ↑58%
	Day 15 (week 2)	9.30	9.20	**14.40***** ↑55%	**13.00**** ↑40%
	Day 29 (week 4)	10.02	**12.08**** ↑21%	**15.65***** ↑56%	**15.02***** ↑50%

*$p<0.05$, ** $p<0.01$ and *** $p<0.001$.*

Liver histopathology

Parameters		♂ Dose level (ppm)			
		0	600	3000	9000
		Incidence (mean severity)			
LIVER HISTOPATHOLOGY					
Hepatocellular hypertrophy centrilobular (pale eosinophilic or granular cytoplasmic appearance)	Day 8 (week 1)	0/5	**5/5 (1.4)**	**5/5 (3.0)**	**5/5 (3.4)**
	Day 15 (week 2)	0/5	**5/5 (1.6)**	**4/4 (3.5)**	**5/5 (3.6)**
	Day 29 (week 4)	0/5	**5/5 (1.2)**	**5/5 (3.0)**	**5/5 (3.8)**

After 1 or 2 weeks of dietary administration of doses ≥ 3000 ppm (≥205.7 mg/kg bw/day), enzyme induction in the liver was demonstrated with increased levels of Cytochrome P450 2B (as determined by the PROD and BROD assay), Cytochrome P450 3A (as determined by the BROD and 7-BQ assay) and UDP glucuronosyltransferase. Cytochrome P450 3A was induced at 9000 ppm (509,4 mg/kg bw/day) at both time points and at 3000 ppm at the second week only.

At 9000 ppm on day 15 there were an increase in activity of UDPGT (3.3 fold), PROD (18 fold), BROD (32 fold) and 7BQ (2.4 fold).

No induction of liver enzymes was found at a dose levels of 600 ppm

[5] References: *Gopinath C. and Mowat V., 2014; Greaves P., 2012*

Type of study, laboratory, guideline, GLP, test substance(purity), route administration, strain, dose levels, no animals/group, acceptability	Results [Effects statistically significantly and dose-related unless stated otherwise as not significant (n.s.) or not dose-related (ndr) or not clearly dose-related (ncdr)]	Reference
	(see below)	

Enzyme induction in the liver

Parameters		Marker	Day necropsy	Dose level (ppm) 0	600	3000	9000
				Activity (pmol/mg/min) (↑fold)			
Cyto-chrome P450	CPY 2B	PROD	Day 8	1.7	5.6 ↑3.2	25.2 ↑14.6	22.8 ↑13.2
			Day 15	1.5	7.0 ↑4.7	10.6 ↑7.1	26.3 ↑17.8
	CPY 2B CPY 3A	BROD	Day 8	6.6	25.9 ↑3.9	116.9 ↑17.7	136.2 ↑20.6
			Day 15	5.9	39.6 ↑6.7	68.4 ↑11.6	187.5 ↑31.8
	CPY 3A	7-BQ	Day 8	922.0	620.7 ↑0.9	1211.6 ↑1.3	1517.7 **↑1.6**
			Day 15	649.6	892.6 ↑1.4	1931.6 **↑3.0**	1582.7 **↑2.4**
UDPGT		UGT multi-enzime sustrato	Day 8	20.1	26.8 ↑1.3	44.9 ↑2.2	47.0 ↑2.3
			Day 15	11.3	16.1 ↑1.4	41.5 ↑3.7	37.4 ↑3.3

Thyroid weight was not affected although thyroid follicular cell hypertrophy at a minimum to moderate severity was seen at all dose levels. Thyroid follicular cell hypertrophy was observed dose- and time- dependently at 3000 ppm and 9000 ppm in week 1 and 2. At week 4 all dose levels were affected, however, no difference in this finding was seen between 3000 and 9000 ppm groups. An increase in the severity of this finding were seen in each study day allocation and in each test item group, except for 3000 ppm and 9000 ppm groups of animals subjected to necropsy at day 29 in which the same incidence and mean severity values were determined. Moreover, thyroid follicular colloid depletion at a minimum severity was seen at dose ≥3000 ppm at study days 15 and 29.

Absolute thyroid / parathyroid weight

Parameters		Dose level (ppm) 0	600	3000	9000
		♂			
THYROID / PARATHYROID					
Absolute (g)	Day 8 (week 2)	0.0161	0.0164	0.0193	0.0163
	Day 15 (week 2)	0.0198	0.0173	0.0183	0.0162
	Day 29 (week 4)	0.0225	0.0255	0.0262	0.0219

**p<0.05, ** p<0.01 and *** p<0.001.*

Type of study, laboratory, guideline, GLP, test substance(purity), route administration, strain, dose levels, no animals/group, acceptability	Results [Effects statistically significantly and dose-related unless stated otherwise as not significant (n.s.) or not clearly dose-related (ndr) or not clearly dose-related (ncdr)]	Reference
	Thyroid histopathology (table below)	

Thyroid histopathology

Parameters		♂ Dose level (ppm) 0	600	3000	9000
		Incidence (mean severity)			
THYROID					
Follicular cell hypertrophy	Day 8 (week 2)	0/5	0/5	**2/5 (1.0)**	**3/5 (1.3)**
	Day 15 (week 2)	0/5	0/5	**3/4 (1.3)**	**4/5 (2.0)**
	Day 29 (week 4)	0/5	**3/5 (1.0)**	**5/5 (2.4)**	**5/5 (2.4)**
Follicular colloid depletion	Day 8 (week 2)	0/5	0/5	0/5	0/5
	Day 15 (week 2)	0/5	0/5	**1/4 (1.0)**	**2/5 (1.0)**
	Day 29 (week 4)	0/5	0/5	**3/5 (1.0)**	**3/5 (1.0)**

Slightly and transiently increased T_4 at 600 ppm in week 3 and 4 (40% and 36% respectively) and increased T_4 at 9000 ppm in week 3 were seen (24%). An increased TSH at 600 and 9000 ppm in week 3 only (54% and 62% respectively) were observed (but not at 30000 ppm).

Mean total T3, total T4 and TSH

Parameters		Dose level (ppm) 0	600	3000	9000
		Males			
Total T_3 (ng/ ml)	Pre-treatment	0.32	0.34	0.25	-
	Week 1	-	-	-	-
	Week 2	-	-	-	-
	Week 3	0.21	0.26	-	-
	Week 4	0.88	0.66	-	-
Total T_4 (nmol/L)	Pre-treatment	85.17	85.86	82.07	83.45
	Week 1	67.21	77.46 ↑15%	65.07	72.74
	Week 2	70.68	81.87 ↑16%	84.73	84.31
	Week 3	67.40	**94.38**** ↑40%	79.90	**83.72 ↑24%**
	Week 4	63.02	**85.92**** ↑36%	74.52	69.64
TSH: Thyrotrophin (ng/ ml)	Pre-treatment	1.06	1.04	1.07	1.14
	Week 1	1.25	1.32	1.55	1.39
	Week 2	1.64	1.65	1.86	1.62
	Week 3	1.26	**1.94 ↑54%**	1.09	**2.05 ↑62%**
	Week 4	1.32	1.41 ↑7%	1.35	1.34

*$p<0.05$, ** $p<0.01$ and *** $p<0.001$.*

Liver effects, such as liver weight increase and liver enzyme induction, occurred just after clofentezine treatment and are a requirement for the observed thyroid effects. Overall it can be concluded that clofentezine leads to increased liver weight associated with hepatocellular hypertrophy at all dose level. The thyroid weight was not affected

Type of study, laboratory, guideline, GLP, test substance(purity), route administration, strain, dose levels, no animals/group, acceptability	Results [Effects statistically significantly and dose-related unless stated otherwise as not significant (n.s.) or not dose-related (ndr) or not clearly dose-related (ncdr)]	Reference
	although thyroid follicular cell hypertrophy was seen at all dose levels. Furthermore, clofentezine caused liver enzyme induction especially of which induction of UDPGT is known to lead to an increased metabolism of thyroid hormones and thyroid hormone reductions. This further leads to a feedback reaction consisting of an increased TSH release. Continuous TSH stimulation is known to stimulate thyroid tissues which after lifetime can lead to thyroid tumours in rats. The study provides evidence that the observed tumorigenic response in liver and thyroid is highly likely rodent-specific. *(Dellarco et al 2006)*	

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

Two long-term toxicity/oncogenicity studies were conducted with clofentezine, one in rats and one in mice (Anonymous 42, 1985a; Anonymous 45, 1985).

In a 2-year long-term toxicity and carcinogenicity study in rats *(*Anonymous 42, 1985a) tested dose levels were 0, 10, 40 and 400 ppm equivalent to 0, 0.43, 1.72 and 17.3 mg/kg bw/day for males and 0, 0.55, 2.18 and 22.1 mg/kg bw/day for females.

The rationale for selection of dose levels was as follow: the low dose of 10 ppm was selected to approximate the minimum useful dose in relation to the likely crop residues, the intermediate dose of 40 ppm was a dose at which minimal changes (i.e. slight centrilobublar hepatocyte enlargement) were observed in one of the 90-day study in rat (Anonymous 74, 1981) and the high dose of 400 ppm was selected since it provided comparative data at the same dose found in the same 90-day study in rat to evoke a syndrome of toxic effects (i.e. centrilobular hepatocyte enlargement, together with increased liver, spleen and kidney weight, reduced haemoglobin and elevation of plama cholesterol).

No mortality or clinical signs were associated to treatment.

No persistent treatment-related effect was observed with respect to haematology parameters. A statistically significant decrease (>20%) was observed at 400 ppm in females in the total WBC in months 6 and 12 and in the total no. lymphocites on month 12 and in males in the total no. neutrophils in month 27. These changes are considered random and of an overall doubtful toxicological relevance.

Blood chemistry revealed an increased statistically significant and dose-dependent in free thyroxine (T4) in males at 400 ppm in month 27. Other parameters of thyroid function were not affected. Other statistically significant differences of the biochemical parameters compared with controls at 400 ppm were only marginal and not dose-dependent.

The absolute liver weight was increased in month 27 from 10 ppm in males and at 400 ppm in females though clearly dose-related only in males. Relative liver weights were increased in both sexes in month 12 and 27 at 400 ppm though the variation was greater than 10% only in males and dose-dependancy was not clear.

Histopathology revealed observations in male liver at the high dose level of 400 ppm with significant (pairwise and dose-trend) centrilobular hepatocyte vacuolation (observed at interim sacrifice and in the total no. of animals including the incidence at interim sacrifice, interim deaths and terminal sacrifice) and centrilobular hepatocyte enlargement (interim sacrifice, terminal sacrifice and total no. of animals). It was also observed focal cyst degeneration of hepatocytes and fat deposits in non-specific distribution dose-trend significant for the total no. of animals and focal hepatocyte necrosis

dose-trend significant for interim sacrifices and the total no. of animals. It has to be noted the presence of telagiectasis in both sexes but only dose-trend significant in females for the total no. of animals.

Effects in male thyroid were manifested at 400 ppm by dose-trend significant agglomeration of colloid in males at interim sacrifice, terminal sacrifice and for the total no. of animals that was also pairwise significant for terminal sacrifices. Besides, follicular cell hyperplasia was observed from 40 ppm in males but not dose-related and not statistically significant.

Other histopathological findings at 400 ppm pairwise and dose-trend significant were glomerular nephropathy in females for interim deaths and interstitial mononuclear infiltration of Harderian gland in males at interim sacrifice and total no. of animals.

At 400 ppm in males, there was a slight increase in the number of follicular cell tumours (combined adenomas and carcinomas) in the thyroid at terminal sacrifice (8/50 vs. 2/50). This may have been associated with the pairwise and dose-trend significant increase of agglomeration of colloid at terminal sacrifice (18/21 vs 12/24). These tumors are not pairwise significant but exhibited dose-trend significance (p<0.01). The notifier has not provided historical control data in line with Regulation 283/2013 that set out the data requirements for active substances, in accordance with regulation 1107/2009. No historical control range was presented in the report. The only background data included in the report were obtained from a single concurrently run study performed at the same conductig laboratory with the same housing conditons, animal husbandry practices, same procedure, strain, diet and pathologist with time period of data collection from December 1983 to March 1085. The incidence of thyroid follicular cell tumours in high dose males treated with clofentezine was only marginally higher than the incidence in control group from this other concurrently run study (8/50 vs. 6/49). Besides, it should be emphasized that the spontaneous rate of thyroid tumour development in rats increases rapidly after the animal exceeded 2 years of age. It has to be noted that the mechanism of action (MoA) thoroughly developed in "Section Thyroid tumours mode of action" shows that follicular cell tumours are not relevant for humans.

Table 23: Incidence of thyroid follicular cell tumours in male rats in two studies with clofentezine

Thyroid follicular cell tumours in males	Doses											
	0 ppm 0 mg/kg bw/day			**10 ppm 0.43 mg/kg bw/day**			**40 ppm 1.72 mg/kg bw/day**			**400 ppm 17.3 mg/kg bw/day**		
	Time of death											
	I	**D**	**T**	**I**	**D**	**T**	**I**	**D**	**T**	**I**	**D**	**T**
Rat carcinogenicity study for 27 months (March 1982-June 1984); *(Anonymous 42, 1985a)*												
No animals examined	20	26	24	20	26	24	20	23	27	20	29	21
Benign	0	1	0	0	0	1	0	0	0	0	0	3
Probably malignant	0	0	0	0	0	0	0	0	0	0	0	2
Malignant	0	1	0	0	0	1	0	1	1	0	0	3
Total tumours (D+T)	2/50†			2/50			2/50			**8/50**		
TOX 82074 (1982-4) Study conducted at the same laboratory (December, 1982-March 1985)												
No animals examined	25	32	17	24	35	15	24	37	13	25	35	15
Benign	0	2	2	0	0	1	0	1	0	1	5	0
Probably malignant	0	0	0	0	1	0	0	0	1	0	1	0
Malignant	0	1	1	0	1	0	0	1	2	0	0	0
Total tumours (D+T)	**6/49**			**3/50**			**5/50**			**6/50**		

I: Interim sacrifice, D: Interim deaths during treatment, T: Terminal sacrifice
†Positive after trend analysis

Additionally, it should be noted the occurrence of several rare tumour incidence, none of them statistically different from the concurrent control and regarded as not relevant.

Malignant mixed glioma: single occurrence was restricted to the mid and high dose group interim decedent male. Historical control data from the same laboratory were not available. Although no HC data was presented in the report, according to the notifier contemporary historical control data were obtained from where the stock animals originated (Charles River laboratories historical control data 2004 @ 104 weeks of age, 30 studies, 2146 males and 2344 females examined) with an incidence that ranged from 0.91% to 1.92% for males. In the clofentezine carcinogenicity rat study the incidence at the two highest doses in males (2%, 1/50) was only slightly above the background range stated by the notifier. Besides, these effects were considered not to be treatment related, but rather incidental in their occurrence for the following reasons: the incidences were not statistically different from the concurrent control, these effects were not replicated in females, and there was no evidence of a dose response.

Astrocytoma: an incidence of 2 (1 in interim deaths, 1 for terminal deaths), 1 (interim death) and 1 (interim death) were observed in the low, mid and high dose group males. In females an incidence of 2 and 1 were observed in the low and high dose groups, with all instances observed at termination. Historical control data from the same laboratory were not available. Although no HC data was presented in the report, according to the notifier contemporary historical control data were obtained from where the stock animals originated (Charles River laboratories historical control data 2004 @ 104 weeks of age; 30 studies, 2146 males and 2344 females examined) with an incidence that ranged from 0.87% to 4.29% in males and from 1.67% to 2.31% in females. In the clofentezine carcinogenicity rat study the incidence of this malignant tumour type in males of the low dose (4%, 2/50), mid dose (2%, 1/50) and high dose (2%, 1/50) and in females of the high dose (2%, 1/50) were within the background range stated by the notifier. Besides, these effects were considered not to be treatment related, but rather incidental in their occurrence for the following reasons: the incidences were not statistically different from the concurrent control and there was no evidence of a dose response.

Leydig cell tumours, testes: a single incidence of this malignant tumour type in the high dose group males (1/50; 2%). The notifier has not provided historical control data in line with Regulation 283/2013 that set out the data requirements for active substances, in accordance with regulation 1107/2009. No historical control range was presented in the report. The only background data included in the report were obtained from a single concurrently run study performed at the same conductig laboratory with the same housing conditons, animal husbandry practices, same procedure, strain, diet and pathologist with time period of data collection from December 1983 to March 1085. The incidence of malignant leyding cell tumours in high dose males treated with clofentezine was below the incidence in control group from this other concurrently run study (2/50; ~ 4%) and is not considered to be treatment-related.

Since no evidence of carcinogenicity was observed at tested dose levels **NOAEL** for carcinogenicity was established at >**400 ppm** equivalent to **17.3 and 22.1 mg/kg bw/day** for males and females respectively. NOAEL for toxicity was **40 ppm** corresponding to **1.72/2.18 mg/kg bw**/day for males and females respectively.

In a 2-year carcinogenicity study in mice (Anonymous 45, 1985) tested dose levels were 0, 50, 500 and 5000 ppm equivalent to 0, 5.0, 50.7 and 543.4 mg/kg bw/day for males and 0, 5.3, 56.9 and 557.1 mg/kg bw/day for females.

The rationale for selection of dose levels was not provided in the report.

The overall survival in male and female is summarised in the following tables:

Table 24: Overall of female survival data

	Female Dose (ppm)			
	0	50	500	5000
Number of animals	52	52	52	52
No.of survivors-wk 60 % survivors- wk 60	44 85%	50 96%	51 98%	43 83%
No. of survivors-wk 72 % survivors- wk 72	40 77%	48 92%	47 90%	36 69%
No. of survivors-wk 78 % survivors- wk 78	37 71%	43 83%	45 87%	32 62%
No. of survivors-wk 90 % survivors- wk 90	33 64%	37 71%	39 75%	24 46%
No. of survivors-wk 105 % survivors- wk 105	25 48%	28 54%	27 52%	10 **19%**

Table 25: Overall of male survival data

	Male Dose (ppm)			
	0	50	500	5000
Number of animals	52	52	52	52
No.of survivors-wk 60 % survivors- wk 60	45 87%	44 85%	43 83%	40 77%
No. of survivors-wk 72 % survivors- wk 72	38 73%	39 75%	40 77%	37 71%
No. of survivors-wk 78 % survivors- wk 78	35 67%	36 69%	37 71%	34 65%
No. of survivors-wk 90 % survivors- wk 90	24 46%	29 56%	26 50%	27 52%
No. of survivors-wk 105 % survivors- wk 105	14 27%	17 33%	17 33%	12 23%

The statistical analysis of the mortality data at the end of the treatment period, revealed that the 5000 ppm female group had significantly more deaths than the control group ($p<0.01$) and this was supported by the test for trend ($p<0.001$). The higher proportion of deaths in females at 5000 ppm was observed during the latter part of the study and amyloidosis was a major contributory factor to death in a greater number of female mice from the group receiving 5000 ppm compared to the controls and the other treated groups. There was no effect on longevity in male mice.

The percentage of survival in male of all doses was within the provided historical control range (23.1-53.8%) obtained from 26 studies with CD1 mice of duration between 92 and 109 weeks conducted at Huntigdon Research Centre (1980-83). Only in females of high dose, the percentage of survival (19%) was slightly lower than the provided historical control range (23.9-55.8%) obtained from 26 studies with CD1 mice of duration between 92 and 115 weeks conducted at Huntigdon Research Centre (1980-83).

According to the OECD nº116 guidance document on the conduct and design of chronic toxicity and carcinogenicity studies, mice are generally exposed to the test chemical for 18 to 24 months, with the higher durations being used for strains having greater longevity or lower spontaneous tumour rate. This guidance further states that for specific strains of mice, e.g CD-1 strain, for which documentation exists showing that a duration of 18 months may be more appropriate (e.g., Giknis and Clifford, 2010), a reference to this information is sufficient for the justification of using a duration shorter than 24 months. It should be noted that amyloidosis is a common age-related condition in CD-1 mice with a tendency to be a frequent cause of death in these aged CD-1 mice. Termination of the clofentezine carcinogenicity mice study was conducted at week 105, rather than the guideline recommendation of 78 weeks for CD-1 mice.

The survival of all groups in the mice carcinogenicity study with clofentezine fulfilled the OECD 453 criteria for combined chronic toxicity/carcinogenicity studies (not < than 50% at 18 months for mice). Besides, according to the OECD nº116 guidance document study termination may occur when the number of survivors in the lower dose or control groups has declined to 25%. In the mice

carcinogenicity study with clofentezine the number of survivors in the lower dose or control groups has not declined under 25%. Survival was also in line with the requirements of the relevant EPA guideline 870.4200 for carcinogenicity studies that specify that survival in any group should not fall below 50% at 15 months and 25% at 18 months in mice. In addition, the WHO (1990) recognizes a further type of carcinogenicity study that continues until mortality in the most susceptible group reaches a fixed level, usually 80%.

Besides, for assessing long-term/carcinogenicity studies, it is also crucial to know the time point at which survival falls bellows 50%. The Food and Drug Administration (FDA, 2001) gave a `rule of thumb´that a 50% survival rate at about weeks 80 to 90 of the 50 initial animals in any treatment group is considered adequate. The percentage can be lower or higher if the number of animals used in each treatment/sex group is larger or smaller than 50, but between 20 to 30 animals should be still alive during these weeks (Lin and Ali, 1994). In the clofentezine carcinogenicity mouse study the number of animal still alive at week 90 are higher than 20 in all dose levels and the difference in survival in high dose female became apparent only from approximately week ~ 90 onwards.

On overall, mortality observed in CD1 female mice in the clofentezine study that ran for an exposure period of 2 years in CD-1 mice should not unduly compromised the validity of the study and the study can be considered acceptable.

Bodyweight and food consumption were not affected by treatment. Only bodyweight gain at 5000 ppm was slightly reduced in males mainly during the first half of the study. No effects were observed from week 52.

The only significant effects on haematology were observed at 5000 ppm in males on week 52 with decrease in red blood cells (12%) not seen at terminal sacrifice.

Analysis of organ weights for mice killed after 105 weeks of treatment revealed slightly increase of absolute liver weights in females (18%) at 5000 ppm that was not clearly dose-related. This increase may be correlated with a dose-related increased incidence of foci/areas of altered hepatocytes (eosinophilic) noted from 500 ppm in females that was found above historical controls. A slight increased incidence of foci/areas of altered hepatocytes above historical controls was seen in males for decedent animals from 500 ppm. The increased incidence of this lesion in terminal males was not dose-related.

Table 26: Incidence of foci/areas of altered hepatocytes (eosinophilic)

Non neoplastic findings liver		Males				HC	Females				HC
		Dose level (ppm)					Dose level (ppm)				
		0	50	500	5000		0	50	500	5000	
Number of animals examined	D	**39**	**35**	**36**	**41**	**1775**	**27**	**24**	**25**	**42**	**1773**
	T	**13**	**17**	**16**	**11**		**25**	**28**	**27**	**10**	
Eosinophilic hepatocytes	D	2 (5.1%)	3 (8.6%)	4 (11.1%)	8 (19.5%)	46 0.0-9.8%	1 (3.7%)	1 (4.1%)	2 (8%)	4 (9.5%)	35 0.0-9.1%
	T	1 (7.7%)	4 (23.5%)	4 (25%)	2 (18.2%)		2 (8%)	2 (7.1%)	5 (18.5%)	5 (50%)	

D: Animals dying or killed during study
T: Animals killed at termination
HC (Huntingdon Research Centre between March 1980 and July 1983). Study duration between 92 and 115 weeks.

At 5000 ppm an increase incidence amyloidosis in females was observed (19/42 *vs* 6/27 of controls). There was no evidence of this effect in males.

A higher number of benign liver cell tumors were observed in females at 5000 ppm. The incidence (7/52; 13.5%) was slightly higher than the concurrent control incidence in females in this study (4/52; 7.7%) and outside the provided historical control range (0-7.7%) obtained from 26 studies with CD1

mice of duration between 92 and 115 weeks conducted at Huntigdon Research Centre (1980-83). The incidence was not significant after pairwise comparison (p>0.05) but showed a positive trend after trend analysis (p<0.01). It has to be noted that the incidence in controls is in the upper HCD value of 7.7%. There was an increase in the malignant hepatic tumors (1/52; 1.9%) in females with respect to controls (0/52) however it was within the range of historical controls and was not statistically significant. The combined analysis of benign and/or malignant hepatic tumours in females (8/52 *vs*. 4/52 of controls) was significant after pairwise comparison (p<0.05) and showed a positive trend after trend analysis (p<0.01).

The toxicological relevance of these liver tumors in females seems doubtful since they correspond to non-significant (pairwise) benign tumors occurring in one sex and one species and at high dose levels of treatment (557.1 mg/kg bw/day for females). However, the increase in liver tumours cannot be dismissed as non-relevant to humans as the mechanism for formation of liver tumours in female CD-1 mice developed in "*Section liver tumours mode of action* remain unclear.

Table 27: Hepatocellular tumours in female mice treated orally (via diet) with clofentezine

Neoplastic findings liver		**Females**				**HC Mean/range**
		Dose level (ppm)				
		0	**50**	**500**	**5000**	
Number of animals examined	D	**27**	**24**	**25**	**42**	
	T	**25**	**28**	**27**	**10**	
Benign tumour	D	0	1	0	3	**63/1875 (3.36%) 0-7.7%**
	T	4	2	1	2	
Benign tumour (two)	D	0	0	0	1	
	T	0	0	0	0	
Benign tumour (multiple)	D	0	0	1	0	
	T	0	0	1	1	
Benign tumour sub-total	D [time of death]	0	1 [98 week]	1 [95 week]	4 (9.5%) [93, 97, 97 and 102 week]	
	T	4 (16%)	2 (7%)	2 (7.4%)	3 (30%)	
Benign tumour overall total		4/52† (7.7%)	3/52 (5.8%)	3/52 (5.8%)	7/52 (13.5%)	
Malignant tumour (two)	D	0	0	0	1 (2.4%)	**22/1875 (1.17%) 0-3.8%**
	T	0	0	0	0	
Total malignant tumour		0/52	0/52	0/52	1/52 (1.9%)	

†Significant after trend analysis.
HC (Huntingdon Research Centre between March 1980 and July 1983). Study duration between 92 and 115 weeks.
D: Animals dying or killed during study (time of occurrence of liver tumor was ≥93 week in all animals) T: Animals killed at termination

No evidence of any thyroid effects was seen in mice.

In conclusion, **NOAEL** for carcinogenicity was **500 ppm** corresponding to **50.7/56.9 mg/kg bw/day** for males and females respectively based on increase of incidence of benign liver cell tumors in females at 5000 ppm equivalent to 543.4/557.1 mg/kg bw/day for males and females respectively. NOAEL for toxicity was **50 ppm** corresponding to **5/5.3 mg/kg bw**/day for males and females respectively.

Thyroid tumours mode of action

In a long-term study in Sprague-Dawley rats which were fed a diet containing 0, 10, 40 or 400 ppm (equivalent to 0, 0.43, 1.72 and 17.3 mg/kg bw/ day for males and 0, 0.55, 2.18, 22.1 mg/kg bw/ day for females) of clofentezine for 27 months *(Anonymous 42, 1985a),* a slight increase in the number of follicular cell tumors (combined adenomas and carcinomas) in the thyroid at the highest dose of 400 ppm in males (but not in females) was noted at terminal sacrifice (8/50 vs. 2/50 control). These tumors are not pairwise significant but exhibited dose-trend significance and were slightly above control data from a concurrently study at the same conducting laboratory (8/50 vs 6/49) as it can be observed in Table 23. No thyroid tumors were seen in the 2-year mouse oncogenicity study fed higher (up to 5000 ppm, equivalent to 543.4 and 557.1 mg/kg bw/day for males and females respectively) doses *(Anonymous 45, 1985).*

Thyroid tumours in male rat were associated with toxicity in liver (increased absolute and relative liver weight, centrilobular hepatocyte enlargement or centrilobular hepatocyte vacuolation) and thyroid (slight follicular cell hyperplasia and agglomeration of colloid). The studies with clofentezine did not indicate irreversible organ damages (cytotoxicity) and neither direct carcinogenic-genotoxic potential. In all other apical studies no evidence of thyroid effects was obvious.

The results of both apical and mechanistic studies with clofentezine provide some evidence which supports the view that the slight increase in incidence of thyroid tumours in male rats might be mediated via a well characterized, non-genotoxic, rodent-specific phenobarbital-like MoA, which is not relevant to humans.

It can be seen that in all studies with thyroid findings, changes in thyroid function are not seen in the absence of liver changes and enzyme induction, suggest the liver effects as a prerequisite for thyroid effects which is an evidence of this MoA.

In order to elucidate the mode of action, the effect of clofentezine on rat thyroid gland, thyroid hormone levels and hepatic thyroxine UDP glucuronosyltransferase activity was investigated.

In the guidance provided by ECHA, they recommend that the IPCS framework (IPCS, 2007) be followed when evaluating MoA data for carcinogenicity findings in animals and their relevance to humans.

All data is then evaluated according to the International Programme on Chemical Safety (IPCS) Mode of Action (MoA) human relevance (species concordance) Framework *(Boobis Alan R. et al., 2006)* using a weight-of-evidence approach based on the Bradford Hill criteria.

THE IPCS CONCEPTUAL MOA FRAMEWORK FOR EVALUATING ANIMAL CARCINOGENESIS:

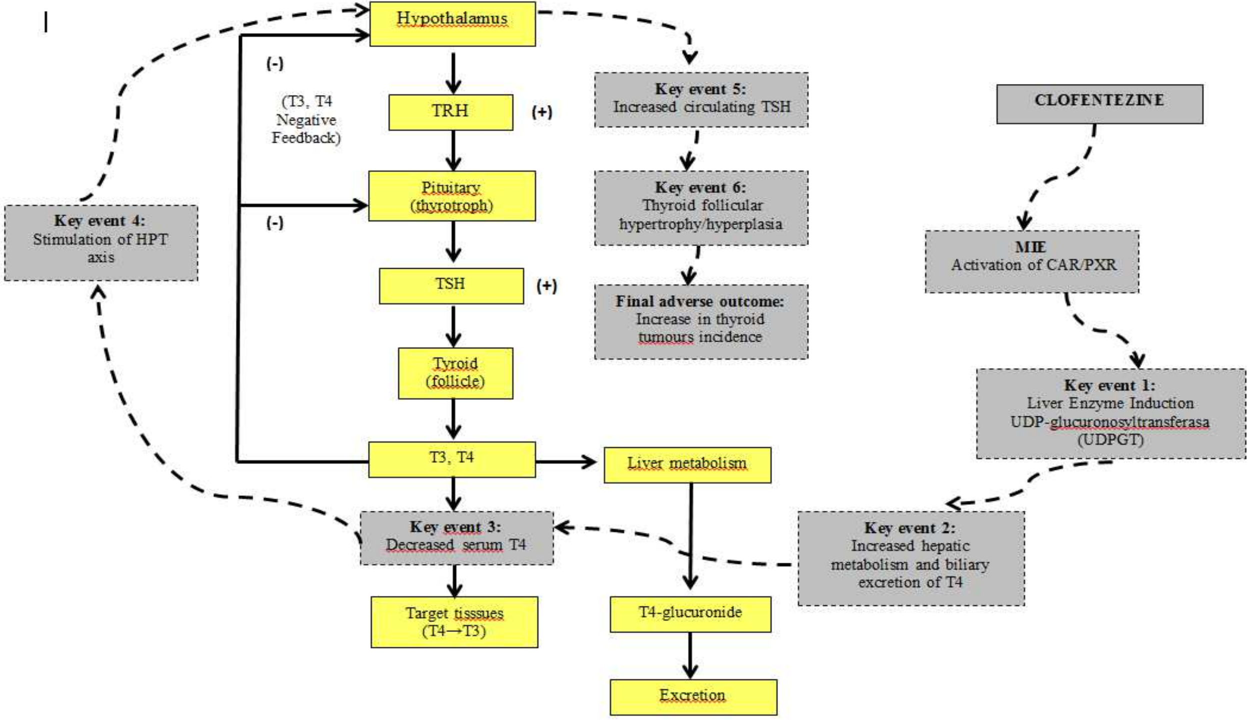
1. Postulated MoA (theory of the case)
2. Key events
3. Concordance of dose-response and Temporal Association
4. Strength, consistency and specificity of association of tumour response with key events.
5. Biological plausibility and coherence
6. Other modes of action
7. Uncertainties, Inconsitencies, and Data Gaps assessment of postulated mode of action.
8. Assessment of postulated mode of action

1- Postulated MoA for the induction of thyroid follicular cell tumours in rats

The postulated MoA for effects on the thyroid and induction of thyroid follicular tumours in rats by clofentezine can be summarised as follows. Briefly, activation of the CAR/PXR nuclear receptors by clofentezine leads to induction of hepatic UDP-glucuronosyltransferase (UDPGT) resulting in increased conjugation and excretion of thyroxine (T4) and decrease in

serum T4 levels. A compensatory increase in thyroid stimulating hormone (TSH) levels secreted via hypothalamus-pituitary-thyroid (HPT) axis results in the chronic proliferative stimulus of thyroid follicular cells by TSH prompting hypertrophy and hyperplasia, and eventually progress to form follicular cell adenomas and/or carcinomas. (Fig. 1)

Figure 3: Proposed Clofentezine MoA for thyroid follicular cell tumors



This figure shows the normal steps in functioning of the HPT axis (yellow boxes and solid lines,), as well as the proposed clofentezine MoA for thyroid follicular cell tumors (gray boxes and dotted arrows).

2- <u>Listing of key events identified in experimental animals</u>

The Key Events are the onward biological consequences that result from the molecular initiating event. These are measurable events that are critical to the induction of the adverse effect (Formation of thyroid follicular tumours).

Associative events are biological processes that are themselves not causal necessary key events for the MoA, but are reliable indicators or markers for key events.

The key events and associative events in this process have been observed and measured in male rats in short-term and MoA studies and a carcinogenicity study *(Anonymous 42, 1985a)*. They are presented below (Table 28).

Table 28: Key Events and associative events for a specific Mode of Action of clofentezine.

Key Events	Associative events
MIE (Molecular Initiating Event) : Activation of CAR/PXR activation	
Key event 1: Induction of hepatic UDP-glucuronosyltransferase (UDPGT)	
Key event 2: Increased hepatic metabolism, increased glucuronidation and biliary excretion of T4	Increased liver weight Liver histopathology
Key event 3: Decreased serum T4 half-live and concentration	
Key event 4: Stimulation of HPT axis	Increased pituitary weight Pituitary histopathology
Key event 5: Increasing circulating TSH concentration	Increased Thyroid weight Thyroids enlargement /Hypertrophy
Key event 6: Increased thyroid folicular cell proliferation (hyperplasia)	Increased colloid depletion Increased mitotic activity follicular cells (lining)
Final adverse outcome (AO): Increase in thyroid tumours incidence	

The key events “activation of CAR/PXR” and “increase in thyroid tumours incidence” are terms decribed as “Molecular Initiating Event” (MIE) and “Adverse Outcome” (AO) respectively in the AOP wiki nomenclature.

3- Concordance of dose-response and Temporal Association

The dose response relationships and temporal association for the Key Events measured in the studies in rats are presented below (Table 29). The responses for the Key Events are shown as *Observed Effect* (OE), *Observed Associate Effect (OAE), No Observed Effect (NOE), No Determined (ND)* and *No Applicate (NA)*. Each box “OE” shows the time (month, week or day) in which the effect is observed. Each box “OAE” shows the time (month, week or day) in which one/some associated effect/s is/are observed. Each box “NOE” shows the time (month, week or day) in which the effect is measured but not observed. Quantification (degree of change) is not shown in order to keep the tables clearer. Final adverse outcome (Formation of thyroid tumours) is generally not applicable to subchronic studies and therefore is labelled as “No Applicate (NA)” in the tables, although the histopathological outcome was measured. A sex difference for final adverse outcome was evident (males having a higher tumour incidence than females), but is also not distinguished in the tables for reasons of clarity. Further details on the studies can found in the main document.

Table 29: Concordance of dose-response and temporal relationships for Key Events in studies in rats.

Reference study	Dose mg/kg bw/day)	Key event 1: Induction UDPGT	Key event 2: ↑ Hepatic metabolism and biliary excretion of T4[1]	Key event 3: Decreased blood T4 levels	Key event 4: Stimulation of HPT axis[2]	Key event 5: Increasing circulating TSH	Key event 6: ↑Thyroid follicular cell proliferation (hyperplasia)[3]	Final adverse outcome: ↑ Thyroid tumours incidence
	Ordered from low to high dosage	Key events shown in order from earliest event to later (left to right). Results show the time that the event was observed. Quantitative changes in severity are not shown.						
Anonymous 42 (1985a)	1.72/ 2.18♂♀	ND	**OAE** 12 and 27 months	**NOE** 6, 12, 18 and 27 months	**NOE** 12 and 27 months	**NOE** 6, 12, 18 and 27 months	**OE** 12 and 27 months	**NOE**[a] 27 months
Anonymous 42 (1985a)	17.3/ 22.1♂♀	ND	**OAE** 12 and 27 months	**NOE** 12 and 27 months (↑T4 at 27 months)	**NOE** 12 and 27 months	**NOE** 6, 12, 18 and 27 months	**OE** 12 and 27 months	**OE** 27 months
Anonymous 59 (1989)	22.69♂	**OE** (5, 8, 15 and 29 days)	**OAE** (15 and 29 days)	ND	ND	ND	**OE** minimum 14 days	NA
Anonymous 74, (1981)	26.2♂/ 29.3♀	ND	**OAE** 13 weeks. Reversible (recovery week 19)	ND	**NOE** 13 weeks	ND	**NOE** 13 weeks	NA
Anonymous 55 (1990)	28.9♂	**OE** (4, 8 and 13 weeks)	**OAE** (4 and 13 weeks)	**NOE** weeks 4 , 8 and 13	**NOE** (4, 8 and 13 weeks)	**NOE** (4, 8 and 13 weeks)	**NOE** (4, 8 and 13 weeks)	NA
Anonymous 60 (2016)	36.4♂	**NOE** 1, 2 weeks	**OAE** 1, 2 and 4 weeks	**NOE** week 1, 2, 3 and 4 (↑T4 wk 3 and 4)	ND	**OE** week 3	**OE** Day 29	NA
Anonymous 51 (1986)	40♂♀	ND	**OAE** week 6	**NOE** week 6 (↑T4)	**OAE** Week 6	**NOE** week 6	**NOE** week 6	NA
Anonymous 53 (1986)	40 ♂	ND	ND	ND	**OAE** Minimum week 6	ND	ND	NA
Anonymous 59 (1989)	169.4♂	ND	**OAE** 5, 8, 15 and 29 days)	ND	ND	ND	**OE** minimum 14 and 28 days	NA
Anonymous 63 (1982b) Anonymous 64 (1988)	202♂/ 221♀	ND	**OAE** 13 weeks. Reversible (recovery week 17)	ND	**NOE** 9 and 13 weeks	ND	**NOE** 9 and 13 weeks	NA
Anonymous 60 (2016)	205.7♂	**OE** 1, 2 weeks	**OAE** Wks 1, 2 ,4	**NOE** wks 1, 2, 3, 4	ND	**NOE** wks 1, 2, 3, 4	**OAE** wks 2 and 4	NA
Anonymous 74 (1981)	265♂/ 292♀	ND	**OAE** 13 weeks. Reversible (recovery week 19)	ND	**NOE** 13 weeks	ND	**NOE** 13 weeks	NA
Anonymous 60 (2016)	509.4♂	**OE** 1, 2 weeks	**OAE** 1, 2 and 4 weeks	**NOE** wks 1, 2, 3, 4 (↑T4 wk 3)	ND	**OE** Week 3	**OE** 1, 2 ,4 weeks	NA
Anonymous 63 (1982b) Anonymous 64 (1988)	602♂/ 662♀	ND	**OAE** 13 weeks Reversible (recovery week 17)	ND	**NOE** 9 and 13 weeks	ND	**OAE** 13 weeks	NA
Anonymous 47 (1988)	1500♂	ND	**OE** 2 weeks	ND	ND	ND	ND	NA
Anonymous 48 (1988)	1500♂	ND	**OE** 5 weeks	ND	ND	ND	ND	NA
Anonymous 59 (1989)	1635♂	**OE** (5, 8, 15 and 29 days)	**OAE** 5, 8, 15 and 29 days)	ND	ND	ND	**OE** 7, 14, 28 days	NA
Anonymous 63 (1982b) Anonymous 64 (1988)	1892♂/ 1992♀	ND	**OAE** 13 weeks. (Reversible week 17)	ND	**NOE** 9 and 13 weeks	ND	**OAE** 13 weeks	NA

Reference study	Dose mg/kg bw/day)	Key event 1: Induction UDPGT	Key event 2: ↑ Hepatic metabolism and biliary excretion of T4[1]	Key event 3: Decreased blood T4 levels	Key event 4: Stimulation of HPT axis[2]	Key event 5: Increasing circulating TSH	Key event 6: ↑Thyroid follicular cell proliferation (hyperplasia)[3]	Final adverse outcome: ↑ Thyroid tumours incidence
	Ordered from low to high dosage	**Key events shown in order from earliest event to later (left to right). Results show the time that the event was observed. Quantitative changes in severity are not shown.**						
Anonymous 58 (1988)	**1915♂**	ND	**OAE** (3 and 5 days and 1 and 2 weeks)	**NOE** (24 h and 2 and 4 days and 1 and 2 weeks)	ND	**OE** 4 days and 1 and 2 weeks)	**OE** (1 and 2 weeks)	NA
Anonymous 55 (1990)	**2250♂**	**OE** weeks 4, 8 and 13	**OAE** 4 and 13 weeks	**NOE** weeks 4 , 8 and 13 (↑T4 at wks 8 and 13)	**OAE** Week 4	**OE** 4, 8 and 13 weeks	**OAE** week 4	NA
Anonymous 46 (1985)	**3000♂**	ND	ND	**OE** week 4	ND	ND	ND	NA
Anonymous 51 (1986)	**3000♂**	ND	**OAE** week 6	**NOE** week 6 (↑T4)	**OAE** week 6	**OE** week 6	**OE** week 6	NA

OE: Observed effect (Brown boxes); OAE: Observed associate effect (green boxes). NOE: No observed effect (yellow boxes); ND: No determinate (white boxes); NA: No applicate (white boxes)
[1]OAE for Key Event 2 are referred to associated effects (liver weight/histopathology)
[2]No data for Key Event 4. The observations correspond to associated effects (pituitary weight/histopathology)
[3]OAE for Key Event 6: Hyperplasia was not observed, although thyroid activity was seen (hypertrophy, colloided depletion ...). Events such as the thyroid follicular cell hypertrophy and hyperplasia in most studies do not have sufficient time points to distinguish temporally between these, although hypertrophy usually precedes hyperplasia in this MoA
[a] Tumors were observed but with an incidence similar to the control.

There are two relationships of interest in this MoA evaluation. First, whether the Key Events show a sequential (temporal) relationship such that Key Events 1 and 2 precede Key Events 3 and 4, which occur before Key Events 5 and 6 and the second relationship examines dose-response, and whether Key Events show an incidence and severity consistent with doses.

a. Temporal Association

When analyzing all the data together of the different effects observed in the short-term, carcinogenicity and MoA studies, it can be observed how the different events show a good temporal (sequential) relationship such as Key Events 1 and 2 precede Key Events 3 and 4, which occur before Key Events 5 and 6.

In the study of carcinogenicity in rat (*Anonymous 42, 1985a*), an increase in hepatic metabolism (key event 2) was observed at 17.3 mg/kg bw/day in males. This effect was characterized by an increased liver weight associated with histopathology (centrilobular hepatocyte enlargement or centrilobular hepatocyte vacuolation). An increase in thyroid tumours incidence "hyperplasia" (key event 6) was also observed. These effects were measured at 12 months and preceded the formation of tumours observed at month 27 (final adverse outcome). No effects were observed in the alteration of the thyroid hormones (T4 and TSH) because these effects were measured after 6 months and their variation is noticeable before (approximately 4 weeks). Results of studies carried out with phenobarbital support it *(McClain et al 1989, Capen, 1992)*, T4 levels decreased after 4 weeks of treatment but after T4 levels returned to near normal due to compensation by the HPT axis. In the MoA study presented by *Anonymous 46 (1985)* is showing decrease in T4 (6.8%) in week 4.

In the MoA study of *Anonymous 59 (1989)*, the effects on liver enzyme induction and liver weight were seen by 5 days, indicating that the liver changes were early effects. In the same study alterations in the thyroid weights / histopathology were observed, this occurred after changes in liver (15 days at 22.69 mg/ kg bw/day and 7 days at 1635 mg/kg bw/day). Thyroid hormones alterations were not monitored and formation of thyroid tumours is generally not applicable to subchronic studies.

In a similar way, in study of *Anonymous 58 (1988*), at 1915 mg/kg bw/day the effects on liver weight were seen by 3 days, an increase of TSH (key event 5) was observed by 4 days and an increase in thyroid follicular cell proliferation (key event 6) was observed by 7 days (slight incidence) and 14 days (marked incidence).

In the study of *Anonymous 51 (1986),* an increase of the hepatic metabolism (Key event 2), a stimulation of HPT axis (Key event 4), an increase in circulating TSH (Key event 5) and an increase in thyroid follicular cell proliferation (Key event 6) were observed at 3000 mg/kg bw/day at 6 weeks of treatment (a single sampling time).

b. Concordance of dose-response

In relation to the second relationship, Key Events shows a consistent dose-response increased incidence and severity of effects with increasing duration of exposure.

Thyroid tumor formation is a progression from follicular cell hypertrophy to hyperplasia to tumors; thyroid histopathologic changes were increased in incidence and severity with a higher dose of treatment with clofentezine, contributing to the biological plausibility of this MoA. In the rat carcinogenicity study, *Anonymous 42 (1985a)*, at 1.72 mg/kg bw/day in males tumors were observed but with an incidence similar to the control (2/50 *vs* 2/50) however, at 17.3 mg/kg bw/day a slight increase in the incidence of follicular cell tumors (combined adenomas and carcinomas) in the thyroid in reference to the control was noted at terminal sacrifice (8/50 *vs*. 2/50 control).

In study of *Anonymous 59 (1989)*, at 22.69 mg/kg bw/day the effects on liver (increase absolute and relative weight) were seen from 15 days and hyperplasia of thyroids was observed on 14 days but with minimal incidence (3/20 *vs* 2/20 control). However, at 1635 mg/kg bw/day the effects on liver (increase absolute and relative weight) were seen from 5 days and hyperplasia of thyroids was observed from 7 days and with a incidence higher (6/20 *vs* 0/20 control) than at 22.69 mg/kg bw/day.

Thyroids effects were only seen at doses at which also liver effects occurred. In MoA study, *Anonymous 60 (2016),* at 205.7 mg/kg bw/day the effects on liver enzyme induction and liver weight with histopathology were seen, however no variation on thyroid weight was observed (at this dose hypertrofia is observed but with low incidence). At the highest dose tested, 509.4 mg/kg bw/day, effects on liver (liver enzyme induction and liver weight) and thyroids effects (increased thyroid weight and histopathological) were seen. In addition, both incidence and severity of the effects observed in liver and thyroid were greater increasing dose level.

4- Strength, consistency and specificity of association of tumour response with key events.

In evaluating the clofentezine data set, the profile of effects was examined for the strength of association, consistency and specificity to determine whether key events occurred consistently across clofentezine studies, whether these key events were linked in a biologically plausible manner, and whether these key events exhibited the expected concordance across dose–response and temporal relationships. Thus, repeat dose guideline studies, which included clofentezine exposures for 13-weeks (subchronic studies), 6-, 12- and 27-months (chronic toxicity/oncogenicity study) and specific MoA studies, were examined for evidence to support the indirect UGT-mediated MoA for clofentezine.

The experimental data (data supporting the proposed MoA for clofentezine´s effects on the HPT axis) evaluated for strength, consistency, and specificity are given below:

MIE (Molecular Initiating Event): Activation of CAR/PXR activation

No data

Key event 1: Induction of hepatic UDP-glucuronosyltransferasa (UDPGT)

Reference Study	Type	Species	Dose (ppm)	Duration	Induction of hepatic UDPGT
Anonymous 59 (1989)	MoA	male rats	0, 10, 400, 3000 or 30000 ppm (equivalent to 0, 0.58, 22.69, 169.4 and 1635 mg/kg bw/day).	4 weeks Sampling times: 5, 8, 15 and 29 days	30000 ppm: ↑ five fold *vs* control from 5 days 400 ppm: ↑ two fold *vs* control from 5 days *Not measured at 3000 ppm*
Anonymous 55 (1990)	MoA	male rats	0, 10, 40, 400 or 30000 ppm (equivalent to 0, 0.71, 2.88, 28.9, or 2250 mg/kg bw/day)	13 weeks Sampling times: 4, 8 and 13 weeks	At doses ≥ 400 ppm there was an increase in liver microsomal UDPGT at 4, 8 and 13 weeks
Anonymous 60 (2016)	MoA	Male rats	0, 600, 3000 and 9000 ppm (equivalent to 0, 36.4, 205.7 and 509.4 mg/kg bw/day)	4 weeks Sampling times: 8 and 15 days	↑ Activity UDP glucuronosyltransferase enzymes (at 9000 ppm: approx. 2 and 3-fold for day 8 and day 15 respectively *vs* control and at 3000 ppm 2 and 4-fold for day 8 and 15 respectively *vs* control)

Key event 2: Increased hepatic metabolism, increased glucuronidation and biliary excretion of T4

Reference Study	Type	Species	Dose (ppm)	Duration	Hepatic metabolism and biliary excretion of T4
Anonymous 47 (1988)	MoA	Male rat	0, 30000 ppm (equivalent to 0, 1500 mg/kg bw/day)	2-3 weeks Sampling times:0, 30, 60, 120, 180 and 240 min	Bile flow rate: ↑ doubled *vs* control Excretion in bile: ↑ marked Clearance: ↑ initial ↑ Excretion thyroxine glucuronide
Anonymous 48 (1988)	MoA	Male rat	0, 30000 ppm (equivalent to 0, 1500 mg/kg bw/day)	5 weeks Sampling times: 3, 6, 9, 12, 24, 28, 32, 48, 54, and 72h.	Excretion: ↓ Urinary and ↑ faecal elimination elimination. Slight ↑ in the total excretion.

Associative event 1: Increase liver weight and histopathology

Reference Study	Type	Species	Dose (ppm)	Duration	glucuronidation/ weight and histopathology liver
Anonymous 74 (1981)	Subchronic study	Male and female rats	0, 40, 400 and 4000 ppm (equivalent to 0, 2.65/2.91, 26.2/29.3, 265/292 ♂/♀ mg/kg bw/day)	90-day with 6 week recovery period	≥400 ppm ↑Absolute and relative liver weight associated with centrilobular hepatocyte enlargement (13 weeks). Reversible after recovery (week 19). At 4000 ppm the increase of relative liver weight was not completely reversible in ♂.

Reference Study	Type	Species	Dose (ppm)	Duration	glucuronidation/ weight and histopathology liver
Anonymous 42 (1985a) *Anonymous 43 (1985-1988)* *Anonymous 44(1988)* *Seamons, M.C. & Crofts, M. (1985)*	Combined chronic toxicity/ carcinogenicity	Male and female rats	0, 10, 40 and 400 ppm (equivalent to 0, 0.43/0.55, 1.72/2.18 and 17.3/22.1 ♂/♀ mg/kg bw/day)	27 month Sampling times: 6, 12, 18 and 27 months	↑ Absolute and relative liver weight in both sexes at dose of 400 ppm associated with centrilobular hepatocyte enlargement or centrilobular hepatocyte vacuolation in ♂
Anonymous 51 (1986) *Anonymous 52 (1988)* *Anonymous 53 (1986)* *Anonymous 54(1989)*	MoA	Male and female rats	0, 400, 30000 ppm (equivalent to 0, 40 and 3000 mg/kg bw/day)	6 weeks	↑ Absolute and relative liver weight in both sexes at 30000 ppm and in males only at 400 ppm
Anonymous 63 (1982b) *Anonymous 64 (1988)*	Subchronic study	Male and female rats	0, 3000, 9000 and 27000 ppm (equivalent to 0, 202/221, 602/662, 1892/1992 ♂/♀ mg/kg bw/day)	90-day with 4 week recovery period	≥3000 ppm ↑Absolute and relative liver weight associated with centrilobular hepatocyte enlargement (13 weeks). Reversible after recovery (week 17). A 9000 ppm ↑relative liver weight was not totally reversible in ♀.
Anonymous 58(1988)	MoA	Male rats	0, 30000 ppm (equivalent to 1915 mg/kg bw/day)	2 weeks Sampling times: 2, 3, 5, 8 and 15 days.	↑ Absolute and relative liver weight (abs 16% and rel 17%) following 3 days of treatment rising to 60% after 5 days and remaining elevated throughout the treatment period.
Anonymous 59 (1989)	MoA	Male rats	0, 10, 400, 3000 or 30000 ppm (equivalent to 0, 0.58, 22.69, 169.4 and 1635 mg/kg bw/day)	4 weeks Sampling times: 5, 8, 15 and 29 days	↑Absolute and relative liver weight from 5 days until the end of treatment period (29 days) at doses ≥3000 ppm. At 400 ppm ↑Absolute and relative liver weight was from 15 days until the end of the treatment period.
Anonymous 55 (1990)	MoA	Male rats	0, 10, 40, 400 or 30000 ppm (equivalent to 0, 0.71, 2.88, 28.9, or 2250 mg/kg bw/day)	13 weeks Sampling times: 4 and 13 weeks.	↑ Relative liver weight at dose ≥ 400 ppm at 4 and 13 weeks.
Anonymous 60 (2016)	MoA	Male rats	0, 600, 3000 and 9000 ppm (equivalent to 0, 36.4, 205.7 and 509.4 mg/kg bw/day)	4 weeks Sampling times: 8, 15 and 29 days	At 600 ppm ↑Absolute liver weight on 29 day and at ≥3000 ppm ↑Absolute liver weight from 8 days until the end of treatment period (29 days) At ≥ 600 ppm hepatocellular hypertrophy (increased incidence and severity dose dependent) from day 8.

Key event 3: Decreased serum T3 and T4 half-live and concentration

Reference Study	Type	Species	Dose (ppm)	Duration	Serum T4 half-live and concentration
Anonymous 46 (1985)	MoA	Male rats	0, 30000 ppm (equivalent to 0, 3000 mg/kg bw/day)	4 weeks	↓T4 half-life. Thyroid iodine uptake by thyroid ↑ Rapid and significant at 6 h after dosing.
Anonymous 51 (1986) *Anonymous 52 (1988)* *Anonymous 53 (1986)* *Anonymous 54(1989)*	MoA	Male and female rats	0, 400, 30000 ppm (equivalent to 0, 40 and 3000 mg/kg bw/day)	6 weeks	↑ T4 at dose 30000 ppm (28% ♂ and 31%♀) and 400 ppm (♂15%)
Anonymous 58 (1988)	MoA	Male rats	0, 30000 ppm (equivalent to 0, 1915 mg/kg bw/day)	2 weeks Sampling times: 24 h, 2 days, 4 days, 7 days and 14 days	↓ T3 (20%) following 2 days of treatment and after 7 days (25%). After 14 days, T3 levels had returned to control. No variations in T4
Anonymous 55 (1990)	MoA	Male rats	0, 10, 40, 400 or 30000 ppm (equivalent to 0, 0.71, 2.88, 28.9, or 2250 mg/kg bw/day)	13 weeks Sampling times: 4, 8 and 13 weeks	↑ T4 at dose 30000 ppm on 8 and 13 weeks
Anonymous 60 (2016)	MoA	Male rats	0, 600, 3000 and 9000 ppm (equivalent to 0, 36.4, 205.7 and 509.4 mg/kg bw/day)	4 weeks Sampling times: 1, 2, 3 and 4 weeks	At 9000 ppm ↑ T4 week 3. At 3000 ppm there were no biologically relevant differences in serum T4 with controls. At 600 ppm ↑ T4 weeks 3 and 4.
Anonymous 42 (1985a) *Anonymous 43 (1985-1988)* *Anonymous 44(1988)* *Seamons, M.C. & Crofts, M. (1985)*	combined chronic toxicity/ carcinogenicity	Male and female rats	0, 10, 40 and 400 ppm (equivalent to 0, 0.43/0.55, 1.72/2.18 and 17.3/22.1 ♂/♀ mg/kg bw/day)	27 month Sampling times: 6, 12, 18 and 27 months	At 400 ppm ↑T4 (49%) in males month 27

Key event 4: Stimulation of HPT axis

No data

Associative event 2: Increased pituitary weight and histopathology pituitary.

Reference Study	Type	Species	Dose (ppm)	Duration	Weight and histopathology pituitary.
Anonymous 51 (1986) *Anonymous 52 (1988)* *Anonymous 53 (1986)* *Anonymous 54(1989)*	MoA	Male and female rats	0, 400, 30000 ppm (equivalent to 0, 40 and 3000 mg/kg bw/day)	6 weeks	Histopathology anterior pituitary: 30000ppm: Minimal hypertrophy and dilatation of rough endoplasmic reticulum in some of the cells that produce TSH (thyrotrophs) from 5/5 males. Occasional secretory granules within the cisternae of the rough endoplasmic reticulum in some thyrotrophs (4/5 male rats). Secondary lysosomes were seen in the cells of two of these rats. 400ppm: Minimal hypertrophy and dilatation of rough endoplasmic reticulum in thyrotrophs (1/4 males).
Anonymous 55 (1990)	MoA	male rats	0, 10, 40, 400 or 30000 ppm (equivalent to 0, 0.71, 2.88, 28.9, or 2250 mg/kg bw/day)	13 weeks Sampling times: 4, 8 and 13 weeks	Focal hypertrophy (7/10 *vs* 1/10) of pituitary thyrotrophs at 30000 ppm and week 4 *No tissues were examined from animals killed at 8 and 13 weeks*

Key event 5: Increasing circulating TSH concentration

Reference Study	Type	Species	Dose (ppm)	Duration	TSH concentration
Anonymous 51 (1986) *Anonymous 52 (1988)* *Anonymous 53 (1986)* *Anonymous 54(1989)*	MoA	Male and female rats	0, 400, 30000 ppm (equivalent to 0, 40 and 3000 mg/kg bw/day)	6 weeks	↑ TSH at 30000 ppm (♂♀)
Anonymous 58 (1988)	MoA	Male rats	0, 30000 ppm (equivalent to 0, 1915 mg/kg bw/day)	2 weeks Sampling times: 24 h, 2 days, 4 days, 7 days and 14 days	↑ TSH after 4 days of treatment (42%). After 14 days, TSH levels remained elevated (37%).
Anonymous 60 (2016)	MoA	Male rats	0, 600, 3000 and 9000 ppm (equivalent to 0, 36.4, 205.7 and 509.4 mg/kg bw/day)	4 weeks Sampling times: 1, 2, 3 and 4 weeks	At 9000 ppm ↑TSH at week 3 (62%) and 600 ppm at week 3 (54%) At 3000 ppm there were no biologically relevant differences in serum TSH with controls.

Associative event 3: Increased thyroid weight

Reference Study	Type	Species	Dose (ppm)	Duration	Thyroid weight
Anonymous 58 (1988)	MoA	Male rats	0, 30000 ppm (equivalent to 0, 1915 mg/kg bw/day)	2 weeks Sampling times: 2 days, 3 days, 5 days, 8 and 15 days	↑ Abs and rel weight (abs 16% and rel 19%) following 3 days of treatment. After 5 days the thyroid weight was similar to controls
Anonymous 59 (1989)	MoA	male rats	0, 10, 400, 3000 or 30000 ppm (equivalent to 0, 0.58, 22.69, 169.4 and 1635 mg/kg bw/day).	4 weeks Sampling times: 5, 8, 15 and 29 days	30000 ppm: ↑ Abs and rel weight after 7 days until termination (abs 17-41% and rel 25-36%) 3000 ppm: ↑Abs and rel weight approximately 30% after 7 and 15 days.
Anonymous 60 (2016)	MoA	Male rats	0, 600, 3000 and 9000 ppm (equivalent to 0, 36.4, 205.7 and 509.4 mg/kg bw/day)	4 weeks Sampling times: 1, 2, 3 and 4 weeks	No effect on thyroid/parathyroid gland weight.

Associative event 4: Thyroids enlargement /Hypertrophy

Reference Study	Type	Species	Dose (ppm)	Duration	Thyroids enlargement /Hypertrophy
Anonymous 63 (1982b) *Anonymous 64 (1988)*	Subchronic study	Male and female rats	0, 3000, 9000 and 27000 ppm (equivalent to 0, 202/221, 602/662, 1892/1992 ♂/♀ mg/kg bw/day)	90-day with 4 week recovery period	≥3000 ppm: min. to mod. Increase in follicular cell size. Fully reversible, no clear dose relationship
Anonymous 58 (1988)	MoA	Male rats	0, 30000 ppm (equivalent to 0, 1915 mg/kg bw/day)	2 weeks Sampling times: 24 h, 2 days, 4 days, 7 days and 14 days	Hypertrophy were seen at 7 days and were marked at 14 days (7 days: 10/10 *vs* 8/10 control; 14 days: 10/10 *vs* 7/10 control).
Anonymous 51 (1986) *Anonymous 52 (1988)* *Anonymous 53 (1986)* *Anonymous 54(1989)*	MoA	Male rats	0, 30000 ppm (equivalent to 0 and 3000 mg/kg bw/day)	6 weeks	↑Total thyroid area (58%): Enlargement

Reference Study	Type	Species	Dose (ppm)	Duration	Thyroids enlargement /Hypertrophy
Anonymous 59 (1989)	MoA	male rats	0, 10, 400, 3000 or 30000 ppm (equivalent to 0, 0.58, 22.69, 169.4 and 1635 mg/kg bw/day).	4 weeks Sampling times: 4, 7, 14 and 28 days	Hypertrophy from day 7 until the end of treatment period at 400 ppm and from day 4 until the end of treatment period at doses ≥3000 ppm
Anonymous 55 (1990)	MoA	Male rats	0, 10, 40, 400 or 30000 ppm (equivalent to 0, 0.71, 2.88, 28.9, or 2250 mg/kg bw/day)	4, 8 and 13 weeks	↑Follicular cell size (hypertrophy) at 30000 ppm on week 4. *No tissues were examined from animals killed at 8 and 13 weeks*
Anonymous 60 (2016)	MoA	Male rats	0, 600, 3000 and 9000 ppm (equivalent to 0, 36.4, 205.7 and 509.4 mg/kg bw/day)	4 weeks Sampling times: 1, 2, 3 and 4 weeks	↑ Incidence and severity of follicular cell hypertrophy: ✓ Incidence: at 9000 ppm 3/5, 4/5 and 5/5 on days 8, 15 and 29 respectively vs 0/5 control, at 3000 ppm 2/5, 3/4 and 5/5 on days 8, 15 and 29 respectively vs 0/5 control and 600 ppm 3/5 *vs* 0/5 control on day 29 ✓ Severity: at 9000 ppm 1.3, 2.0 and 2.4 on days 8, 15 and 29 respectively *vs* 0 control, at 3000 ppm 1, 1.3 and 2.4 on days 8, 15 and 29 respectively *vs* 0 control and 600 ppm 1.0 *vs* 0 control on day 29.

Key event 6: Increased thyroid follicular cell proliferation (hyperplasia)

Reference Study	Type	Species	Dose (ppm)	Duration	Increased thyroid follicular cell proliferation (hyperplasia)
Anonymous 42 (1985a) ***Anonymous 43 (1985-1988)*** ***Anonymous 44(1988)*** ***Seamons, M.C. & Crofts, M. (1985)***	combined chronic toxicity/ carcinogenicit	Male and female rats	0, 10, 40 and 400 ppm (equivalent to 0, 0.43/0.55, 1.72/2.18 and 17.3/22.1♂♀ mg/kg bw/day)	27 month Sampling times: 12 and 27 months	Slight follicular cell hyperplasia (n.s.) and agglomeration of colloid in ♂ at 400 ppm. At 40 ppm there was only slightly follicular cell hyperplasia (ns) in males.

Reference Study	Type	Species	Dose (ppm)	Duration	Increased thyroid follicular cell proliferation (hyperplasia)
Anonymous 58 (1988)	MoA	Male rats	0, 30000 ppm (equivalent to 0, 1915 mg/kg bw/day)	2 weeks Sampling times: 24 h, 2 days, 4 days, 7 days and 14 days	↑ 7 days and were marked at 14 days. (7 days: 4/10 vs 0/10 control; 14 days: 8/10 vs 0/10 control).
Anonymous 51 (1986) *Anonymous 52 (1988)* *Anonymous 53 (1986)* *Anonymous 54(1989)*	MoA	Male rats	0, 30000 ppm (equivalent to 0 and 3000 mg/kg bw/day)	6 weeks	↑Total number of thyroid follicular cells (47%)
Anonymous 59 (1989)	MoA	male rats	0, 10, 400, 3000 or 30000 ppm (equivalent to 0, 0.58, 22.69, 169.4 and 1635 mg/kg bw/day).	4 weeks Sampling times: 4, 7, 14 and 28 days	Hyperplasia of the follicular lining cells from day 7 until the end of treatment period at 30000 ppm with higher incidence and severity after 28 days. In addition, there was, in general terms, a clear dose response relationship at each time point.

<u>Associative event 5</u>: Increased colloid depletion

Reference Study	Type	Species	Dose (ppm)	Duration	Increased colloid depletion
Anonymous 63 (1982b) *Anonymous 64 (1988)*	Subchronic study	Male and female rats	0, 3000, 9000 and 27000 ppm (equivalent to 0, 202/221, 602/662, 1892/1992 ♂/♀ mg/kg bw/day)	90-day with 4 week recovery period	At ≥ 3000 ppm: min. to mod. thyroid colloid depletion. No completely reversible, no clear dose relationship.
Anonymous 58 (1988)	MoA	Male rats	0, 30000 ppm (equivalent to 0, 1915 mg/kg bw/day)	2 weeks Sampling times: 24 h, 2 days, 4 days, 7 days and 14 days	↑ On 7 days and were marked on 14 days.
Anonymous 59 (1989)	MoA	male rats	0, 10, 400, 3000 or 30000 ppm (equivalent to 0, 0.58, 22.69, 169.4 and 1635 mg/kg bw/day).	4 weeks Sampling times: 4, 7, 14 and 28 days	Marked depletion of colloid, with 20/20 animals at 30000 ppm, 19/20 animals at 3000 ppm and 12/20 animals at 400 ppm on 28 days showing at least a slight response compared with only 4/20 controls.

Reference Study	Type	Species	Dose (ppm)	Duration	Increased colloid depletion
Anonymous 55 (1990)	MoA	Male rats	0, 10, 40, 400 or 30000 ppm (equivalent to 0, 0.71, 2.88, 28.9, or 2250 mg/kg bw/day)	4, 8 and 13 weeks	↑ Colloid depletion at 30000 ppm on week 4 *No tissues were examined from animals killed at 8 and 13 weeks*
Anonymous 60 (2016)	MoA	Male rats	0, 600, 3000 and 9000 ppm (equivalent to 0, 36.4, 205.7 and 509.4 mg/kg bw/day)	4 weeks Sampling times: 1, 2, 3 and 4 weeks	↑ Incidence (at 9000 ppm 2/5 and 3/5 on days 15 and 29 respectively *vs* 0/5 control and 3000 ppm 1/4 and 3/5 on days 15 and 29 respectively *vs* 0/5 control)

Associative event 6: Increased mitotic activity follicular cells (lining)

Reference Study	Type	Species	Dose (ppm)	Duration	Increased mitotic activity follicular cells (lining)
Anonymous 58 (1988)	MoA	Male rats	0, 30000 ppm (equivalent to 0, 1915 mg/kg bw/day)	2 weeks Sampling times: 24 h, 2 days, 4 days, 7 days and 14 days	This cellular division was maximal at 7 days and some activity was still evident at 14 days. (4 days: 5/10 rats *vs* 1/10 control; 7 days: 7/10 vs 0/10 control; 14 days: 5/10 *vs* 0/10 control).
Anonymous 59 (1989)	MoA	male rats	0, 10, 400, 3000 or 30000 ppm (equivalent to 0, 0.58, 22.69, 169.4 and 1635 mg/kg bw/day).	4 weeks Sampling times: 4, 7, 14 and 28 days	30000 ppm: Increase from 4 days until the end of treatment period. 3000 ppm: Increase after 4 days, thereafter declining although still detectable after 28 days. 400 ppm: Increase only until 4 days.

Final adverse outcome: Increase in thyroid tumours incidence

Reference Study	Type	Species	Dose (ppm)	Duration	Increased thyroid follicular cell proliferation (hyperplasia)
Anonymous 42 (1985a) *Anonymous 43 (1985-1988)* *Anonymous 44(1988)* *Seamons, M.C. & Crofts, M. (1985)*	Combined chronic toxicity/ carcinogenicity	Male and female rats	0, 10, 40 and 400 ppm (equivalent to 0, 0.43/0.55, 1.72/2.18 and 17.3/22.1 ♂♀ mg/kg bw/day)	27 month	Slightly increased thyroid tumor incidence in males (8/50 vs. 6/49) at 400 ppm, but not in females

Clofentezine has been shown to increase liver weight and induce hepatocellular hypertrophy in numerous repeat-dose studies. These signs are indicators of clofentezine-induced hepatic enzyme induction. In addition, results from another MoA studies in male CD rats, *(Anonymous 59, 1989; Anonymous 55, 1990; Anonymous 60, 2016)* show that clofentezine markedly increased activation of UGT enzymes indicating a possible activation of the Constitutive Androstane Receptor (CAR) pathway.

The effect of the clofentezine on the biliary excretion of thyroxine in the male rat (effect on thyroid hormone turnover) is observed in MoA study (*Anonymous 47, 1988)*. The profile of metabolites in bile indicates that the action of clofentezine on the thyroid gland is caused by an increased turnover of thyroid hormones due to induction of the hepatic enzymes responsible for the catabolism of T4 and resulting in an increased excretion of thyroxine metabolites.

Decreases in serum T4 levels were assessed in the MoA study *(Anonymous 46, 1985a)*, A decrease in T4 (6.8%) in week 4 was shown.

Stimulation of the HPT axis can be demonstrated by pituitary histopathology which corresponds with increased TSH. Pituitary histopathology was assessed in two MoA studies (*Anonymous 53, 1986 and Anonymous 55, 1990).* In both studies an increased in circulating TSH was observed.

Subsequent thyroid changes induced in response to TSH stimulation, which typically include increases in thyroid weight, as well as thyroid histopathological changes (follicular cell hyperplasia/hypertrofia) were assessed in the clofentezine thyroid MoA studies *(Anonymous 51, 1986, Anonymous 59, 1989, Anonymous 55, 1990 and Anonymous 60., 2016*). These results were consistent with the chronic toxicity/oncogenicity study *(Anonymous 42, 1985a)*, where hyperplasia progressed to tumors.

The reversibility is consistent with the proposed MoA where the non-neoplastic cellular changes may be reset by the normal feedback-control systems and reversed. In this analysis there are studies that show effects with reversibility. The 90-day study in male rats *(Anonymous 63, 1982b)* showed reversibility in decreases in body weight gain, increases of liver weight, centrilobular hepatocyte enlargement, liver macroscopic pathology and changes in thyroid. Besides, in the other 90-day study in male rats *(Anonymous 74, 1981)* there was also reversibility in decreases in body weight gain, centrilobular hepatocyte enlargement and liver weight.

Thus, overall, the weight-of-evidence assessment across studies is judged to be strong, consistent and specific, indicating that the liver is the primary target organ and that it responds to clofentezine by hepatic enzyme induction and increased metabolic activity generating catabolism of T4 and increase excretion of thyroxine metabolites which it involves an turnover of thyroid hormones. Stimulation of the HPT axis generates an increase in TSH so that increases in thyroid weight and hyperplasia/hypertrofia progress to tumors.

5- <u>Biological plausibility and coherence</u>

The proposed MoA for clofentezine could be consistent with phenobarbital-induced thyroid tumors, which also shows induction of hepatic enzymes (UGT, coupled with increases in liver weight and hepatocellular hypertrophy), decreased serum T4, increased circulating TSH, hypertrophy of the pituitary, and subsequent thyroid effects (increased weight, hypertrophy and hyperplasia progressing to thyroid tumors) *(Capen, 1997; McClain et al., 1989).* However, there are a number of uncertainties, data gaps and other potential alternative mode of action for the thyroid tumours that has to be taken into account in the weigh-of –evidence assessment.

The tumour response elicited by clofentezine is typical of a rodent thyroid carcinogen, in that thyroid follicular cell tumours are found in male rats but not in female rats or mice. Rats tend to be more sensitive to thyroid carcinogenesis than mice, and male rats are frequently found to be more sensitive

than female rats with respect to the proportion of chemicals that induce thyroid tumours *(Hurley et al., 1998).* In keeping with this, TSH levels are typically higher in male rats than in females *(Hill et al., 1989).*

6- Other modes of action

Direct MoA as genotoxicity can be excluded, since clofentezine was non-mutagenic in a series of studies. A battery of *in vitro* genotoxicity studies, including the bacterial reverse mutation test (Ames test), a mammalian chromosome aberration test, and a mammalian cell gene mutation test and gene conversion and mitotic recombination test in yeast, demonstrated that clofentezine does not cause gene mutations or chromosome aberrations *(McConville 1980, Bowles 2005, Bootman and Rees 1982, Wallner. 2015a, Allen, Brooker, Godfrey, 1987* and *Riach and McGregor 1983).* In addition, two *in vivo* mouse micronucleus assay *(Anonymous 39 1982 and Anonymous 40, 1987)* showed that clofentezine does not induce micronucleus in somatic cells. Therefore, the available evidence indicates that mutagenesis is not an alternative MoA for clofentezine.

There are multiple alternative indirect MoAs operating via hormonal imbalance in alterations in thyroid function; however, it can be difficult to differentiate which of the thyroid MoAs is operating. The presentation of altered thyroid function, which can lead to thyroid tumors in rat chronic studies, has similarities in some key events across several different MoAs (e.g., decreased serum T4, increased TSH, increased thyroid weight and/or histopathology) as the HPT axis strives to reestablish thyroid hormone homeostasis. However, there are no data specifically supporting these alternate hypotheses. Across the clofentezine studies, there is ample evidence that the liver is a primary target organ. Changes in thyroid function are not seen in the absence of liver changes and enzyme induction. Besides, the reversibility of the liver effects and subsequently the thyroid effects supports the postulated MoA of clofentezine.

The possible MoAs for thyroid tumors and clofentezine's evidence for or against each MoA are presented below (Table 30):

Table 30: Alternative MoAs resulting in thyroid tumors.

MoA	Data relating to clofentezine	Conclusion
Direct MoA		
Mutagenic (interacts with DNA)	Clofentezine is negative for mutagenicity in *in vitro* and *in vivo* mutagenicity assays	**Unlikely**
Cytotoxicity	No evidence of a cytotoxic mode of action in the thyroid.	**Unlikely**
Indirect MoA operating via hormonal imbalance		
Iodide deficiency; Blockage of Iodine Uptake by Thyroid	Perchlorate (ClO4)-like MoA Generally requires anions similar in size to iodide; clofentezine is too large a molecule	**Unlikely**
Inhibition of thyroid peroxidase (TPO)	Thioamides, aniline derivatives and substituted phenols-like MoA There are no data specifically supporting this alternate hypothesis, but changes in thyroid function are not seen in the absence of liver enzyme induction which further supports this MoA.	**Plausible but no probable**

MoA	Data relating to clofentezine	Conclusion
Increased hepatic T4 metabolism and biliary elimination	Phenobarbital- like MoA Based on the HPT axis and thyroid hormone feedback; consistent based on supporting data (i.e., clofentezine's liver effects, including hepatic UGT induction, and measured increases in T4 metabolism and elimination…)	**Plausible and coherent**
TSH secreting pituitary tumor	Histopathology: no treatment-related pituitary tumors	**Unlikely**
Inhibited TSH synthesis	Clofentezine increases TSH levels across studies (TSH not decreased)	**Unlikely**
Excess iodine	Clofentezine is not an iodine source	**Unlikely**
Inhibition of Type I or II Deiodinase inhibition	There are no data specifically supporting this alternate hypothesis	**Plausible**
Interference with iodide uptake into the thyroid via the sodium/iodide symporter (NaIS)	There are no data specifically supporting this alternate hypothesis	**Plausible**

7- <u>Uncertainties, Inconsistencies and data Gaps assessment of postulated mode of actio</u>

i. Only in a study of MoA with clofentezine the decrease of T4 was observed at week 4 *(Anonymous 46, 1985*) whereas in other three MoA studies *(Anonymous 51, 1986, Anonymous 55, 1990 and Anonymous 60, 2016)* besides the combined chronic toxicity/ carcinogenicity study *(Anonymous 42, 1985a)* an increase in T4 was observed. This may be due to the times in which the measurement of hormones has been made. Phenobarbital-treated rats develop a characteristic pattern of changes in circulating thyroid hormone levels. After Phenobarbital treatment, serum T4 is markedly decreased after 1 week and remains decreased for 4 week. Afterward, levels return to near normal due to compensation by the hypothalamic- pituitary-thyroid axis *(McClain et al 1989).*

ii. In this MoA, thyroid tumours in rats are preceded by hyperplasia and the incidence of tumours is always lower than earlier effects. These results were no consistent with the values obtained in chronic toxicity/oncogenicity study *(Anonymous 42, 1985a).* At 400 ppm in males (17.3 mg/kg bw/day) the incidence of follicular cell hyperplasia was 7.1% (5/70 animals) while the incidence in tumors was 16% (8/50 animals).

iii. There is a similar profile of effects for multiple alternative MoAs that alter thyroid hormone homeostasis (i.e., decreased serum T4, increased TSH, activation of the HPT axis, thyroid stimulation, resulting in increased thyroid weight and follicular cell hypertrophy/hyperplasia). Due to similarity in the presenting signs for altered thyroid function, not all thyroid MoAs can be readily dismissed (e.g., thyroid peroxidase inhibition).

No other uncertainties, inconsistencies or data gaps have been identified.

8- <u>Assessment of postulated mode of action</u>

Most of the key events of the postulated MoA can be identified in regulatory and mechanistic studies. Data from short-term studies rats and carcinogenicity, as well as from several MoA studies, were

analyzed in order to identify the MoA resulting in the formation of thyroid tumors in rats and their relevance to humans.

a) Qualitative concordance (evidence in rats *vs* evidence in humans)

Key event	***Evidence in rats***	***Evidence in humans***
Activation of CAR	**No direct evidence. Indirect experimental evidence** is observed in the MOA studies *in vivo* in rats with increased PROD and BROD and BQ activity and increased of CYP2B and CYP3A enzymes. Potency of clofentezine was lower compared to phenobarbital.	No data available for clofentezine, but microsomal enzyme induction is **plausible**.
Induction of hepatic UDP-glucuronosyltransferase (UDPGT)	**Direct** experimental evidence In three MoA studies increase of induction hepatic UDPGT was observed.	No direct data on clofentezine in humans, but **plausible** given that other microsomal enzyme inducers (phenobarbital) have been shown to induction of hepatic UDP-glucuronosyltransferase (UDPGT) in humans.
Increased hepatic metabolism, increased glucuronidation and biliary excretion of T4	**Direct** experimental evidence. In short-term, chronic rat and MOA studies in rats, the liver is found to be the most sensitive target, and evidence of increased T4 hepatic clearance is provided by studies on T4-hepatic UGT activity, T4 half-life, T4 biliary elimination, liver weights, and liver hypertrophy/hyperplasia.	No direct data on clofentezine in humans, but **plausible** given that other microsomal enzyme inducers (phenobarbital) have been shown to increased hepatic metabolism, increased glucuronidation and biliary excretion of T4 in humans.
Decreased serum T4	**Direct** experimental evidence Only in a study of MoA with clofentezine the decrease of T4 was observed at week 4, whereas in other three MoA studies besides the combined chronic toxicity/ carcinogenicity study an increase in T4 was observed. This may be due to the measurement time.	No direct data on clofentezine in humans, but **plausible** given that other microsomal enzyme inducers (phenobarbital) have been shown to reduce T4 in humans.
Stimulation of HPT axis	**Indirect** experimental evidence. In two MOA studies in rats hypertrophy of pituitary thyrotrophs was observed.	No data available for clofentezine but other microsomal enzyme inducers have been extensively studied (phenobarbital) and they have not been shown to stimulation of HPT axis.
Increased TSH levels	**Direct** experimental evidence. In three MOA studies in rats, an increased TSH levels was observed	No data available for clofentezine, but other microsomal enzyme inducers have been extensively studied (phenobarbital) and they have not been shown to increase TSH levels even when T4 is decreased.
Increases thyroid cell proliferation	**Direct** experimental evidence. Thyroid follicular cell hyperplasia and hypertrophy with increased colloid deposition were observed in sub-acute, sub-chronic and lifetime studies in rats	No data available for clofentezine, but induction of thyroid cell proliferation secondary to hypothyroidism is remote in humans, given the quantitative differences in thyroid function/homeostasis.

Key event	***Evidence in rats***	***Evidence in humans***
Tumour formation	**Direct** experimental evidence. Clofentezine induced a slight increase in thyroid follicular cell adenomas in male rats in a lifetime dietary study. Thyroid tumours were associated thyroid cell proliferation and thyroid weight increases.	No data available for clofentezine, but induction of thyroid follicular cell tumours secondary to hypothyroidism is remote in humans, given the quantitative differences in thyroid function/homeostasis. Occurrence of thyroid cancer is rare even in severely hypothyroid individuals.

b) Quantitative concordance (rats *vs* humans)

There are important differences between the physiology of the thyroid between rats (rodents) and humans. Humans may be quantitatively less sensitive than rats to chemicals reducing T4 levels. The biochemical response to TSH between rats (rodents) and humans is also quantitatively different.

In the rat serum thyroxine (T4) is bound to post-albumin albumin and pre-albumin, in human T4 is bound to TBG, albumin and post-albumin. The binding affinity of TBG for T4 is approximately 1000 times greater than pre-albumin so the proportion of T4 available unbound is therefore lower in animals with high TBG (human) (Reference to Table 31). Increased hormone binding is associated with a slower metabolic degradation and the half-life in human is thus 5 to 9 days compared with 12 to 24 hours in the rat and constitutive levels of TSH are about 25-fold higher in rats compared to humans. *(Dohler, K.D. et al 1979; Hill et al., 1989 and McClain, 1992).*

Table 31: T4-binding to serum proteins in selected species. (From *Dohler, K.D. et al 1979*).

Species	T_4-binding globulin	Postalbumin	Albumin	Prealbumin
Human	++	—	++	+
Monkey	++	—	++	+
Dog	+	—	++	—
Mouse	—	++	++	—
Rat	—	+	++	+
Chicken	—	—	++	—

Codes: + or ++ = degree of T_4 binding to serum proteins; — = absence of binding of T_4 to serum protein.

The rodent thyroid is far more dynamic than that of humans. The follicular cells appear to be in active synthesis compared to humans where they are quiescent. The low binding capacity and affinity of T4 for plasma proteins in the rat results in more rapid hormone turnover and thus a higher level of thyroid/pituitary activity. Increased hormone clearance in the rat, as a consequence of increased systemic clearance, thus leads to greatly increased activity of the thyroid, pituitary, and hypothalamic axis. The consequence of these differences is that the thyroid of rodents is a much more active organ than that of humans. This means that the compensatory reaction in the rats towards a T4 deficiency is much more pronounced than in humans. This is also reflected in the histological appearance of the thyroid *(Dellarco, 2006).* Whereas in humans the thyroid follicular epithelium is composed of short cuboidal cells (indicative of their quiescent nature), the rat follicular cells are tall cuboidal and appear to be continuously active in synthesis. It can, therefore, be envisaged that alterations in the TSH levels can easily lead to an overstimulation of already active cells in rats resulting in the observed hypertrophy/hyperplasia, whereas in the human system it would merely activate a quiescent cell *(Choksi et al.2003, Junguee L., 2016)*

Thyroid tumours are a relatively common finding in rat long-term studies, whilst the only known human thyroid carcinogen is ionizing radiation. Several analyses have been conducted to investigate the human relevance of rodent thyroid follicular tumours and have concluded that the relevance is low. *(Hill et al, 1989; Hurley, 1998; Hard, 1998).*

Moreover, unlike the situation in human, the kinetics of hormones which regulate thyroid control in the rat are different between males and females. Thus basal TSH levels and the TSH response to TRH are greater in adult males than in age matched females and the difference is androgen-mediated. This finding is borne out by a study of radiation-induced follicular cell carcinomas of the rat thyroid where male rats were found to be twice as likely as females to develop this type of tumour. The circulating TSH concentrations were similarly elevated. This strongly suggests that the greater incidence of thyroid tumours in intact males must be due to a combination of the effects of TSH and circulating male sex hormone. (*Winterhoff, H and Sourgens, H, 1979; Christianson D., et al, 1981, Chen, H.J 1984, Choksi et al.2003).*

Conclusion on thyroid tumours

Clofentezine, caused an increased incidence of follicular cell tumors (combined adenomas and carcinomas) in a rat carcinogenicity study. However, this increase has low relevance to humans due to the following reasons:

1. Clofentezine has been investigated for genotoxicity, and tested negative in a battery of standard *in vitro* and *in vivo* studies so, clofentezine is considered a non-genotoxic agent. The mechanism behind tumour formation in the rat as genotoxic can be excluded.
2. There are some experimental evidence supporting a PB like MoA for the induction of thyroid tumours in male rats. One finding related with a PB-like response consists of the induction of CYP450 of the 2B/3A families and hepatic UDP-glucuronosyltransferase (UDPGT). Other findings consistent with this MoA are increased liver weight and centrilobular hepatocellular hypertrophy, increased conjugation and excretion of thyroxine (T4) and decrease in serum T4 levels, increase in thyroid stimulating hormone (TSH) levels and thyroid hypertrophy and hyperplasia. This MoA is not relevant for humans but a definitive conclusion on the similarity of the mode of action between the Phenobarbital and Clofentezine cannot be established regarding the number of uncertainties, inconsistencies and data gaps of postulated mode of action. It has to be remarked that the MoA could have been confirmed with a CAR-knock-out mouse study.

   All the same, the human relevance of the specific mechanism (s) that produces perturbation of the hypothalamic–pituitary–thyroid (HPT) axis is not considered relevant to humans based on quantitative species differences:

   - Numerous microsomal enzyme inducers have been studied in humans (e.g., phenobarbital) that do not produce alterations in TSH.
   - Thyroid hormone reserves are smaller in rats than humans, making the HPT axis in rats more sensitive to perturbations. Humans have a greater buffering capacity for thyroid hormone changes than rats.
   - Rats have a shorter thyroid hormone half-life due to the absence of thyroxine-binding globulin (T4 half-life is 5– 9 days in humans *vs.* 0.5–1 day in rats; therefore, the rat HPT axis is activated more easily.
   - The increased rate of T4 clearance results in a more ‘‘functionally active’’ thyroid in rats than humans, which is reflected in different thyroid histopathology between the two species (i.e., tall cuboidal follicular cells in rats *vs.* short cuboidal follicular cells in humans). Rat follicular cells are more likely to undergo hyperplasia in response to TSH than humans due to the already stimulated state of the rat’s follicular cells.
   - Humans have a very low incidence of thyroid tumors, whereas rats frequently develop thyroid tumors during chronic studies.

   These differences make rats markedly more sensitive than humans to thyroid perturbations.

In the ECB C&L guidance document on thyroid tumours (EC, 1999, ECBI49/99-Add1.Rev2) it is concluded that when a non-genotoxic substance produces a low/medium potency perturbation on the thyroid-pituitary axis the mechanism of action is not relevant for humans and do not need to be classified for carcinogenicity.

3. The incidence of follicular cell tumors (combined adenomas and carcinomas) at 400 ppm (17.3 mg/kg bw/day) in males [8/50 (16%)] were not pairwise significant (although, exhibited dose-trend significance) and were only slightly above control data from a concurrently study at the same conducting laboratory [6/49 (12%)].
4. Thyroid tumours appeared after an exposure period of 2 years (27 months). Older rat have more probability of tumours due to ageing.
5. Thyroid tumours are a relatively common finding in rat long-term studies, whilst the only known human thyroid carcinogen is ionizing radiation. Several analyses have been conducted to investigate the human relevance of rodent thyroid follicular tumours and have concluded that the relevance is low. *(Hill et al, 1989; Hurley, 1998; Hard, 1998).*
6. These were only neoplastic lesions in one species (rat), but not in dog and mouse.

Liver tumours mode of action

In the mice carcinogenicity study (Anonymous 45, 1985) following dietary administration for up to 2 years, a higher number of benign liver cell tumors were observed in females at 5000 ppm (557.1 mg/kg bw/day), the highest dose administrated. The incidence of benign liver cell tumors in females was not significant after pairwise comparison (p>0.05) but showed a positive trend after trend analysis (p<0.01). This was accompanied by a statistically significant after pairwise comparison (p<0.05) increase in the incidence combined of benign and malignant tumour in females (8/52 vs. 4/52 of controls) at the highest dose level that showed a positive trend after trend analysis (p<0.01). The incidence of benign hepatocellular tumours in females dosed with 5000 ppm (7/52; 13.5%) was only slightly outside the historical control range (0-7.7%) for this strain and sex of mouse in the same laboratory. In addition, the incidence of benign hepatocellular tumours in high dose females was only slightly higher than the incidence in control females in this study (4/54, 7.7%), and there was no clear dose response (incidence in controls, low, mid and high dose females was 4/52, 3/52, 3/52 and 7/52 respectively), despite the wide range of doses studied, and the magnitude of the high dose. There was an increase in the malignant hepatic tumors (1/52; 1.9%) in females with respect to controls (0/52) but it was not statistically significant and was within the range of historical controls (0-3.8%). Therefore, there was no significant treatment-related effect on the incidence of benign liver cell tumors in male mice or on the incidence of malignant liver cell tumors in either sex.

Mortality was increased in high dose females (42 *vs.* 27 of control), for which amyloidosis was regarded the cause of death. It should be noted that amyloidosis is a common age-related condition in CD-1 mice with a tendency to be a frequent cause of death in CD-1 mice and the incidence varies widely within laboratories *(Frith, C.H and Manik Chandra, 1991; Majeed S.K., 1993).* It is also pertinent to note that the carcinogenicity study in mice with clofentezine ran for 105 weeks (2 years approx.) and as amyloidosis is a disease of advanced age mice, the probability of a higher incidence of mortality is therefore bigger in studies of such prolonged exposure. At the same dose of 5000 ppm, only absolute liver weights were increased in females and body weight gains of males were reduced mainly during the first half of the study (up to 22%) but no effect was seen at the end of treatment. Therefore, although the higher incidence of hepatocellular adenomas exceeded those of the controls only at the highest dose level tested, it seems that tumours were not related to excessive toxicity. The mortality observed in CD-1 female mice at this dose was not considered due to treatment with clofentezine and rather a common age-related condition in CD-1 mice with a tendency of develop amyloidosis, which is a frequent cause of death in CD-1 mice.

The treatment related findings seen in the long-term studies reflected the same effects in the short-term studies, with the liver as a principle target of clofentezine toxicity. Effects on the liver were noted in both rats and mice, as an evident increase in liver weight, hypertrophy as well as centrilobular hepatocyte enlargement in both sexes. This was accompanied by focal cystic degeneration, fatty change and telangiectasis of hepatocytes in the rat and foci/ areas of altered hepatocytes (eosinophilic) in both sexes and benign adenomas female in the mouse. Histologically, the cytoplasm of affected hepatocytes was increased in area and electron microscopy demonstrated an association with the proliferation of smooth endoplasmic reticulum *(Anonymous 74, 1981).*

The MoA studies showed that clofentezine treatment in rats led to liver effects, including liver enzyme induction, increased liver weight and hepatocellular centrilobular hypertrophy. The most recent mode of action study in rats using dose levels of 0, 600, 3000 and 9000 ppm over a time course experiment of 29 days (Anonymous 60, 2016), looked more broadly at liver enzyme induction. It was seen effects on levels of Cytochrome P450 2B activity (as determined by the PROD[6] and BROD[7] assay), Cytochrome P450 3A activity (as determined by the BROD and 7-BQ[8] assay) and levels of UDPGT at the top two dose levels of 3000 and 9000 ppm – dose levels spanning the 5000 ppm dose level used on the carcinogenicity study at which a slight increase in benign liver tumours in mice was seen. It was seen effects on levels of Cytochrome P450 2B activity (as determined by the PROD and BROD assay), Cytochrome P450 3A activity (as determined by the BROD and 7-BQ assay): at 9000 ppm there were increase in activity of PROD (18 fold), BROD (32 fold), 7BQ (2.4 fold) and UDPGT (3.3 fold) and at 3000 ppm there were increase in activity of PROD (15 fold), BROD (18 fold), 7BQ (3 fold) and UDPGT (3.7 fold). The macroscopic finding of enlarged liver from 3000 ppm was also reflected in markedly higher liver weights, the latter was also seen at 600 ppm. These increases were associated with centrilobular hepatocellular hypertrophy at all dose levels and at all time of sacrifice (day 8, 15 and 29) with increase in severity in a dose-dependent manner. Hypertrophied hepatocytes were characterized by pale eosinophilic or granular cytoplasmic appearance, which is typically found in liver of rats treated with phenobarbital-type agents.

The results of both apical and mechanistic studies with clofentezine provide some evidence which supports the view that the slight increase in incidence of liver tumours in female mice might be mediated via a well characterized, non-genotoxic, rodent-specific phenobarbital (liver CYP2B inducer/CAR activator)-like MoA.

It is postulated by the applicant that clofentezine induces liver tumours by a non-genotoxic mechanism which involves the induction of certain cytochrome P450 iso-forms as CYP 2B and 3A, known to be under the regulation of the constitutive androstane receptor (CAR), induction of mitogenic hepatocyte proliferation, increase in the severity of hepatocellular hypertrophy leading to pronounced hepatomegaly and enlargement and dose-related increase in liver weight. After prolonged *in vivo* treatment eosinophilic altered hepatic foci were observed and finally hepatocellular tumours were formed.

The CAR activation MoA mimics that of phenobarbital (PB), a mode of action which is accepted to be of no relevance for humans and to be rodent-specific *(Elcombe et al, 2014; Holsapple, 2006).*

Peffer et al, 2018, cited the published proceedings of a nuclear receptor workshop on the CAR/PXR MoA *(Elcombe et al., 2014),* in which the authors could not identify a suitable non-genotoxic PXR activator for which carcinogenicity data were available and hence a MoA was not developed for liver

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[6] PROD (CYP2B): Pentoxyresorufin-O-depentylase;

[7] BROD (CYP2B/CYP3A): Benzyloxyresorufin-O-debenzylase;

[8] 7BQ (CYP3A): 7-Benzyloxyquinoline-O-debenzylase

tumor formation by PXR activators. CAR and PXR are often cited together regarding potential MoAs for a specific chemical agent, because extensive cross-talk between these two nuclear receptors has been described *(Stanley et al., 2006)*, and some agents can activate both CAR and PXR in a particular species *(Elcombe et al., 2014).* In fact, PXR is activated by a large array of diverse chemical substances, far more than those that activate CAR *(Martin et al., 2010; Timsit and Negishi, 2007; Willson and Kliewer, 2002).* Those chemicals that are pure PXR activators have been shown to increase liver weight after activation but do not increase cell proliferation in the same way that activators of CAR or PPARα do (*Shizu et al., 2013; Thatcher and Caldwell, 1994).* Recently, co-administration of a PXR activator along with known activators of other nuclear receptors has shown that while PXR does not produce an increase in cell proliferation on its own, it may enhance the proliferative signals of CAR or PPARαactivators *(Shizu et al., 2013).* Given the lack of actual tumorigenic key events due to PXR activators alone, the rest of this paper will focus on the CAR MoA by itself.

Some of the effects of CAR activators observed in rodent liver can also be demonstrated in human liver. For example, PB and other CAR activators can induce CYP enzymes in both rodent liver and in human liver *(Elcombe et al., 2014).* Treatment with PB has been shown to increase liver size in humans, which is due to hepatocyte hypertrophy and proliferation of the smooth endoplasmic reticulum *(Aiges et al. 1980; Piettiaho et al., 1978, 1982).* However, in terms of the human relevance, the key species difference is that while CAR activators are mitogenic agents in rodent hepatocytes, they do not appear to stimulate replicative DNA synthesis in human hepatocytes.

For phenobarbital it has been shown *in vitro* that there is a difference in ability between rodent and human hepatocytes in producing cell proliferation through CAR activation. Studies with cultured human hepatocytes have demonstrated that phenobarbital was able to induce CYP2b forms in both rat and human hepatocytes, but cell proliferation only in rat hepatocytes *(Hirose et al., 2009; Parzefall et al, 1991).* Apparently a similar result has been observed for mouse versus human hepatocytes, given the results reported for phenobarbital *(Elcombe et al, 2014).*

Next to indications that human hepatocytes are refractory to the hyperplastic effects of PB, there is strong epidemiological data (as a sedative, hypnotic and antiepileptic drugs for many years at doses comparable to those in rodent bioassays) supporting non-carcinogenicity of PB in humans *(Monro, 1993, La Vecchia & Negri, 2014).*

The key role of increased cell proliferation in a CAR activator MOA for mice liver tumour formation has been demonstrated in studies performed in mice lacking CAR. In such CAR knockout mice, PB does not stimulate replicative DNA synthesis in hepatocytes and does not promote liver tumours *(Huang et al., 2005; Wei et al., 2000; Yamamoto et al., 2004).* Besides, *in vivo* studies with humanized CAR (hCAR) mice exposed to phenobarbital confirmed the absence of cell proliferation.

It is broadly recognized that the PB-like MOA for induction of rodent liver tumor is qualitatively not plausible for humans due to differences in rodent and human responses to CAR activation, in particular as to hepatocellular proliferation, a critical event in the development of liver tumours. Thus, compounds that cause rat or mouse liver tumors through this CAR-mediated MOA, similar to PB, would not be expected to increase the risk of liver tumor development in humans.

Assessment and evidences of the postulated MoA for liver tumours induced by clofentezine using the framework developed by IPCS and ILSI/HESI or the OECD guidance for Adverse Outcome Pathway development. Modified Bradford Hill Considerations.

In the guidance provided by ECHA, they recommend that the IPCS framework (IPCS, 2007) be followed when evaluating MoA data for carcinogenicity findings in animals and their relevance to humans.

This postulated mode of action and the human relevance for the clofentezine-induced liver tumours is assessed by applying the MoA/Human Relevance Framework (HRF) developed by the International Programme on Chemical Safety (IPCS) of the World Health Organization (WHO) *(Sonich-Mullin et al., 2001; Boobis et al., 2006)* and the International Life Science Institute (ILSI/HESI) *(Meek, M.E. et al, 2003; Meek, M.E. et al, 2014, Holsapple et al., 2006).* This framework considers systematically data on apical and mode of action for effects regarding this mechanism of hepatic tumour formation and their relevance to humans and use a weight-of–evidence approach based on the Bradford Hill criteria.

THE IPCS CONCEPTUAL MOA FRAMEWORK FOR EVALUATING ANIMAL CARCINOGENESIS:

1. Postulated MoA (theory of the case)
2. Key events
3. Concordance of dose-response and Temporal Association
4. Strength, consistency and specificity of association of tumour response with key events.
5. Biological plausibility and coherence
6. Other modes of action
7. Uncertainties, Inconsitencies, and Data Gaps assessment of postulated mode of action.
8. Assessment of postulated mode of action

This approach is also consistent with the Adverse Outcome Pathway (AOP) process developed by the OECD, for which a CAR Liver Tumor AOP has been described *(Peffer et al., 2017)* (http://aopwiki.org/aops/107). The OECD encourages scientists to capture these AOPs in an online tool known as AOP wiki as part of the AOP process *(Kleinstreuer et al., 2016; OECD, 2013; OECD, 2016).* It should be noted that in the MoA proposed in the current document, the initial step KE1 (CAR activation) can also be described by the equivalent term "Molecular Initiating Event" (MIE) in the recommended nomenclature of an AOP (*OECD, 2016; Peffer et al., 2017*). Similarly, the final step of KE5 (increase in hepatocellular adenomas/ carcinomas) can also be described as an "Adverse Outcome" (AO) in the AOP wiki nomenclature.

A MoA consists of a series of key event (KEs), which are integral to tumor formation, providing the dose is sufficiently high and the duration of exposure is sufficiently long. A MoA can also include associative events (AEs), which are not required for tumor development, but can be used as markers for certain required KEs. In addition, modulating factors (ModFs) may be identified that are not necessary for tumor development, but can modulate the severity or dose response kinetics of KEs leading to tumor development.

1. Postulated MoA for the induction of hepatocellular tumours in mice

The proposed mode of action for clofentezine liver tumours consists on the activation of the Constitutive Androstane Receptor (CAR) in the liver. CAR activation conduces to increased expression of pro-proliferative and anti-apoptotic genes in the liver and an early, transient, increase in hepatocellular proliferation. Over time, the increased hepatocellular foci as a result of clonal expansion of spontaneously mutated cells in the mouse results in slight increases in liver tumour incidence compared to concurrent controls.

2. Listing of key events identified in experimental animals

A recent review captured the state of the science for the CAR MoA *(Elcombe et al., 2014);* it provided a review of the evidence that mouse or rat liver tumors that occur via a CAR MoA are not relevant to humans based on qualitative differences between the species. This review paper is used as a basis for defining the key events and associative events that are part of this MoA. The CAR activation is the molecular initiating event for the cellular pathway ultimately leading to the apical adverse outcome of liver tumours in PB treated rodents. In this evaluation to analysis the human

relevance of PB-induced rodent liver tumour MoA (non-genotoxic) mediated through CAR activation the following key events were identificated: KE1: CAR activation, KE2: altered gene expression specific to CAR activation, KE3: increased cell proliferation, KE4: clonal expansion leading to altered foci and KE5: liver adenomas/carcinomas. It has to be mentioned that, in a more recent publication *(Peffer et al., 2018),* the author notes that altered foci at tumorigenic doses are not observed with all CAR activators, so this author considers that demonstration of this key event is not critical. In addition to these key events in the pathogenesis of hepatocellular tumors in rodents, reversibility of hepatic effects upon discontinuance of treatment is considered as a necessary data to support this MoA.

Associative events for this MoA, although do not constitute direct evidence of causality of CAR-mediated MoA, they provide associative support of a CAR-mediated MoA and are commonly seen following exposure to PB-like xenobiotic compounds. Altered gene expression leads to several associative events, out of which the following ones have been considered as the most feasible to demonstrate as part of a regulatory dataset *(Peffer et al., 2018):* AE1: Increased Cyp2b, Cyp3a enzyme activity and/or protein, AE2: Hepatocellular hypertrophy in the centrilobular region of the liver, AE3: Increased liver weight. Additional associative events are: decreased apoptosis and altered epigenetic changes specific to CAR activation and with inhibition of gap junctional intercellular communication and oxidative stress being modulating factors *(Elcombe et al., 2014).*

PB treatment first led to early observable key and associative events (e.g CAR activation, altered gene expression, cell proliferation, enzymatic activation, apoptosis suppression, hypertrophy, and liver weight increase). While effects on some key and associative events including CAR activation, altered gene expression, CYP induction and hypertrophy are observed from early time points throughout the period of PB treatment, the stimulation of cell proliferation in normal hepatocytes is only observed at early time points. For most CAR activators, the stimulation of cell proliferation, assessed as the labelling index (i.e. the percentage of hepatocyte nuclei undergoing replicative DNA synthesis), in rat and mouse liver is transient and not sustained, primarily observed in the first 1-3 weeks after treatment begins, and then returns to a similar rate as in control animals *(Kolaja et al., 1996a; Orton et al., 1996; Philips et al., 1997; Whysner et al., 1996).* However, while hepatocyte labelling index returns to control levels with sustained treatment, overall cell proliferation is still enhanced due to the increase in the total number of hepatocytes per animal. Increased cell proliferation is also important in the growth of altered hepatic foci. At longer treatment times, rates of cell proliferation are enhanced in altered hepatic foci, which typically develop relatively late in long-term studies. Short-term mechanistic studies in mice with PB or CAR-associated compounds typically do not develop hepatocellular foci for months *(Goldsworthy and Fransson-Steen 2002).* In promotion studies where altered hepatic foci were produced by initiation with diethylnitrosamine (DEN), PB was found to increase replicative DNA synthesis within the foci *(Kolaja et al.; 1996b,c; Elcombe et al., 2014).* Clonal expansion leading to altered foci and liver adenomas/carcinomas are only observed after chronic treatment with PB.

In the Table 32 are showed the key and associative events of the CAR activation MOA *(Elcombe et al., 2014; Peffer et al., 2018)*

Table 32: Key events and associative events in the MoA

Key events	Associative events
Key event 1: CAR nuclear receptor activation	
Key event 2: Altered gene expression specific to CAR activation	Enzyme induction (CYP2B) Hepatocellular hypertrophy Liver weight increase Inhibition of apoptosis Epigenetic changes
Key event 3: Increased cell proliferation	
Key event 4: Clonal expansion leading to foci/areas of altered hepatocytes (eosinophilic)	
Key event 5: Liver adenomas/carcinomas	
Modulating factor	
Gap junctional intercellular communication	
Oxidative stress	

In evaluating the clofentezine data set, the profile of effects was examined for the strength of association, consistency and specificity to determine whether key events occurred consistently across clofentezine studies, whether these key events were linked in a biologically plausible manner, and whether these key events exhibited the expected concordance across dose-response and temporal relationship. Thus, repeat dose guideline studies, which include subcronic, chronic toxicity/oncogenicity studies and studies to the reproduction as well as specific MoA studies, were examined for evidence to support the CAR-mediated MoA for clofentezine.

The table below shows the experimental evidences for the key and associative events of a CAR-mediated induction of liver tumours in rats, mice and dogs studies with Clofentezine. Regarding to evidences in humans there are not data available in this analysis, studies in cultured human hepatocytes have not been included.

Table 33: Evidences for the key and associative events of a CAR-mediated induction of liver tumours in mice, rats and dogs

Key events	Rats	Mice	Dogs
CAR activation	Not determined	Not determined	Not determined
Altered gene expression specific to CAR activation	Not determined	Not determined	Not determined
Increased cell proliferation	*In vitro*: Not determined in cultured hepatocytes *In vivo*: Not observed/ reported	*In vitro*: Not determined in cultured hepatocytes *In vivo*: Not observed/ reported	*In vitro*: Not determined in cultured hepatocytes *In vivo*: Not observed/ reported
Clonal expansion leading to altered foci	Not observed/ reported	YES There are increases in foci/areas of altered hepatocytes (eosinophilic) in male and female mice, in the long-term oral study (Anonymous 45, 1985).	Not observed/reported

Key events	Rats	Mice	Dogs
Liver adenomas/carcinomas	Not observed/ reported	YES- The incidence of mouse benign liver tumours (13.5%) is slightly higher than the historical control range for females (0-7.7%), (Anonymous 45, 1985).	Not observed/ reported
Associative events	**Rats**	**Mice**	**Dogs**
Enzyme induction (CYP2B)	YES There are increases in hepatic enzymes levels, especially CYP2B, CYP3A and UDPGT in rats. (Anonymous 60 2016), (Anonymous 59 1989) (Anonymous 55, 1990)	Not determined	Not determined
Hepatocellular hypertrophy	YES (Anonymous 63, 1982b) (Anonymous 74, 1981) (Anonymous 42, 1985a) (Anonymous 60, 2016).	YES (Anonymous 70, 1982) (Anonymous 77, 1982)	Not observed/ reported
Increased liver weight	YES (Anonymous 68, 1980) (Anonymous 63, 1982b), (Anonymous 74, 1981) (Anonymous 42, 1985a) (Anonymous 51, 1986) (Anonymous 55, 1990) (Anonymous 58, 1988) (Anonymous 59, 1989) (Anonymous 60, 2016) (Anonymous 65, 1982) (Anonymous 61, 1984)	YES (Anonymous 70, 1982) (Anonymous 77, 1982) (Anonymous 45, 1985)	YES (Anonymous 71, 1981) (Anonymous 73, 1983) (Anonymous 78, 1981) (Anonymous 79, 1984)
Inhibition of apoptosis	Not determined	Not determined	Not determined
Epigenetic changes	Not determined	Not determined	Not determined

Mice apical studies show key events of this MoA as liver tumours in females, increases in foci/areas of altered hepatocytes (eosinophilic) in both sexes and also associative events as increased liver weight and centrilobular hepatocellular hypertrophy. Increased liver weight and centrilobular hepatocellular hypertrophy were also seen in apical and mechanistic studies in rat. However, in apical studies with clofentezine hyperplasia was not reported in any specie and was not determined in mechanistic studies with cultured hepatocytes.

Regarding, the induction of hepatic enzymes typical of PB-like MoA, this associative event was only experimentally evaluated in male rat mechanistic studies with clofentezine, where activation of CYP 2B, CYP 3A and UDPGT were observed. At this regards, it is well known that for compounds acting

through CAR activation, a similar MoA operates for both mice and rats and therefore, the activation of hepatic enzymes typical of PB-like MoA could be plausible in mice.

However, there are not experimental evidences for all key events in mice because of lack of studies. The role of CAR in mice hepatocellular tumours has not been confirmed experimentally. No studies in altered gene expression specific of CAR activation were carried out. Besides, hyperplasia was not reported in apical studies and no additional study was carried out to determining whether clofentezine induces cell proliferation in the liver of CD-1 mice. Therefore, there is no clear evidence that the tumorigenic action of clofentezine in the liver of female CD1 mice is the result of a phenobarbital-like MoA acting through liver enzyme induction via CAR receptor activation.

3. Concordance of dose-response and temporal association

The dose response and temporal relationships for the Key and associative Events measured in the studies in rats, mice are presented below (Table 34 and Table 35) The responses for the Key and associative events are shown as positive or negative, quantification (degree of change) is not shown in order to keep the tables clearer. Key Event 4 (foci) and key event 5 (Formation of liver tumours) are generally not applicable to subchronic studies and therefore is labelled as “not applicable” in the tables, although the histopathological outcome was measured.

Table 34: Concordance of dose-response and temporal relationships in studies in mice

Reference	**Dose (mg/kg bw/day)** ♂/♀	**Associative event**[1] Enzyme induction	**Associative event**[1] Hepatocellular hypertrophy	**Associative event**[1] Increased liver weight	**Key event 4:** Clonal expansion leading to altered foci	**Key event 5:** Liver adenomas/ carcinomas
	Ordered from low to high dosage	**Key and associative events are shown in order from earliest event to later (left to right). Results show the time that the event was observed. Quantitative changes in severity are not shown.**				
MICE						
(Anonymous 45, 1985)	**5/5.3**	ND	- 105 weeks	- 105 weeks	+ 105 weeks (♂)	- 105 weeks
(Anonymous 45, 1985)	**50.7/56.9**	ND	- 105 weeks	- 105 weeks	+ 105 weeks (♂/♀)	- 105 weeks
(Anonymous 77, 1982)	**151.4/176.5**	ND	- 13 weeks	+ 13 weeks (Rel in ♂/♀)	NA	NA
(Anonymous 45, 1985)	**543.4/557.1**	ND	- 105 weeks	+ 105 weeks (abs in ♀)	+ 105 weeks (♂/♀)	+ 105 weeks (♀)
(Anonymous 77, 1982)	**757.1/884.9**	ND	+ 13 weeks [a] (♂)	+ 13 weeks (Abs in ♂ Rel in ♂/♀)	NA	NA
Anonymous 70, 1982	**766/912**	ND	+ 6 weeks [b] (♂)	+ 6 weeks (abs and rel in ♂)	NA	NA
Anonymous 70, 1982	**5149/5395**	ND	+ 6 weeks [b] (♂)	+ 6 weeks (abs and rel in ♂)	NA	NA

- : negative response, + : positive response, ND: Not determined; NA: Not applicable [a]:Observed as centrilobular hepatocyte enlargement; [b]: observed as centrilobular hepatocytomegaly.
[1]Associative events are referred to key event 2 (Altered gene expression specific to CAR activation)

Table 35: Concordance of dose-response and temporal relationships in studies in rats

Reference	Dose (mg/kg bw/day) ♂/♀	Associative event[1] Enzyme induction	Associative event[1] Hepatocellular hypertrophy	Associative event[1] Increased liver weight	Key event 4: Clonal expansion leading to altered foci	Key event 5: Liver adenomas/ carcinomas
	Ordered from low to high dosage	Key and associative events are shown in order from earliest event to later (left to right). Results show the time that the event was observed. Quantitative changes in severity are not shown.				
RAT						
Anonymous 42, 1985a	**0.43/0.55**	ND	- 108 weeks	+ 108 weeks (abs in ♂)	- 108 weeks	- 108 weeks
Anonymous 42, 1985a	**1.72/2.18**	ND	- 108 weeks	+ 108 weeks (abs in ♂)	- 108 weeks	- 108 weeks
Anonymous 61, 1984	**3.55 (♂) F_2 (Parental toxicity)**	ND	- 82-84 days	+ 82-84 days (Rel in ♂)	NA	NA
Anonymous 42, 1985a	**17.3/22.1**	ND	+ 48 weeks [a] (♂) + 108 weeks [a] (♂)	+ 48 weeks (rel in ♂/♀) + 108 weeks (abs and rel in ♂/♀)	- 108 weeks	- 108 weeks
Anonymous 68, 1980	**20/20**	ND	- 17 days	+ 17 days (abs and rel ♀)	ND	ND
Anonymous 59, 1989	**22.69 (♂)**	+(UDPGT) 5, 8 days 2, 4 weeks	ND	+ 2, 4 weeks (Abs and rel)	ND	ND
Anonymous 74, 1981	**26.2/29.3**	ND	+ 13 weeks [a, b] (♂), Reversible after recovery (week 19)	+ 13 weeks (Abs and rel in ♂/♀) Reversible after recovery (week 19)	NA	NA
Anonymous 55, 1990	**28.9 (♂)**	+(UDPGT) 4, 8, 13 weeks	ND	+ 4 weeks (rel) + 13 weeks (abs and rel)	ND	ND
Anonymous 61, 1984	**36.1 (♂) F_2 (Parental toxicity)**	ND	- 82-84 days	+ 82-84 days (Rel in ♂)	NA	NA
Anonymous 61, 1984	**36.1 (♂) F_1 (Parental toxicity)**	ND	+ 88 days [a] (♂)	+ 88 days (Rel in ♂)	NA	NA
Anonymous 60, 2016	**36.4 (♂)**	- (CYP2B/ 3A) 4 weeks	+ 8 days, 2, 4 weeks	+ 4 weeks (Abs)	NA	NA
Anonymous 51 1986	**40/40**	ND	ND	+ 6 weeks (Abs and rel in ♂)	ND	ND
Anonymous 68, 1980	**80/80**	ND	- 17 days	+ 17 days (Abs in ♀ Rel in ♂/♀)	ND	ND
Anonymous 59, 1989	**169.4 (♂)**	ND	ND	+ 5,8 days 2, 4 weeks (Abs and rel)	ND	ND
Anonymous 63, 1982b	**202/221**	ND	+ 13 weeks [a] (♂/♀) Reversible after recovery (week 17)	+ 13 weeks (Abs and rel in ♂/♀) Reversible after recovery (week 17)	NA	NA
Anonymous 60, 2016	**205.7 (♂)**	+(UDPGT) + (CYP2B/ 3A) 8 days, 2 weeks	+ 8 days, 2, 4 weeks	+ 8 days, 2, 4 weeks (Abs)	NA	NA
Anonymous 74, 1981	**265/292**	ND	+ 13 weeks [a, b] (♂/♀) Reversible after recovery (week 19)	+ 13 weeks (Abs and rel in ♂/♀) Reversible after recovery (week 19) ♀	NA	NA

Reference	Dose (mg/kg bw/day) ♂/♀	Associative event[1] Enzyme induction	Associative event[1] Hepatocellular hypertrophy	Associative event[1] Increased liver weight	Key event 4: Clonal expansion leading to altered foci	Key event 5: Liver adenomas/ carcinomas
	Ordered from low to high dosage	**Key and associative events are shown in order from earliest event to later (left to right). Results show the time that the event was observed. Quantitative changes in severity are not shown.**				
Anonymous 68, 1980	**320/320**	ND	- 17 days	+ 17 days (Abs in ♀ Rel in ♂/♀)	ND	ND
Anonymous 60, 2016	**509.4 (♂)**	+(UDPGT) + (CYP2B/ 3A) 8 days, 2 weeks	+ 8 days, 2, 4 weeks	+ 8 days, 2, 4 weeks (Abs)	NA	NA
Anonymous 63, 1982b	**602/662**	ND	+ 13 weeks [a] (♂/♀) Reversible after recovery (week 17)	+ 13 weeks (Abs and rel in ♂/♀) Reversible after recovery (week 17) in♂	NA	NA
Anonymous 68, 1980	**1280/1280**	ND	- 17 days	+ 17 days (Abs and rel in ♂/♀)	NA	NA
Anonymous 65, 1982	**1280 (♀) (Maternal toxicity)**	ND	- Gestation days 7-20 pc	+ Gestation days 7-20 pc (Rel ♀)	NA	NA
Anonymous 59, 1989	**1635 (♂)**	+(UDPGT) 5, 8 days 2, 4 weeks	ND	+ 5,8 days 2, 4 weeks (Abs and rel)	ND	ND
Anonymous 63, 1982b	**1892/1992**	ND	+ 13 weeks [a] (♂/♀) Reversible after recovery (week 17)	+ 13 weeks (Abs and rel in ♂/♀) Reversible after recovery (week 17) in♂	NA	NA
Anonymous 58, 1988	**1915 (♂)**	ND	ND	+ 3,5,8 days, 2 weeks (Abs and rel)	ND	ND
Anonymous 55, 1990	**2250 (♂)**	+(UDPGT) 4, 8, 13 weeks	ND	+ 4 weeks (abs and rel) + 13 weeks (abs and rel)	ND	ND
Anonymous 51, 1986	**3000/3000**	ND	ND	+ 6 weeks (Abs and rel in ♂/♀)	ND	ND
Anonymous 65, 1982	**3200 (♀) (Maternal toxicity)**	ND	+ Gestation days 7-20 pc [a]	+ Gestation days 7-20 pc (Rel ♀)	NA	NA

- : negative response, + : positive response, ND: Not determined; [a]:Observed as centrilobular hepatocyte enlargement
[b]: histologically, the cytoplasm of affected hepatocites was increased in area and electron microscopy demonstrated an association with the proliferation of smooth endoplasmic reticulum
[1]Associative events are referred to key event 2 (Altered gene expression specific to CAR activation)

In mice, data are available for the effect of treatment of male and female with clofentezine at various time points ranging from 6 weeks to 105 weeks (Table 34). In rats, data are available for the effect of treatment of male and female with clofentezine at various time points ranging from 17 days to 108 weeks (Table 35).

The temporality of the different events of the MoA proceeds in the expected order. If a key event (or events) is essential element for carcinogenesis, it must precede the appearance of tumours. Clofentezine treatment first led to early events as enzymatic activation, hypertrophy and liver weight increase. The final adverse outcome effect (Key Event 5) of formation of hepatocellular tumours and the key event 4 of formation of eosinophilic foci only occurs in mice. At same doses these two effects are always late events, only observed at 104 weeks (no evidence at 52 weeks). Although eosinophilic

foci and hepatocellular tumours do not have sufficient time points to distinguish temporally between both, the incidence of foci occurred at lower doses at which tumours have not been developed yet. A sex difference for final adverse outcome was evident (female mice having a higher tumour incidence than males).

Effects of clofentezine on a number of the key and associative events showed similar dose-dependency with the incidence of tumours only observed at the highest dose in female mice. The incidence of severity (quantitative response) was in most cases also consistent.

Overall, the key and associative events observed in mice and rats receiving clofentezine occurred in a logical temporal sequence and in a dose-dependent manner. However, as said before, there is no evidence for all key events of this MoA.

4. Strength, consistency and specificity

The weight of evidence linking the key and associative events with the toxicological response is consistent with the hepatic effects observed in many apical and mechanistic studies in rats, mice and dogs. The succession of key and associative events, including liver enzyme induction (particularly CYP2B family but also CYP3A and UDPGT), proliferation of smooth endoplasmic reticulum, increased liver weight with associated histopathological hepatocellular changes (hypertrophy, centrilobular enlargement or centrilobular hepatocytomegaly and vacuolization), foci/ areas of altered hepatocytes (eosinophilic) and liver benign tumours in mice is consistent with a PB-like mechanism.

Reversibility is also consistent with the proposed CAR MoA where the non-neoplastic cellular changes may be reset by the normal feedback-control systems and reversed. Liver effects observed might be considered as an adaptive effect if they are caused by induction of enzyme activities; if it is not associated to any other liver toxicity; and if it is a transient phenomenon, which is fully reversible. In this sense, there are studies with clofentezine that show effects with reversibility. The 90-day study in rats *(Anonymous 63, 1982b)* showed reversibility in the increases of liver weight, liver macroscopic pathology and histopathology changes (centrilobular hepatocyte enlargement). Besides, in the other 90-day study in rats *(Anonymous 74, 1981)* there was also reversibility in the increase of liver weight and the centrilobular hepatocellular enlargement.

The incidence of hepatocellular tumours in mice is lower than the incidence of earlier effects. Hepatic tumours in mice are preceded by foci/ areas of altered hepatocytes (eosinophilic) that occurs at lower doses and at a higher incidence than seen for tumours. In the chronic toxicity/ oncogenicity study *(Anonymous 45, 1985)* at 5000 ppm in female mice (557 mg/kg bw/day), the incidence of hepatocellular adenomas was 13.5% (7/52 animals) while the incidence in foci of animal dying or killed during study and at termination was 17% (9/52 animals). These observations also fit with the MoA, where, at similar doses, the incidence/severity of later key events would not be expected to be greater than that of earlier key events.

The same pattern of response among events has been seen in different apical and mechanistic studies and species. The key and associative events occurred in a logical temporal sequence and in a dose-dependent manner and are reversible when exposure is discontinued. At similar doses, the incidence of later key events is not greater than that of earlier key events. All these factors provide support for the proposed MoA. However, there is no evidence for all key events of this MoA and the strength, consistency and specificity of association of the hepatic tumor response with key events suggested that the MoA is only partially convincing.

5. Biological plausibility and coherence

The CAR activation could be a plausible MOA for liver tumour formation in rodents caused by clofentezine. The succession of key and associative events are consistent with the PB-like mechanism. This mechanism is biologically plausible and consistent with our current understanding of liver tumor formation by non-genotoxic mitogenic agents that can activate nuclear receptors. The reversibility of early key events upon cessation of treatment with clofentezine is also consistent with the proposed MoA, where the non-neoplastic cellular changes may be reset by the normal feedback-control systems and reversed. However, there are a number of uncertainties, data gaps and other potential alternative mode of action for the liver tumours that has to be taken into account in the weigh-of –evidence assessment.

6. Other modes of action

To define a MoA in liver, it is critical to ensure that other MoAs do not contribute significantly to hepatocarcinogenesis. In addition to CAR activation, other mechanisms may be involved in clofentezine-induced tumourgenesis in mice liver.

Several MoAs have been identified for liver carcinogenesis and those applicable to the rodent model are listed in publications by *Cohen (2010)* and *Klaunig et al. (2012).* These include DNA reactive and non- DNA reactive mechanisms (genotoxic and non genotoxics MoAs). Several nongenotoxic mechanisms for hepatocarcinogenesis in rodents include sustained cytotoxicity, oxidative stress/damage, inflammation, infection, iron (copper) overload, increased apoptosis, immunosuppression, porphyria or receptor-mediated. MoAs for hepatocellular carcinogens that causes receptor mediated hepatocellular proliferation include: aryl hydrocarbon receptor (AhR), constitutive androstane receptor (CAR), pregnane X receptor (PXR), or peroxisome proliferator-activated receptor alpha (PPAR- α) activation, as well as estrogens and statins (*Boobis et al., 2009; Holsapple et al., 2006; Klaunig et al., 2003; Meek et al., 2003; Williams, 1997a*). Alternative MoAs for clofentezine-induced hepatocellular carcinogenesis are discussed below:

- DNA reactivity and mutagenicity: it is important to ensure that DNA reactivity is not the source of the tumour findings. In this sense, there is no data, such as DNA adducts analysis in liver cells, to assess whether hepatocellular tumours seen are attributable to specific mutagenic events. However, DNA reactivity can be excluded since the genotoxicity testing *in vivo* and *in vitro* of clofentezine gave no evidence of a genotoxic potential and there is no evidence for clofentezine accumulation in the liver.

- Citotoxicity and regenerative hyperplasia: Some concern is raised by the fact that clofentezine was shown to be toxic to isolated mice and rat liver cells whereas phenobarbital was not. In a 42-day study in mice *(Anonymous 70, 1982)* localized areas of hepatic necrosis were observed in male at 5149 mg/kg bw/day. Besides, slight incidence of degenerative lesions (vacuolization, focal cyst degeneration of hepatocytes and focal hepatocyte necrosis) were reported in males of the 27 month cronic toxicity rat study *(Anonymous 42, 1985a)* at 17 mg/kg bw/day. Whereas tumours observed in the chronic/carcinogenicity study in mice were observed in female at dose levels of 557 mg/kg bw/day.

A small increase in the incidence of mild to moderate single-cell necrosis can sometimes occur, particularly after longer-term treatment of mice with CAR activators. However, more severe/diffuse necrosis in the liver suggests that an alternative MoA via cytotoxicity might be operative (Hall et al., 2012). The limited amount of hepatic necrosis (single cell or focal) observed in the *in vivo* mouse and rat treated with clofentezine studies is in contrast with the pattern of effects seen with classic cytotoxic carcinogens that cause a diffuse necrosis (widespread multifocal hepatocyte death) in the

liver that progressed to a sustained regenerative hyperplasia, as is the case of chloroform and carbon tetrachloride.

Besides, there were not increases in other necrosis indicators (e.g., alanine aminotransferase [ALT], aspartate aminotransferase [AST], alkaline phosphatase [ALP]) and, in contrast with classic cytotoxic carcinogens, there was reversibility in early key events (hepatocellular hypertrophy and increased liver weight). In addition, the localized areas of hepatic necrosis were seen in male in both species, whereas hepatic tumours were seen only in female mice. Additionally, despite the similar toxic effects that were observed in rats, no tumours occurred in this specie. Therefore, it is not considered that cytotoxicity was an additional MOA involved in the hepatocellular tumour formation.

- There was no structural similarity with estrogens, no changes in clinical chemistry parameters & no hepatic necrosis or other histological changes suggestive of other receptor (Estrogen, statins) and non-receptor-mediated (apoptosis, infections or metal accumulation) involvement. Evaluation of data indicate that these alternative possible MoAs are not likely to be relevant.

- Peroxisome proliferator-activated receptor alpha (PPARα) activation and Aryl Hydrocarbon Receptor (AhR) activator MoAs: these MoAs have not been experimentally ruled out since there are no measures in lauric acid 12α-hydroxylase activity and Cyp4a protein levels to determinate PPARα activation, neither no measures in EROD activity and Cyp1a protein levels to determine AhR activation in mice liver microsomes.

- Immunosuppresion: No changes in the immune system or immune cells were detected in clofentezine studies.

Overall, several possible MoAs for the hepatocellular carcinogenesis observed in mice can be dismissed, because the results of the available studies indicated lack of plausibility and/or coherence. However, there are other MoA, for which experimental data to rule them out is not available (like e.g. PPARα, AhR).

7. Uncertainties, inconsistencies and data gaps

The following data gaps has been founded:

- Data for concordance analysis with PB are limited, in spite of the fact that phenobarbital-like liver effects were observed *in vivo* after clofentezine treatment (Cyp2B-induction, proliferation of smooth endoplasmic reticulum, hypertrophy, increased liver weight, vacuolisation, eosinophilic foci and liver tumours). There are no studies in mice liver *in vivo* and hepatocytes *in vitro* comparing the pattern of responses to clofentezine and phenobarbital. Microarray transcriptional studies to identify activated genes and pathways underlying the proliferative processes and to confirm similarities among phenobarbital and clofentezine are not available.

- A gene expression biomarker signature assessment comparing effects in wild-type and CAR-null mice was not performed in order to find if clofentezine produce liver effects (increases liver weights, hypertrophy) in a CAR-dependent manner.

- An S-Phase Response Study (using BrdU Stained cells) to determining whether Clofentezine induces cell proliferation in the liver of CD-1 mice was not carried out. Hepatic cell proliferation was neither investigated in humanized and knockout PXR/CAR mice. Besides, the evidence for cell proliferation has not been analyzed in an *in vitro* comparative cell proliferation study in cultured CD1 mouse, rat and human hepatocytes. Therefore, the CAR dependency of this effect has not been established.

- There are no studies to assess effects of clofentezine on drug metabolizing enzymes in the livers of CD1 mice. The profile of liver enzyme induction was assessed only in dietary male rat studies and refers only to Cytochrome P450 2B activity (as determined by the PROD and BROD assay) and

Cytochrome P450 3A activity (as determined by the BROD and 7-BQ assay) and levels of UDPGT. Other activities of cytochrome P-450 enzymes, epoxide hydrolase, and glutathione S-transferase were not assessed. No studies in cultured human, rat or mouse hepatocytes were done to determinate the induction of Cytochrome activities, neither induction of mRNA levels for Cyp2b and Cyp3a activities in any specie. Besides, no study using CAR-knockout mice hepatocytes was done to assess if CAR-activation is required for clofentezine-induced CYP2B isoforms following Clofentezine exposure. Consequently, the role of CAR in hepatocellular tumours in female CD1 mice has not been confirmed.

- It has not been investigated other associative events as inhibition of apoptosis in the liver of mice or altered epigenetic changes and there is no data on effects of clofentezine on modulating factors as gap junctional intercellular communication and oxidative stress.

8. Assessment of postulated mode of action

When proposing a CAR MoA for liver tumours induced by a test compound, there are critical parameters to be included in the final mechanistic data package, which should (at a minimum) include demonstration of the molecular initiating event (CAR activation, KE1) and the obligatory key event of increased cell proliferation (KE3) *(Peffer et al., 2018).* However, in the analysis of postulated MoA for hepatocellular tumour caused by clofentezine, there are not experimental evidences for these crucial key events as CAR activation (KE1) and increased cell proliferation (KE3).

Therefore, there is no clear evidence that CAR receptor activation is involved in tumorigenic action of clofentezine in the liver of CD-1 mice. The absolute certainty on CAR involvement in the MoA could have been confirmed with a CAR-knock-out mouse study and the evidence for cell proliferation could have been strengthened with an *in vitro* comparative cell proliferation study (mouse, rat, human). Further enzyme induction studies might have also been done.

Conclusion on liver tumours

The tumour profile has some factors that reduce considerably the level of concern regarding the clofentezine carcinogenicity for humans. These factors are:

1. The lack of evidence of progression into malignancy of the adenomas.
2. Benign liver tumours appeared only in one species (mice) and one sex (females).
3. A long time of latency: the higher incidence of hepatocellular adenomas was only observed at the end of the study (105 weeks). Whereas, no significant incidence of adenomas was reported after 52 weeks of exposure.
4. The incidence of benign hepatocellular tumours in high dose females (13.5%) was only slightly higher than the incidence in control females in the mouse carcinogenicity study (7.7%).
5. The liver tumours incidence was not significant after pairwise comparison. Although, there was a positive trend after trend analysis.
6. There was no clear dose response despite the wide range of doses studied, and the magnitude of the high dose.
7. The incidence of hepatocellular adenomas in high dose females (13.5%) was only slightly higher than the historical control range (0-7.7%).
8. The liver tumours incidence in controls of the carcinogenicity mice study (7.7%) was in the upper value of the historical control data (0-7.7%)
9. The liver tumours appeared only at the highest dose level and after an exposure period of 2 years. Older mice have more probability of tumours due to ageing.

Clofentezine promoted formation of spontaneous liver adenomas with low potency to develop malignancy, only in one species (mice) and in one sex (female); the low incidence of adenomas observed at the highest dose was not pairwise statistically significantly increased (although, there was a positive trend after trend analysis) and was only slightly above the contemporary historical control; there was no dose-response relationship and adenomas were seen only at the highest dose of treatment (557.1 mg/kg bw/day for females) after a period of exposure of 2 years. All these considerations reduce considerably the concern of the liver tumours in mice and lead the applicant to propose no classification of clofentezine for carcinogenicity.

Besides, there are some experimental evidence supporting a PB like MoA for the induction of liver tumours in female mice. One finding consistent with a PB-like response are the induction of CYP450 of the 2B/3A families. Other findings consistent with a PB-like response are observations of increased liver weight and centrilobular hepatocellular hypertrophy. Presence of proliferation of smooth endoplasmic reticulum and vacuolisation are also findings consistent with a PB-like response. The development of eosinophilic altered hepatic foci is also a key event in the MoA for Phenobarbital-induced liver tumors.

Like with PB, the appearance of such foci, like adenomas, occurred only after chronic administration of Clofentezine. Besides, hepatocellular effects observed after short term clofentezine treatment showed reversibility, which is consistent with the known hepatic effects of other mitogenic rodent liver CAR activators *(LeBaron et al., 201; Osimitz and Lake, 2009; Yamada et al., 2014).*

The results of the studies indicate that clofentezine and phenobarbital may share some common mechanisms but a definite conclusion on the similarity of the mode of action between the two substances cannot be established. Data for concordance analysis with PB are limited. There are a number of data gaps, such as the lack of available data regarding CAR involvement in the induction of CYP isoforms following clofentezine exposure, no experimental evidence of hepatic cell proliferation and there is no data regarding the concordance of key events between rodents and humans.

The overall assessment has elements of uncertainty that cannot be overruled. The applicant also considered that the PB-like MoA still has its uncertainties as stated that the absolute certainty on CAR involvement in the MoA could have been confirmed with a CAR-knock-out mouse study and the evidence for cell proliferation could have been strengthened with an *in vitro* comparative cell proliferation study (mouse, rat, humans).

Therefore, for this compound there is not robust data for a PB-like MoA. There is no clear evidence that CAR receptor activation is involved in tumorigenic action of clofentezine in the liver of CD-1 mice. Also, there is not a satisfactory demonstration that other molecular mechanisms are not relevant. Therefore, based on the data available, no mode of action for formation of liver tumours in rodents could be established with certainty and hence, the relevance for humans cannot be excluded.

10.9.2 Comparison with the CLP criteria

<u>Comparison with criteria for Category 1A classification:</u> In accordance with the criteria in the CLP regulation, classification for carcinogenicity Category 1A is reserved for substances known to have carcinogenic potential in humans. In the absence of human data, category 1A is not triggered.

<u>Comparison with criteria for Category 1B classification:</u> In accordance with the criteria in the CLP regulation, classification for carcinogenicity Category 1B is reserved for substances that are presumed to be carcinogenic in humans, and is largely based on data from animal studies where there is sufficient evidence to demonstrate animal carcinogenicity (presumed human carcinogen).

There are two different types of tumors (liver and thyroid) in two different species (mice and rats). In order to assess the strength of evidence and to conclude whether clofentezine triggers cat.1B, cat.2 or

no classification, the Guidance on the Application of the CLP Criteria (version 5.0, July 2017) in section 3.6.2.2.2. establishes certain important factors which may be taken into consideration when assessing the overall level of concern. These factors are displayed in the Table 36 below.

Table 36: Factors to be taken into consideration in assessing the overall level of concern of the clofetenzine-induced liver and thyroid tumours

	Liver tumours	Thyroid tumours
Genotoxicity	Clofentezine is not genotoxic	
Tumour type and background incidence	Mouse: Hepatocellular tumours (benign) in female mice. The incidence was slightly higher than the historical control range. Rat: There were not liver tumours.	Mouse: There were not thyroid tumours. Rat: follicular cell tumors (combined adenomas and carcinomas) in male rats. The incidence was only slightly higher than the control of contemporaneus study.
Multi-site responses	Liver in female mice and thyroid in male rat.	
Progression of lesions to malignancy	No	
Reduced tumour latency	No, liver tumours in mice occurred at a later stage of the study, at terminal necropsy after 105 weeks treatment in high dose group females.	No, thyroid tumours in rat occurred at a later stage of the study, at terminal necropsy after 27 months treatment in high dose group males.
Whether response single or several species	Liver tumours only in single species: mice.	Thyroid tumours only in single species: rat.
Whether response is in single or both sexes	Liver tumours only in a single sex: females.	Thyroid tumours only in a single sex: male.
Structural similarity to a substance(s) for which there is good evidence of carcinogenicity	Not noted	
Routes of exposure	Only experimental studies by oral route are available. Dietary oral (relevant for humans)	
Comparison of absorption, distribution, metabolism and excretion between test animals and humans	No human data available.	
Possibility of a confounding effect of excessive toxicity at test doses	NO: In ♀ mice at 557.1 mg/kg bw/day after 105 weeks, it was observed a high proportion of deaths (42 *vs.* 27 of controls) attributed to amyloidosis. It should be noted that amyloidosis is a common age-related condition in CD-1 mice with a tendency to be a frequent cause of death in these CD-1 mice. Therefore, the mortality observed in CD1 female mice in a study that ran for a exposure period of 2 years is considered not due to treatment.	NO: In ♂ rat at 400 ppm (equivalent to 17.3 and 22.1 mg/kg bw/ day for males and females respectively) of clofentezine for 27 months thyroid tumours in male rat were associated with toxicity in liver (Increased absolute and relative liver weight, centrilobular hepatocyte enlargement or centrilobular hepatocyte vacuolation) and thyroid (slight follicular cell hyperplasia and agglomeration of colloid).
Mode of action and its relevance for humans.	Mechanistic studies in rats provide some experimental evidence supporting a PB MoA for liver tumours in female mice. This mode of action is rodent-specific and not regarded as relevant to humans. However, CAR involvement in the induction of CYP isoform following	Based on the available data, it is considered that the induction of hepatic UGT activity is sufficiently supported This MoA might give rise to thyroid tumours in rodents. The relevance of such a MoA based on enhancement of the metabolism and excretion of thyroid hormone by the liver, largely

	Liver tumours	Thyroid tumours
	clofentezine exposure was not established. No genotoxic and no cytotoxicity MoA. Evaluation of data indicate that some possible MoAs are not likely to be relevant (immunosuppresion, estrogen, statins, apoptosis, infections or metal accumulation) No experimental data about other alternative modes of action (like e.g. PPARα, AhR).	through induction of UGT enzymes, is considered not to be relevant to humans. The mechanism (s) that produces perturbation of the hypothalamic–pituitary–thyroid (HPT) axis is not considered relevant to humans based on quantitative species differences

Thyroid tumors in male rat: mechanistic data suggest that these tumours are not relevant to humans and therefore not considered for classification. MoA supported: HPT disturbance by induction of T4 excretion, subsequent TSH increase, thyroid follicular cell hypertrophy and hyperplasia. This mode of action closely mimics that of phenobarbital, is rodent- specific and non-relevant for humans.

ECHA CLP Guidance (2017) states that “certain thyroid tumours in rodents mediated by UDP glucuronyltransferase (UGT) induction (IARC, 1999; EU Specialised Experts, 1999)” as not relevant to humans.

In the ECB C&L guidance document on thyroid tumours (EC, 1999, ECBI49/99-Add1.Rev2) it is concluded that when a non-genotoxic substance produces a low/medium potency perturbation on the thyroid-pituitary axis the mechanism of action is not relevant for humans and do not need to be classified for carcinogenicity.

On overall, thyroid tumours are not considered to be of relevance to humans.

Liver tumors in female mouse: With respect to the carcinogenic potential in the liver, clofentezine was shown to have a weak carcinogenic effect in female CD-1 mice. After a period of exposure of two years, the increase in the incidence that only involved adenomas was relatively small and was limited to the highest dose, without statistical significance, and with no dose-response at lower doses. Also, clofentezine was not genotoxic in a battery of in-vitro and in-vivo studies.

However, in our view a treatment-related tumour response cannot be excluded. Although the increase in the incidence of adenomas at the highest dose in female mice was not statistically significant, a significant positive trend after trend analysis was observed. Also, the increase was above the historical control, although only slightly. The combined analysis of benign and malignant hepatic tumours in females of the highest dose was also significant after pairwise comparison and showed a positive trend after trend analysis. Besides, although the higher incidence of hepatocellular adenomas exceeded those of the controls only at the highest dose level tested, it seems that tumours were not related to excessive toxicity. The mortality observed in CD-1 female mice at this dose was not considered due to treatment with clofentezine and rather a common age-related condition in CD-1 mice with a tendency of develop amyloidosis, which is a frequent cause of death in CD-1 mice.

Given the limited evidence in mice, although a treatment-related tumour response cannot be excluded there are insufficient grounds for a category 1B classification for carcinogenicity. The choice is between a category 2 classification and no classification, depending on the mode of action that could account for the liver effects in mice and their relevance to humans.

Comparison with criteria for Category 2 classification: In accordance with the criteria in the CLP regulation, classification for carcinogenicity Category 2 is reserved for substances where there is

evidence obtained from human and/or animal studies but which is not sufficiently convincing to place the substance in Category 1.

The comparison of clofentezine carcinogenicity data with the corresponding classification criteria is not trivial. Data are some kind of borderline and the criteria leave a margin for different interpretations. All the considerations mentioned before reduce considerably the concern and it might be possible that the benign tumours in the liver of female mice were chance observations due to aging. However, a treatment-related tumour response cannot be excluded considering that the results from the supplementary studies are not sufficient to eliminate the concern for the relevance of these tumours to humans.

Whereas the thyroid tumours are considered not relevant to humans, the increase in liver tumours cannot be dismissed as non-relevant to humans as the mechanism for formation of liver tumours in female CD-1 mice remains unclear.

There is evidence for a non-genotoxic mechanism that bear similarities to a phenobarbital-like mode of action. However, for this compound, there is not robust data for a PB-like MoA. Data are not sufficient to conclude that CYP mediated CAR activation is the only critical key event and there is not a satisfactory demonstration that other molecular mechanisms are not relevant.

On overall, the mechanistic data provided were ultimately insufficiently robust to dismiss the elements of uncertainty in the liver tumour profile in mice and contribution of other MoA than CAR. Consequently, the Spanish CA considers that the overall available evidence is deemed to best match the criteria for classification as category 2 and proposes to classify clofentezine as **Carc. 2; H351 - Suspected of causing cancer**.

10.9.3 Conclusion on classification and labelling for carcinogenicity

Carc. 2; H351 - Suspected of causing cancer.

10.10 Reproductive toxicity

10.10.1 Adverse effects on sexual function and fertility

Table 37: Summary table of animal studies on adverse effects on sexual function and fertility

For more detailed information see RAR B6 (AS) chapter 6.6.1.1

Method, guideline, deviations if any, species, strain, sex, no./group	Test substance, dose levels duration of exposure	**Results** [Effects statistically significantly and dose-related unless stated otherwise as not significant (n.s.) or not dose-related (ndr) or not clearly dose-related (ncdr)]	Reference
Multigeneration study in the rat Laboratory: Schering Agrochemicals Limited Guideline: OECD 416 GLP: No (prior to GLP enforcement) Rat strain: Charles River Sprague Dawley Crl CD BR No. animals (groups): F0: 30 rats/sex/dose (0, 4, 40 ppm) and 40 rats/sex/dose (400 ppm) F1: 25 rats/sex/dose F2: 20 rats/sex/dose **Study acceptable**	Purity: 97.9 – 99.3% Oral (diet) Study scheme F0 →F1A and F1B ↙ F1 →F2A and F2B ↙ F2 No data on range-finding study Doses: 0, 4, 40 or 400 ppm equivalent to: F_0→ F1A and F1B ♂: 0, 0.28, 2.79, or 27.8 mg/kg bw/day ♀: 0, 0.33, 3.22, 31.7 mg/kg bw/day. F_1→ F2A and F2B ♂: 0, 0.35, 3.57, or 36.1 mg/kg bw/day ♀: 0, 0.39, 3.85 or 38.5 mg/kg bw/day F_2 ♂: 0, 0.36, 3.55, or 36.1 mg/kg bw/day ♀: 0, 0.38, 3.85 or 39.3 mg/kg bw/day. Exposure: Pre-mating treatment: F0 (74 days) →F1A F1 (88 days) →F2A Treatment continued in F1 and F0 throughout gestation and lactation. 14 days after weaning, animals of F0 and F1 were remated again to give F1B and F2B. Treatment did not stop until weaning of the 2nd generation. ↓ Total treatment for both sexes: F0 (32 weeks) and F1 (33 weeks) F2 (maturation of 82-84 days).	**PARENTAL ANIMALS** ***Parental toxicity*** *Mortality* ***F0:*** 2 ♀ died during the breeding phase: 1 ♀ (0 ppm) during gestation of the F_{1A} litter and 1 ♀ (400 ppm) during parturition of the F_{1B} litter. 1 ♂ (40 ppm) was killed *in extremis* following weight loss. This animal was found to have to a fractured upper jaw. ***F1:*** 2 ♀ (40 ppm) died during their first breeding phase: during parturition and 1 ♀ killed in extremis on day 3 *post partum* after total litter loss. 1 ♂ (400 ppm) died during the maturation period a 1 ♂ (4 ppm) during lactation of F2A offspring. **400 ppm** ***F0:*** ▪ ↓ bw gain in ♀ between days 4-7 *post coital* to give F_{1B} (33%). ***F1:*** ▪ ↓ bw in ♂ at week 1 and 5 of pre-mating period (11 and 7% respectively) and in ♀ following the birth of F_{2A} (10% and 7% on days 10 and 14 of *post partum*) and F_{2B} litters (6-7% days 4-14 *post coital* and 7-9% days 4-21 *post partum*). ▪ ↓ bw gain in ♀ at week 12 of pre-mating period (*ndr* 43%) and on days 4-10 *post partum* (37%) following the birth of F_{2A}. ▪ ↓ Terminal bw in ♀ (10%). ▪ ↑ Relative liver weight (16%) in ♂ and ↓ absolute liver weight (*ndr* 11%) in ♀ ▪ ↑ Relative ovaries weight (*ndr* 15%) in ♀. ▪ Increased incidence of minimal centrilobular hepatocyte enlargement in ♂ (4/10 *vs* 0/10 in controls). ***F2 (maturation):*** ▪ ↓ bw in ♂ at week 1 (12%), week 2 (11%), week 3 (10%) and week 6 (7%) and in ♀ at week 2 and 3 (10% and 7% respectively). ▪ ↑ Relative liver weight (14%) in ♂. In the absence of histopathological change, this marginal effect is no toxicological relevance. **40 ppm** ***F0:*** There were no treatment-related effects. ***F1:*** ▪ ↓ bw gain in ♀ at week 12 (*ndr* 43%) and on days 4-10 *post partum* (33%) following the birth of F_{2A} (33%). ▪ ↓ Absolute liver weight (*ndr* 11%) in ♀. ▪ ↑ Relative ovaries weight (*ndr* 18%) in ♀. ***F2:*** ▪ ↑ Relative liver weight (9%) in ♂. 4 ppm ***F0:*** There were no treatment-related effects.	**Anonymous 61 (1984)** **Seamons M.C. & Crofts. M., (1984) (dietary concentrations)** **Anonymous 62 (1986) (report addendum)** **(AS) B.6.6.1.1**

	Parameters observed: *F_0, F_1 and F_2 parental*: Mortality, clinical signs, bodyweights, food consumption, organ weights, gross pathology, histopathology and reproductive parameters (mating, fertility and pregnancy index and gestation period). *F_0→F_{1A}/F_{1B} and F_1→F_{2A}/F_{2B} litter*: Mortality, clinical signs, body weights, litter size, pup developmental (pinna detachment, tooth eruption and eye opening), organ weights, gross pathology and histopathology	***F1:*** ▪ ↓ Bw gain in ♀ following the birth of F_{2A} (*ndr* 41%) on days 4-10 *post partum*. ▪ ↓ Absolute liver weight (*ndr* 12%) in ♀. ▪ ↑ Relative ovaries weight (*ndr* 15%) in ♀. ***F2:*** There were no treatment-related effects. NOAEL $_{\text{parental toxicity}}$: **40 ppm** (equivalent to approx. **4 mg/kg bw/day**) **REPRODUCTIVE PARAMETERS** ***F0 and F1*** All reproductive parameters were similar to controls NOAEL $_{\text{reproductive toxicity}}$ **> 400 ppm (>27.8 mg/kg bw/day)** **LITTER DATA** **400 ppm** ***F_0→F_{1A} and F_{1B}:*** There were no treatment-related effects. ***F_1→F_{2A} and F_{2B}*** ***F_1→F_{2A}*** ▪ ↓ Pup weights (17%) at day 21 *post partum.* ***F_1→F_{2B}*** ▪ ↓Mean litter size: born pups (12%), live pups (16% on day 1 *post partum* and 18% on day 21 *post partum*). ▪ ↓ Litter weights between day 4 to day 21 *post partum* (16-18%). **40 ppm** ***F_0→F_{1A} and F_{1B}.*** There were no treatment-related effects. ***F_1→F_{2A} and F_{2B}*** ***F_1→F_{2A}*** ▪ ↓ Pup weights (11%) at day 21 *post partum.* This slight reduction reflected the slightly higher mean litter size at this dose and is of no toxicological concern ***F_1→F_{2B}:*** There were no treatment-related effects. **NOAEL** $_{\text{neonatal toxicity}}$**: 40 ppm** (equivalent to approx. **4 mg/kg bw/day**)	

Table 38: Summary table of human data on adverse effects on sexual function and fertility

Type of data/report	Test substance	Relevant information about the study	Reference
No data reported of adverse health effects in humans			

Table 39: Summary table of other studies relevant for toxicity on sexual function and fertility

Type of study/data	Test substance,	Relevant information about the study	Reference
Oral 90-day dietary study in rat Method: broadly comparable to OECD 408 GLP: No (prior to GLP enforcement) Rat strain: Sprague Dawley 20 rats/sex/dose Interim kill: up to 5 rats/sex/dose Recovery period (week 17): up to 5 rats/sex/dose **Study acceptable as supporting information**	Purity: 98.8-100% Oral (diet) Doses of 0, 3000, 9000 and 27000 ppm equivalent to 0, 202/221, 602/662, 1892/1992 mg/kg bw/day for ♂/♀	It was observed an increase statistically significant in relative testes weight (18%) at week 13 to 27000 ppm equivalent to 1892 mg/kg bw/day in males.	**Anonymous 63 (1982b)** Anonymous 64 (1988) (Additional thyroid gland examination) **(AS) B.6.3.2.1.1-01**

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

The potential effects of clofentezine on fertility and reproductive performance have been investigated in a standard 2-generation study in rat (B.6.6.1.1) at doses up to 400 ppm (approximately equivalent to the interval 27.8-39.3 mg/kg bw/day). No dose-range finding study was performed according to the data included in the study. However it has to be noted that after 90-day repeated dose toxicity in rats (B.6.3.2.1.1-02) NOAEL was established in 2.65 mg/kg bw/d for males and 2.91 mg/kg bw/day in females due to toxicity observed at the inmmediate upper dose level of 26.2/29.3 mg/kg bw/day for males and females respectively (see section 10.12) with liver as main target. Consequently, top dose level used in the 2-generation study is known to cause toxicity.

This study is previous to the current test guideline (OECD 416, 2001) and is therefore deficient in some endpoints. In parents, the dietary intervals were larger than those recommended, oestrus cycle length, sperm parameters and anogenital distance were not measured and spleen and pituitary were not weighed. In pups, developmental and functional observations were not undertaken and age of vaginal opening and preputial separation were not determined. However, the conclusions reached are robust and the omissions/deviations are considered unlikely to alter these conclusions.

Parental toxicity was evident in F_1 and F_2 parents at 400 ppm (in F_0 there were no treatment-related effects):

In F_1 parents, body weights were lower than control values in both sexes: throughout the maturation phase in males (11% and 7% at week 1 and 5 respectively) and gestation and lactation period in females [$F_1 \rightarrow F_{2A}$ (10% and 7% on *post-partum* days 0 and 14) and $F_1 \rightarrow F_{2B}$ (6-7% during period 4-14 days *post-coitum* and 7-9% during period 4-21 days *post-partum]*. Besides, in females it was observed a decrease in body weight gain $F_1 \rightarrow F_{2A}$ (37%) during on days 4-10 days *post-partum* and in terminal body weight (10%). In males, an increase in relative liver weight (16%) was seen associated with histopathology (increased incidence of minimal centrilobular hepatocyte enlargement).

In F_2 parents, there were a decrease of bodyweight from week 1 to week 6 in males (7-12%) and at week 2 and 3 (10% and 7% respectively) in females. In males, an increase in relative liver weight (14%) was seen, but this increase was not associated with histopathological changes, so this marginal effect has no toxicological relevance. **NOAEL** for **maternal toxicity** was set at 40 ppm (equivalent to approx. 4 mg/kg bw/day).

Neonatal toxicity was evident in F_2 pups at 400 ppm (in F_1 pups there were no treatment-related effects). In F_{2A} pups a decrease in weights (17%) on day 21 *post-partum* was observed. In F_{2B} pups a decrease in mean litter size [born pups (12%) and live pups (16% and 18% on days 1 and 21 *post-partum* respectively)] and litter weights (16-18% between day 4 to day 21 *post-partum*) were seen. **NOAEL** for **development** was set at 40 ppm (equivalent to approx. 4 mg/kg bw/day).

Clofentezine had no effect on **fertility or reproductive performance**. NOAEL for reproductive toxicity was found to be higher than 400 ppm (>27.8 mg/kg bw/day)

10.10.3 Comparison with the CLP criteria.

Substances are classified in Category 1 for reproductive toxicity when they are known to have produced an adverse effect on sexual function and fertility in humans or when there is evidence from animal studies, possibly supplemented with other information, to provide a strong presumption that the substance has the capacity to interfere with reproduction in humans. The classification of a substance is further distinguished on the basis of whether the evidence for classification is primarily from human data (Category 1A, known human reproductive toxicant) or from animal data (Category 1B, presumed human reproductive toxicant).

According to the CLP criteria a classification of a substance in category 1B is largely based on data from animal studies. Such data shall provide clear evidence of an adverse effect on reproductive toxicity in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effects.

Substances are classified in Category 2 for reproductive toxicity when there is some evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on sexual function and fertility and where the evidence is not sufficiently convincing to place the substance in Category 1. If deficiencies in the study make the quality of evidence less convincing, Category 2 could be the more appropriate classification. Such effects shall have been observed in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of the other toxic effects.

No human information is available regarding effects on the reproductive system by clofentezine. Information from a reliable 2-generation study in rats showed that clofentezine has no effects on fertility and reproductive performance. Consequently, the MSCA is of the opinion that classification is not warranted.

10.10.4 Adverse effects on development

Table 40: Summary table of animal studies on adverse effects on development

<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
[Effects statistically significantly and dose-related unless stated otherwise as not significant (n.s.) or not dose-related (ndr) or not clearly dose-related (ncdr)]</th>
<th>Reference</th>
</tr>
<tr>
<td>Teratology study in rats

Laboratory: Fisons Limited Pharmaceutical Division

Method: "In house method" comparable to OECD 414 (1981) / B.31

GLP: Yes

Oral (gavage)

Rat strain: CD Sprague Dawley

34 or 35 mated females/group

Study acceptable

Deviations: The exposure period was from day 7 to 20 of gestation when it should have begun at least from implantation.

The highest dose tested, 3000 mg/kg bw/day exceeds the dose recommended for a test limit (1000 mg/kg bw/day)</td>
<td>Test substance: Clofentezine (NC 21314, technical material; batch CR 20099/8; purity 100%)

Dose levels were selected after a range-finding study and represents the maximum that could be administered by gavage: 0, 320, 1280, 3200 mg/kg bw/day

Vehicle: 0.5 % carboxymethyl cellulose

Exposure: dosing on gestation days 7-20 gestation</td>
<td>Maternal toxicity

Mortality: During treatment 3♀ were found dead, 2 at 1280 mg/kg bw/day and 1 at 3200 mg/kg bw/day. The cause of these deaths was considered to be mis-dosing into the respiratory system

3200 mg/kg bw/day:
▪ ↓ bw on day 21 for body weight (4%) and corrected bw for uterine contents (5%).
▪ ↓ bw gain during periods days 7-14 (24%) and days 14-21 (9%).
▪ ↑ Relative liver weight (10%) when corrected for the uterine contents, associated with histopathology changes (staining and enlargement of centrilobular hepatocytes)

1280 mg/kg bw/day:
▪ ↑ Relative liver weight (7%) when corrected for the uterine contents. This increase was < 10% and not associated with histopathological changes (not toxicologically relevant).

320 mg/kg bw/day: No effects.

NOAEL $_{maternal}$: 1280 mg/kg/day

Developmental toxicity

3200 mg/kg bw/day:
▪ Skeletal alteration:
↑Incidence fetuses with:
- Incomplete ossification of the hyoid (8.92 vs 2.69).
- One or less sternebrae incompletely ossified (57.28 vs 40.81)
- Two or more ossified caudal vertebrae (78.87% vs 52.47)

Foetal skeletal alterations
<table>
<tr><th rowspan="2">Parameters</th><th colspan="4">Dose level (mg/kg bw/day)</th></tr>
<tr><th>0</th><th>320</th><th>1280</th><th>3200</th></tr>
<tr><td>Foetuses examined</td><td>223</td><td>207</td><td>193</td><td>213</td></tr>
<tr><td>Litters examined</td><td>35</td><td>33</td><td>30</td><td>33</td></tr>
<tr><td>Observations</td><td colspan="4">Foetal incidence% (Litter incidence %)</td></tr>
<tr><td colspan="5">Incomplete ossification or absence of hyoid</td></tr>
<tr><td></td><td>2.69 (8.57)</td><td>5.80 (18.18)</td><td>7.25 (26.67)</td><td>8.92** (18.18)</td></tr>
<tr><td colspan="5">Incomplete ossification of sternebrae - number of bones affected</td></tr>
<tr><td>≤1</td><td>40.81 (85.71)</td><td>46.38 (81.81)</td><td>37.31 (76.67)</td><td>57.28** (96.97)</td></tr>
<tr><td>2</td><td>53.81 (97.14)</td><td>46.86 (87.88)</td><td>53.37 (96.67)</td><td>41.32* (81.82)</td></tr>
<tr><td>≥3</td><td>5.38 (20.00)</td><td>6.76 (24.24)</td><td>9.33 (40.00)</td><td>1.41* (6.06)</td></tr>
<tr><td colspan="5">Number of ossified caudal vertebrae</td></tr>
<tr><td><2</td><td>47.53 (85.71)</td><td>20.77** (57.58)</td><td>17.62** (56.67)</td><td>21.13** (69.70)</td></tr>
<tr><td>≥2</td><td>52.47 (88.57)</td><td>79.23** (96.97)</td><td>82.38** (100.00)</td><td>78.87** (100.00)</td></tr>
</table>
*p≤0.05; **p≤0.01; *** p≤0.001

These increases were not considered to be related to the treatment since:
- No significant difference in the litter incidences of these parameters was detected.
- The number of foetuses with one or less sternebrae incompletely ossified was significantly increased. However, the number of foetuses with 2, 3 or more sternebrae incompletely ossified was reduced at 3200 mg/kg bw/day compared to controls.
- The number of foetuses with two or more ossified caudal vertebrae was significantly increased at all dose levels but these findings were not dose-dependent.

1280 mg/kg bw/day: No effects

NOAEL $_{maternal}$: 250 mg/kg bw/day</td>
<td>Anonymous 65 (1982)

Crofts M., (1982a) (Determination concentrations in suspensions)

(AS) B.6.6.2.1</td>
</tr>
</table>

Method, guideline, deviations if any, species, strain, sex, no./group	Test substance, dose levels duration of exposure	Results [Effects statistically significantly and dose-related unless stated otherwise as not significant (n.s.) or not dose-related (ndr) or not clearly dose-related (ncdr)]	Reference
Teratology study in rabbit Laboratory: Schering Agrochemical limited Method: *"In house method"* comparable to OECD 414 (1981) / B.31 GLP: No (conducted prior to the enforcement of GLP). Oral (gavage) Rabbit strain: New Zealand White 14 or 15 mated females/group **Study acceptable**	Test substance: Clofentezine (NC 21314), Lot/Batch No.: CR 20099/12, Purity: 98.5% *Preliminary study:* 6 animals/dose at dose levels of 250, 1000 and 4000 mg/kg bw/d. *Main study:* Dose levels: 0, 250, 1000 and 3000 mg/kg bw/day Vehicle: 0.5% sodium carboxymethyl cellulose Exposure: from day 7 to 28 of gestation	***Maternal toxicity*** **3000 mg/kg bw/day:** ▪ 1/14 dead treatment-related associated with anorexia, reduced fecal output and weight loss. ▪ ↓ bw change relative to the initiation of dosing on day 7 of gestation on day 10 (90%), 14 (51%), 18 (38%), 22 (33%), 26 (29%) and day 29 (19% but not significant). ▪ ↓ Food consumption (~ 20% from day 7 to day 25). There is no statistical calculation for this effect. ▪ Pink discoloration of the GIT. **1000 mg/kg bw/day:** ▪ 1/14 dead. The clinical signs observed were indicative of respiratory and gastro-intestinal disorder. 1/14 death was observed in the control group with the same associated symptoms and were considered to be coincidental. ▪ ↓ bw change relative to the initiation of dosing on day 7 of gestation on day 10 (85%) and day 18(31%). **250 mg/kg bw/day:** ▪ ↓ bw change relative to the initiation of dosing on day 7 of gestation on day 18 (27%). **NOAEL $_{maternal}$: 250 mg/kg bw/day** ***Developmental toxicity*** **3000 mg/kg bw/day:** ▪ ↓ Mean foetal weight (13%). In consequence, mean litter weights were lower than the control value (12%), although the difference did not attain statistical significance. **1000 mg/kg bw/day:** No effects **NOAEL $_{developmental}$: 1000 mg/kg bw/day**	**Anonymous 66** **(1983)** Anonymous 67 (1983) (Report addendum) Crofts M., (1982b) (Determination concentrations in suspensions) **(AS) B.6.6.2.2**

Table 41: Summary table of human data on adverse effects on development

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No data reported of adverse health effects in humans				

Table 42: Summary table of other studies relevant for developmental toxicity

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No relevant studies				

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

The developmental toxicity of clofentezine was investigated in two prenatal developmental toxicity studies, one performed in rats (B.6.6.2.1) and the other in rabbits (B.6.6.2.2). Both studies predate the current OECD Test Guideline Number 414 (2001) and do not include the recommended extended dosing period (i.e. from implantation to one day prior to the day of scheduled kill). However, both studies are considered adequate and relevant for evaluation of the potential of clofentezine to induce developmental effects. No evidence of teratogenicity was observed in both species.

In the **rat study**, dose levels were selected after a range-finding study and represent the maximum that could be administered by gavage. No more detail of the dose-range finding study was included the study. However, it has to be noted that the maximum tested dose level of 3200 mg/kg bw/day is clearly above the limit dose level of 1000 mg/kg bw/day according to test method B.31. Consequently, tested dose levels are considered appropriate for regulatory purposes.

The highest dose tested of 3200 mg/kg bw/day induced maternal toxicity. Bodyweights were significantly decreased (also when were corrected for uterine content) at day 21 (4%) and also body weight gain between days 7-14 (24%) and 14-21 (9%). Dose-related and significantly increased relative liver weight were observed (also when corrected for uterine content). This increase (10%) was associated with histopathology (staining and enlargement of centrilobular hepatocytes).

There were foetal skeletal alterations in the range of variations considered not related to treatment at this dose level. The number of ossified caudal vertebrae seen along dose levels was not dose-dependent. The incomplete ossification of sternebrae was significant for foetuses but not per litter basis and not dose-dependant. Besides, it was higher in the number of foetuses with ≥ 2 bones affected in controls than the highest treated group. Consequently, no developmental effects were attributable to this dose level.

At the intermediate dose level of 1280 mg/kg bw/day no maternal toxicity or developmental effects were observed. **NOAEL** for **maternal toxicity** was set at 1280 mg/kg/bw/day and **NOAEL** for **development** was considered to be higher than 3200 mg/kg bw/day.

In the **rabbit study**, dose levels were selected from a dose-range finding study with tested dose levels of 250, 1000 and 4000 mg/kg bw/day in which the maximum tested dose level was regarded for impractical dosing. It has to be noted that the maximum tested dose level of 3000 mg/kg bw/day is clearly above the limit dose level of 1000 mg/kg bw/day according to test method B.31. Consequently, tested dose levels are considered appropriate for regulatory purposes.

The highest dose of 3000 mg/kg bw/day was associated with death in one rabbit (with anorexia, reduced fecal output and weight loss), marked reduced body weight gain and reduced food consumption (~ 20% from day 7 to day 25). At the intermediate dose, 1000 mg/kg bw/day, a decrease of body weight gain (85% days 7-10 and ~ 20% from day 10 to day 18) was observed.

Developmental effects were only observed at 3000 mg/kg bw/d with reduced foetal weight (13%) and mean litter weight (12%).

At the lowest dose, 250 mg/kg bw/day, no maternal toxicity or development effects were observed. Effects on bodyweight gain at 1000 mg/kg bw/day are not considered adverse according to CLP Regulation (Annex I, Section 3.7.2.4.4): "*in rabbits, the body weight gain may not be useful indicators of maternal toxicity because of normal fluctuations in body weight during pregnancy*". Consequently, **NOAEL** for **maternal toxicity** was set at 1000 mg/kg/bw/day taking into account reduced food consumption and mortality in one rabbit. **NOAEL** for **development** was set at 1000 mg/kg bw/day.

10.10.6 Comparison with the CLP criteria

Substances are classified in Category 1 for developmental toxicity when they are known to have produced an adverse effect on development in humans or when there is evidence from animal studies, possibly supplemented with other information, to provide a strong presumption that the substance has the capacity to interfere with development in humans. The classification of a substance is further distinguished on the basis of whether the evidence for classification is primarily from human data (Category 1A, known human reproductive toxicant) or from animal data (Category 1B, presumed human reproductive toxicant). There is no human data available on clofentezine therefore classification in Category 1A is not appropriate.

The classification of a substance in Category 1B is largely based on data from animal studies. Such data shall provide clear evidence of an adverse effect on development in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on development is considered not to be a secondary non-specific consequence of other toxic effects.

Substances are classified in Category 2 for reproductive toxicity when there is some evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on development, and where the evidence is not sufficiently convincing to place the substance in Category 1. If deficiencies in the study make the quality of evidence less convincing, Category 2 could be the more appropriate classification. Such effects shall have been observed in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on development is considered not to be a secondary non-specific consequence of the other toxic effects.

In the classification system, adverse effects on development of the offspring include any effect which interferes with normal development of the conceptus, either before or after birth, and resulting from exposure of either parent prior to conception, or exposure of the developing offspring during prenatal development, or postnatally, to the time of sexual maturation.

In the rat teratogenicity study as developmental effects only some skeletal variations not attributable to treatment were observed.

In the rabbit teratogenicity study, decreased foetal and litter weights observed at the highest dose level of 3000 mg/kg bw/day could be considered a consequence of maternal toxicity manifested by reduced food consumption. In any case, isolated foetal and litter weight reductions potentially linked to maternal toxicity are not considered relevant for classification for development.

There were no effects triggering classification for clofentezine due to developmental toxicity and hence classification is not warranted.

10.10.7 Adverse effects on or via lactation

The classification is intended to indicate when a substance may cause harm due to its effects on or via lactation and is independent of consideration of the reproductive or developmental toxicity of the substance. This can be due to the substance being absorbed by women and adversely affecting milk production or quality, or due to the substance (or its metabolites) being present in breast milk in amounts sufficient to cause concern for the health of a breastfed child.

Reproductive study available in section 10.10.1 does not provide evidence of adverse effect in the offspring due to transfer in the milk or adverse effect on the quality of the milk. Toxicokinetics studies do not indicate the likelihood that the substance can be potentially present in breast milk (see section 9).

In conclusion, there were no effects to warrant classification of clofentezine, for effects on or via lactation.

10.10.8 Conclusion on classification and labelling for reproductive toxicity

Not classified (conclusive but not sufficient for classification).

10.11 Specific target organ toxicity-single exposure

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

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. Relevant information for STOT SE is covered by acute toxicity studies in form of clinical observations, and macroscopic and microscopic pathological examination that can reveal hazards that may not be life-threatening but could indicate functional impairment. Acute toxicity studies are included in section 10.1.

STOT SE 3

STOT SE 3 includes narcotic effects and respiratory tract irritation. These are target organ effects for which a substance does not meet the criteria to be classified in Categories 1 or 2.

According to the results of the acute inhalation studies (see Table 43) respiratory tract irritation was not observed after administration of clofentezine.

Narcotic effects were not observed in acute toxicity studies.

STOT SE 1 and 2

STOT-SE Category 1 and 2 is assigned on the basis of findings of ‘significant’ or ‘severe’ toxicity. In this context ‘significant’ means changes which clearly indicate functional disturbance or morphological changes which are toxicologically relevant. ‘Severe’ effects are generally more profound or serious than ‘significant’ effects and are of a considerably adverse nature with significant impact on health. Both factors have to be evaluated by weight of evidence and expert judgement.

Table 43: Summary table of relevant effects for STOT SE below cut-off values for classification

Species, route, dose levels and author	Results
Acute oral toxicity study in rats **Anonymous 26 (1980) (AS) B.6.2.1.1-01** Doses: 0, 800, 1131, 1600, 2261 and 3200 mg/kg bw (3 rats/sex/dose) *Guideline value for classification: ≤ 2000 mg/kg bw (STOT SE 2); ≤ 300 mg/kg bw (STOT SE 1)*	**Clinical signs** Slight salivation in 1♂ at 1131 mg/kg bw. Pink coloration of faeces (attributed to the test chemical) between 20 and 22 hours was seen after dosing in females at all dose levels **Necropsy** No relevant findings.
Acute oral toxicity study in dogs **Anonymous 31 (1981) (AS) B.6.2.1.4-01** Doses: 2 dogs/sex in controls and at 2000 mg/kg bw and 1♂ at 1000 mg/kg bw *Guideline value for classification: ≤ 2000 mg/kg bw (STOT SE 2); ≤ 300 mg/kg bw (STOT SE 1)*	**Clinical signs** Not observed **Necropsy** Slight focal hyperplasia of the renal papillary epithelium was observed amongst treated male and female dogs. Although such changes were not evident in controls, this is a common, spontaneous lesion in laboratory dogs.

10.11.2 Comparison with the CLP criteria

No effects were observed for STOT SE 1 (guidance value for classification: ≤ 300 mg/kg bw).

The only effects observed in the range for STOT SE 2 after oral administration included in Table 43 are not relevant for classification (guidance value for classification: ≤ 2000 mg/kg bw and >300 mg/kg bw):

- Slight focal hyperplasia of the renal papillary epithelium observed amongst treated male and female dogs (2 dogs/sex at 2000 mg/kg bw/day and 1 male at 1000 mg/kg bw/day) is a common and spontaneous lesion in laboratory dogs not regarded for STOT SE.
- In the acute oral toxicity study in rats there was slight salivation in 1 male rat at 1131 mg/kg bw/day regarded as an isloted and not dose-related change, not sufficient for STOT SE. Pink coloration in faeces observed at doses > 800 mg/kg bw/day was attributable to test chemical.

No relevant effects were observed after dermal application or inhalation of clofentezine.

No signs were observed to be regarded for classification for STOT SE 3 according to CLP Regulation (respiratory tract irritation and narcotic effects).

10.11.3 Conclusion on classification and labelling for STOT SE

Clofentezine does not require classification for STOT SE according to CLP Regulation.

10.12 Specific target organ toxicity-repeated exposure

Table 44: Summary table of animal studies on STOT RE
For more detailed information see RAR B6 (AS) chapter 6.3

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results** [Effects statistically significant and dose-related unless stated otherwise as not significant (n.s.) or not dose-related (ndr) or not clearly dose-related (ncdr)]	**Reference**
Oral 17-day range-finding study in rats Non-guideline study GLP: No (prior to GLP enforcement) Rat strain: Charles River CD Sprague Dawley 5 rats/sex/dose **Study acceptable as a range-finding study** *Guideline value for classification extrapolated to 17-day study:* *STOT RE 1 ≤ 52.9 mg/kg bw/day* *STOT RE 2 ≤ 529.4 mg/kg bw/day*	Purity: not stated Oral (gavage): test item suspended in 0.5% gum tracanah (aq). Doses: 0, 5, 20, 80, 320 and 1280 mg/kg bw/day daily for 17 days. Parameters observed: mortality, clinical signs, bodyweight and food/water intake, haematology, biochemistry , organ weights, urinalysis, gross pathology and histopathology	Mortality: Considered incidental: 0 (1♂, 1♀), 80 (2♂), and 320 (2♂ and 1♀) mg/kg/day. **1280 mg/kg bw/day** Clinical signs ▪ Red coloration of faeces ♂/♀. Clinical chemistry: ▪ (↑) cholesterol in ♀ (26% ndr). ▪ (↑) total proteins in ♀ (5% ndr). ▪ (↑) albumin in ♂ (3% ndr). ▪ (↑) bilurrubin in ♂ (100% ncdr). ▪ (↑) calcium in ♂ (4% ndr). ▪ (↑) sodium in ♂/♀ (1% ndr/3% ndr). ▪ (↓) alkaline phosphatase (AP) in ♀ (29% ndr). Organ weights (end of treatment period): ▪ Liver: (↑) abs wt in ♂/♀ (15% ncdr/23%) and (↑) rel wt in ♂/♀ (16% ncdr/21% ncdr). Histopathology: Liver: ▪ Cytoplasmic and nuclear staining intensity of hepatocytes in ♂ (5/5 *vs*. 0/9 in controls). ▪ Isolated periportal and parenchymal chronic in-flammatory cell foci in ♀ (4/5) not relevant since it occurred in controls in ♂ (5/9) and ♀ (6/10). **320 mg/kg bw/day** Clinical signs: ▪ Red coloration of faeces ♂/♀. Organ weights: ▪ Liver: (↑) abs wt in ♀ (16%) and (↑) rel wt in ♂/♀ (7% n.s./10% ncdr). **80 mg/kg bw/day** Organ weights: ▪ Liver: (↑n.s.) abs wt in ♀ (14%) and (↑) rel wt in ♂/♀ (9% ncdr/12% ncdr). **20 mg/kg bw/day** Organ weights: ▪ Liver: (↑) abs wt (14%) and rel wt (9% ncdr) in ♀. **NOAEL**: not derived since it is a range-finding study.	**Anonymous 68** **(1980)** **(AS)** **B.6.3.1.1.1**
Oral 21-day palatability study in rats Non-guideline study GLP: No (prior to GLP enforcement) Rat strain: Charles River CD Sprague Dawley 5 rats/sex/dose **Study acceptable as a palatability study** *Guideline value for classification extrapolated to 21-day study:* *STOT RE 1 ≤ 42.9 mg/kg bw/day*	Purity: not stated Oral (diet) Doses of 0, 10000, 20000 and 30000 ppm equivalent to 0, 914/863, 1880/1763, 2768/2652 mg/kg bw/day for ♂/♀. Parameters observed: mortality and clinical signs, bodyweight gain, water and food consumption and macroscopic evaluation.	Clinical signs: Soiling of the eyes and nose, dullness of eyes, hair loss and abrasions /scratches were noted in some animals of all groups during the first week only. Additionally, pink staining of the coat and tail was seen in the treatment groups. **30000 ppm** (2768♂/2652♀ mg/kg bw/day) Bodyweight and food consumption: ▪ (↓) bw in ♀ [day 11 (6.5%)]. ▪ (↓) bw gain in ♂ [week 1(26% ncdr)] and in ♀ [week 3 (23% ncdr)]. Histopathology: ▪ Spleen: pale (2/5♂) and pitted with nodule (1/5♂). ▪ Liver: discoloration/pallor (3/5♂). **20000 ppm** (1880♂/1763♀ mg/kg bw/day) Bodyweight and food consumption: ▪ (↓) bw gain in ♂ [week 1 (25% ncdr)]. Histopathology: ▪ Spleen: small (2/5♂ ndr since not seen at 30000 ppm). ▪ Liver: discoloration/pallor liver (3/5♂).	**Anonymous 69** **(1982a)** Snowdon, P.J. (1981b) (determinat ion of test conc.) **(AS)** **B.6.3.1.1.2**

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results [Effects statistically significant and dose-related unless stated otherwise as not significant (n.s.) or not dose-related (ndr) or not clearly dose-related (ncdr)]	Reference
STOT RE 2 ≤ 428.6 mg/kg bw/day		**10000 ppm** (914♂/863♀ mg/kg bw/day) ▪ Spleen: small (1/5♂ ndr). **NOAEL**: not derived since it is a palatability study.	
Oral 42-day palatability study in mice Non-guideline study GLP: No (prior to GLP enforcement) **Study acceptable as a palatability study** Mouse strain: Charles River CD-1 5 mice/sex/dose *Guideline value for classification extrapolated to 42-day study:* *STOT RE 1 ≤ 21.4 mg/kg bw/day* *STOT RE 2 ≤ 214.3 mg/kg bw/day*	Purity: not stated Oral (diet) Doses of 0, 50, 500, 5000 and 30000 ppm equivalent to 0, 7.87/8.21, 79.5/91.4, 766/912, 5149/5395 mg/kg bw/day for ♂/♀. Parameters observed: mortality, clinical signs, bodyweight and food/water intake, biochemistry , urinalysis, liver weights, and liver macroscopic examination and histopathology	**30000 ppm** (5149♂/5395♀ mg/kg bw/day) Clinical signs: ▪ Bright pink colouration of the faeces and urine in ♂/♀. Bodyweight and food consumption: ▪ (↓) bw gain in ♂ [week 2-3 (33% ndr)]. Organ weights: ▪ Liver: (↑) in ♂ of abs wt (23%) and rel wt (22%). Urinalysis: ▪ Red crystals and yellow/orange to red coloration attributed to urinary excretion of the test material in ♂/♀. Histopathology: ▪ Liver: centrilobular hepatocytomegaly (3/5♂) and area of necrosis (3/5♂). **5000 ppm** (766♂/912♀ mg/kg bw/day) Clinical signs: ▪ Faint pink coloration of the faeces in ♂/♀. Organ weights: ▪ Liver: (↑) in ♂ of abs wt (16%) and rel wt (8%). Urinalysis: ▪ Red crystals and yellow/orange to red coloration attributed to urinary excretion of the test material in ♂/♀. Histopathology: ▪ Liver: Centrilobular hepatocytomegaly (1/5♂). **500 ppm** (79.5♂/91.4♀ mg/kg bw/day) Histopathology: ▪ Liver: Areas of necrosis (1/5♂ not seen at 5000 ppm). **NOAEL**: not derived since it is a palatability study.	**Anonymous 70** **(1982a)** **(AS)** **B.6.3.1.2.1.**
Oral 17-day range-finding study in dog Non-guideline study GLP: No (prior to GLP enforcement) **Study acceptable as a range-finding study** Dog strain: Beagle 1 dog/sex/dose Statistics not performed *Guideline value for classification extrapolated to 17-day study:* *STOT RE 1 ≤ 52.9 mg/kg bw/day* *STOT RE 2 ≤ 529.4 mg/kg bw/day*	Purity: not stated Oral (gavage) test item suspended in 0.5% aqueous gum tragacanth Doses of 0, 125, 500 and 2000 mg/kg bw/day Parameters observed: mortality, clinical signs, bodyweight and food intake, haematology, clinical chemistry , urinalysis, organ weights, macropathology and histopathology	*Since statistical analysis was not performed only dose-dependent effects are included in the table.* Clinical signs: All treated dogs exhibited red or pink coloration of faeces. Female dog at 2000 mg/kg bw/day regurgitated the dose. Histopathology: A variety of morphological abnormalities was encountered in both sexes from control and treated groups. Within the limitations of the small group size there is no histopathological evidence to suggest that any of the lesions observed could be attributable to treatment with clofentezine. **2000 mg/kg bw/day** Hematology: ▪ (↑n.s.) white cell count (WBC) in ♂ (138%) due to an increase of neutrophils. Biochemical: ▪ (↑ n.s) total protein in ♂ (22%). ▪ (↑ n.s) potassium in ♂ (30%). ▪ (↑ n.s) γ-glutamyl transpetidase in ♂ (383%). ▪ (↑ n.s) alkaline phosphatase (AP) in ♂ (110%). ▪ (↑ n.s) lactate deshidrogense (LDH) in ♂ (34%). ▪ (↑ n.s) hydroxibutyric deshidrogense (HBDH) in ♂ (110%). Organ weights: ▪ Liver: there is a trend of increase abs wt and rel wt especially in ♂ but with not clear dose dependency. ▪ **NOAEL**: not derived since it is a palatability study.	**Anonymous 71** **(1981)** **(AS)** **B.6.3.1.3.1**

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results [Effects statistically significant and dose-related unless stated otherwise as not significant (n.s.) or not dose-related (ndr) or not clearly dose-related (ncdr)]	Reference
Oral 28-day palatability study in dog Non-guideline study GLP: No (prior to GLP enforcement) Dog strain: Beagle 1/2 dog/sex/dose **Study of limited design** Statistics not performed *Guideline value for classification extrapolated to 28-day study:* *STOT RE 1 ≤ 30 mg/kg bw/day* *STOT RE 2 ≤ 300 mg/kg bw/day*	Purity: not stated Oral (diet) Doses of 10000 ppm (1♀), 20000 ppm (1♂&1♀) and 30000 ppm (1♂&1♀) equivalent to 250, 500 and 750 mg/kg bw/day Parameters observed: clinical signs, bodyweight and food consumption.	*Since statistical analysis was not performed only dose-dependent effects are included in the table.* No mortality occurred. The only treatment related effect seen was a red colouration in faeces. **30000 ppm** (750 mg/kg bw/day) ▪ The only observable effect was a decrease in the food intake in the ♀ at 30000 ppm compared to the ♀ at 20000 ppm. **NOAEL**: not derived since it is a palatability study.	**Anonymous 72** **(1980)** **(AS)** **B.6.3.1.3.2**
Oral 28-day range-finding study in dog Non-guideline study GLP: No (prior to GLP enforcement) **Study acceptable as a range-finding study** Dog strain: Beagle 1dog/sex/dose Statistics not performed *Guideline value for classification extrapolated to 28-day study:* *STOT RE 1 ≤ 30 mg/kg bw/day* *STOT RE 2 ≤ 300 mg/kg bw/day*	Purity: not stated Oral (diet) Doses of 200, 2000 and 20000 ppm equivalent to 10, 100 and 1000 mg/kg bw/day (no control group) Parameters observed: mortality, clinical signs, bodyweight and food consumption, biochemistry, haematology, organ weights and gross pathology.	*Since statistical analysis was not performed only dose-dependent effects are included in the table.* **20000 ppm** (1000 mg/kg bw/day) ▪ Increase in ♂/♀ in the liver absolute (37%/20%) and relative weight (34%/34%) with respect to the lowest dose but only clearly dose-related in ♀. **2000 ppm** (100 mg/kg bw/day) ▪ Increase in ♂/♀ in the liver absolute (38%/7%) and relative weight (34%/21%) with respect to the lowest dose but only clearly dose-related in ♀. **NOAEL**: not derived since it is a palatability study.	**Anonymous 73** **(1983)** **(AS)** **B.6.3.1.3.3**

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results [Effects statistically significant and dose-related unless stated otherwise as not significant (n.s.) or not dose-related (ndr) or not clearly dose-related (ncdr)]	Reference
Oral 90-day dietary study in rat Method: broadly comparable to OECD 408 GLP: No (prior to GLP enforcement) **Study acceptable as supporting information** Rat strain: Charles River Sprague Dawley 20 rats/sex/dose Interim kill (week 9): up to 5 rats/sex/dose Recovery period (week 17): up to 5 rats/sex/dose *Guideline value for classification (90 day study):* *STOT RE 1 ≤ 10 mg/kg bw/day* *STOT RE 2 ≤ 100 mg/kg bw/day*	Purity: 98.8-100% Oral (diet) Doses of 0, 3000, 9000 and 27000 ppm equivalent to 0, 202/221, 602/662, 1892/1992 mg/kg bw/day for ♂/♀ Parameters observed: mortality, clinical signs, bodyweight and food/water intake, haematology, clinical chemistry, organ weights, urinalysis, macroscopic examination and histopathology (thyroid gland was re-examined)	**27000 ppm** (1892♂/1992♀ mg/kg bw/day) Mortality: ▪ 1 male sacrificed on day 6 in a moribund state and another one found dead on day 90. Both individuals showed severe congestion and/or hemorrhaging in the region of the bladder and prostate with gross distension of the bladder. A third male dead as a result of errors during blood sampling Clinical signs: ▪ Hair loss in both sexes. Bodyweight and food consumption: ▪ (↓) bw in ♂/♀ [week 4 (10%/4% ndr), week 11 (14%/5% ndr), week 12 (13%/10% ncdr) and week 13 (13%/12% ncdr)] and in ♂ [week 1 (8%)]. (↓) during recovery in ♀ [week 14 (11%), week 15 (9% ndr) and week 16 (10% ndr)]. ▪ (↓) bw gain in ♂ [week 1 (30%), week 2 (15%), week 3 (17%), week 6 (15% ndr), week 7 (25%), week 8 (33%)] and in ♀ [week 2 (26% ncdr), week 9 (75%) and week 11 (50% ndr)]. (↓ n.s.) total bw gain in ♂/♀ during treatment [weeks 1-13 (19%/ 26%)]. ▪ (↓) food conversion in ♂ [week 1 (20%), week 2 (11%), week 3 (13%) and week 8 (36%)]. Haematology: ▪ (↓) Hb in ♂ [week 4 (4% ndr) and week 12 (6% ndr)] and in ♀ [week 12 (5.3% ndr)]. ▪ (↑) red blood cells (RBC) in ♂ [week 4 (7%)]. ▪ (↓) packed cell volume (PCV) in ♂/♀ [week 4 (4% ndr/5% ndr) and week 12 (6% ncdr/5% ndr)]. ▪ (↓) mean corpuscular volume (MCV) in ♂ [week 4 (9%)]. ▪ (↓) mean corpuscular hemoglobin (MCH) in ♂/♀ [week 4 (9%/3% ndr)]. ▪ (↑) platelets in ♂/♀ on [week 12 (31% ndr/25%)]. Clinical chemistry: ▪ (↑) cholesterol in ♂/♀ [week 4 (101% ndr/106%), week 6 (126%/92%), week 12 (79% ndr/112%) and week 17 (22%/19% ndr)]. ▪ (↑) triglycerides in ♂/♀ [week 6 (95%/63%)]. ▪ (↑) total proteins in ♂/♀ [week 4 (9%/7%)] and in ♂ [(week 12 (4% ndr)]. ▪ (↑) albumin in ♂ [week 4 (5% ncdr)]. ▪ (↑) globulins in ♂/♀ on [week 4 (13%/14% ndr)] and in ♂ [week 12 (6% ndr)]. ▪ (↓) albumin/globulin ratio in ♂/♀ [week 4 (8% ndr/15% ndr) and week 12 (10% ndr/8% ndr)]. ▪ (↑) calcium in ♂ [week 4 (5% ndr) and week 12 (11% ndr)]. ▪ (↑) phosphate in ♂ [week 4 (11% ndr) and week 12 (21% ndr)]. ▪ (↑) sodium in ♂ [week 4 (2% ndr) and week 12 (2% ndr)]. ▪ (↓) creatinine in ♂/♀ on week 4 (13%/9% ncdr) and in ♀ [week 12 (9% ndr)]. ▪ (↓) total bilirubin in ♂ [week 12 (34% ndr)]. ▪ (↓) aspartate aminotransferase (AST) in ♂ [week 12 (27%)]. ▪ (↓) alanine aminotransferase (ALT) in ♂ [week 12 (20% ndr)]. ▪ (↓) alkaline phosphatase (AP) in ♂ [week 4 (24%) and week 12 (29%)]. ▪ (↑) LDH in ♂ [week 4 (54%)]. Organ weights: ▪ Liver: (↑) abs wt in ♂/♀ [week 13 (70%/70%)] and (↑) rel wt in in ♂/♀ [week 13 (102%/93%)] and week 17 (15% ndr)]. ▪ Brain: (↑) rel wt in ♂/♀ [week 13 (16%/13% ndr)] and in ♀ [week 17 (13%)]. ▪ Heart: (↑) rel wt in ♂/♀ [week 13 (17% ncdr/9% ndr)]. ▪ Kidneys: (↑) rel wt in ♂/♀ [week 13 (27%/13% ndr)] and in ♀ [week 17 (13% ndr)]. ▪ Testes: (↑) rel wt [week 13(18%)]. Macroscopic pathology: Liver: ▪ Accentuation hepatic architecture in 3/3♂ at interim kill (week 9). ▪ Liver dark in 1/3♂ ncdr and 3/5♀ at interim kill and 1/10♂ and 2/5♀ at terminal kill (week 13); ▪ Liver enlarged in 2/3♂ and 1/5♀ at interim kill and 1/10♂ at terminal kill.	**Anonymous 63** **(1982b)** Anonymous 64 (1988) (Additional thyroid gland examination) Crofts, M. (1981) (dietary concentrations) **(AS) B.6.3.2.1.1-01**

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results [Effects statistically significant and dose-related unless stated otherwise as not significant (n.s.) or not dose-related (ndr) or not clearly dose-related (ncdr)]	Reference
		Histopathology: Liver ▪ Centrilobular hepatocyte enlargement: 4/5♂ and 5/5♀ at interim kill, 9/11♂ and 10/10♀ at terminal kill. Thyroid ▪ Colloid depletion in thyroid in 3/3♂ and 4/5♀ at interim kill, 9/12♂ and 8/10♀ at terminal kill and 4/4♂ and 1/5♀ at recovery kill. ▪ Follicular cell size in thyroid in 3/3♂ and 4/5♀ at interim kill, 10/12♂ and 9/10♀ at terminal kill and 3/4♂ and 1/5♀ at recovery kill. ▪ Central resting follicles in 1/3♂ at interim kill, 1/12♂ and 2/10♀ at terminal kill and 1/4♂ at recovery kill.	
		9000 ppm (602♂/662♀ mg/kg bw/day)	
		Mortality: ▪ 1 male resulted dead as a result of errors during blood sampling and another one was sacrificed due to poor condition. Clinical signs: ▪ Hair loss in both sexes. Bodyweight and food consumption: ▪ (↓) bw in ♂/♀ [week 4 (7%/5% ndr), week 11 (9%/5% ndr), week 12 (8%/10% ncdr) and week 13 (8%/11% ncdr)] and in ♂ [week 1 (5%)]. (↓) during recovery in ♀ [week 16 (11% ndr)]. ▪ (↓) bw gain in ♂ [week 1 (20%), week 2 (10%), week 3 (10%), week 6 (19% ndr), week 8 (21%)] and in ♀ [week 2 (21% ncdr) and week 11 (62% ndr)]. (↓n.s.) total bw gain in ♂/♀ during treatment [weeks 1-13 (14%/17%)]. ▪ (↓) food conversion in ♂ [week 1 (10%) and week 2 (7%)]. Haematology: ▪ (↓) Hb in ♂ [week 4 (6% ndr) and week 12 (6% ndr)] in ♀ [week 12 (7% ndr)]. ▪ (↓) PCV in ♀ [week 4 (5% ndr) and week 12 (7% ndr)]. ▪ (↓) MCV in ♂ [week 4 (6%)]. ▪ (↓) MCH in ♂/♀ [week 4 (8%/4% ndr)]. Clinical chemistry: ▪ (↑) cholesterol in ♂/♀ [week 4 (104% ndr/72%), week 6 (79%/83%), week 12 (100% ndr/81%)]. ▪ (↑) triglycerides in ♂/♀ [week 6 (35%/51%)]. ▪ (↑) total proteins in ♂/♀ [week 4 (8%/6%) and in ♂ [(week 12 (6% ndr)]. ▪ (↑) albumin in ♂ [week 4 (4% ncdr)]. ▪ (↑) globulins in ♂/♀ on [week 4 (13%/17% ndr)] in ♂ [week 12 (7% ndr)]. ▪ (↓) albumin/globulin ratio in ♂/♀ [week 4 (8% ndr/23% ndr) and week 12 (10% ndr/23% ndr)]. ▪ (↑) calcium in ♂ [week 4 (5% ndr)]. ▪ (↑) sodium in ♂ [week 4 (2% ndr)]. ▪ (↓) creatinine in ♀ [week 12 (7% ndr)]. ▪ (↓) total bilirubin in ♂ [week 12(49% ndr)]. ▪ (↓) AST in ♂ [week 12 (24%)]. ▪ (↓) AP in ♂ [week 12 (27%)]. ▪ (↑) LDH in ♂ [week 4 (49%)]. Organ weights: ▪ Liver: (↑) abs wt in ♂/♀ [week 13 (45%/47%)] and (↑) rel wt in ♂/♀ [week 13 (61%/68%)] and in ♀ week 17 (16% ndr)]. ▪ Brain: (↑) rel wt in ♂/♀ [week 13 (11%/13% ndr)] and in ♀ [week 17 (12%)]. ▪ Heart: (↑) rel wt in ♂/♀ [week 13 (10% ncdr/8% ndr)]. ▪ Kidneys: (↑) rel wt in ♂/♀ [week 13 (22%/13% ndr)] and in ♀ [week 17 (17% ndr)]. Macroscopic pathology: Liver: ▪ Liver dark in 1/4 ncdr ♂ at interim kill.	

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results [Effects statistically significant and dose-related unless stated otherwise as not significant (n.s.) or not dose-related (ndr) or not clearly dose-related (ncdr)]	Reference
		Histopathology: Liver ▪ Centrilobular hepatocyte enlargement: 4/5♂ and 5/5♀ at interim kill, 11/11♂ and 10/10♀ at terminal kill. Thyroid: ▪ Colloid depletion in thyroid in 4/4♂ and 4/5♀ at interim kill, 10/10♂ and 10/10♀ at terminal kill and 2/4♂ and 1/5♀ at recovery kill. ▪ Follicular cell size in thyroid in 4/4♂ and 5/5♀ at interim kill, 10/10♂ and 10/10♀ at terminal kill and 2/4♂ and 1/5♀ at recovery kill. ▪ Central resting follicles in 1/5♀ at interim kill, 1/10♂ and 3/10♀ at terminal kill and 2/4♂ and 1/5♀ at recovery kill. **3000 ppm** (202♂/221♀ mg/kg bw/day) Clinical signs: ▪ Hair loss in both sexes. Bodyweight and food consumption: ▪ (↓) bw in ♂/♀ [week 11 (5%/5% ndr), week 12 (5%/7% ncdr)] and in ♀ [week 13 (7% ncdr)]. ▪ (↓) bw gain in ♀ [week 2 (21% ncdr) and week 11 (62% ndr)]. (↓n.s.) total bw gain in ♂/♀ during treatment [weeks 1-13 (4%/14%)]. Haematology: ▪ (↓) Hb in ♂/♀ [week 12 (4% ndr/3% ndr)]. ▪ (↓) PCV in ♀ [week 4 (2% ndr)]. Clinical chemistry: ▪ (↑) cholesterol in ♂/♀ [week 4 (82% ndr/48%), week 6 (53%/50%) and week 12 (82% ndr/39%)]. ▪ (↑) triglycerides in ♀ [week 6 (46%)]. ▪ (↑) total proteins in ♂ [week 4 (5%) and week 12 (6% ndr)]. ▪ (↑) albumin in ♂ [week 4 (3% ncdr)]. ▪ (↑) globulins in ♂/♀ on [week 4 (7%/10% ndr)] in ♂ [week 12 (7% ndr)]. ▪ (↓) albumin/globulin ratio in ♂/♀ [week 4 (8% ndr/15% ndr) and week 12 (10% ndr/15% ndr)]. ▪ (↓) creatinine in ♀ [week 12 (7% ndr)]. Organ weights: ▪ Liver: (↑) abs wt in ♂/♀ [week 13 (40%/28%)] and (↑) rel wt in ♂/♀ [week 13 (47%/40%)]. ▪ Brain: (↑) rel wt in ♀ [week 13 (12% ndr)]. ▪ Heart: (↑) rel wt in ♂/♀ [week 13 (10% ncdr/6% ndr)]. ▪ Kidneys: (↑) rel wt in ♂/♀ [week 13 (14%/13% ndr)]. Macroscopic pathology: Liver ▪ Accentuation hepatic architecture in 1/5♂ interim kill of doubtful toxicological relevance at this dose level and in 1/10♂ ndr at terminal kill. ▪ Liver dark in 1/5♂ ncdr at interim kill. Histopathology: Liver ▪ Centrilobular hepatocyte enlargement: 5/5♂ and 5/5♀ at interim kill, 10/10♂ and 10/10♀ at terminal kill. Thyroid ▪ Colloid depletion in thyroid in 5/5♂ and 4/5♀ at interim kill, 10/10♂ and 10/10♀ at terminal kill and 2/5♂ at recovery kill. ▪ Follicular cell size in thyroid in 5/5♂ and 4/5♀ at interim kill, 10/10♂ and 10/10♀ at terminal kill and 2/5♂ at recovery kill. ▪ Central resting follicles in 1/10♂ and 3/10♀ at terminal kill and 1/5♂ at recovery kill. **0 ppm** Mortality: ▪ 1 male resulted dead as a result of errors during blood sampling **NOAEL**: not achieved. **LOAEL**: <202♂/<221♀ mg/kg bw/day	

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results [Effects statistically significant and dose-related unless stated otherwise as not significant (n.s.) or not dose-related (ndr) or not clearly dose-related (ncdr)]	Reference
Oral 90-day dietary study in rat Method: broadly comparable to OECD 408 GLP: No (prior to GLP enforcement) **Study acceptable** Rat strain: Charles river CD Sprague Dawley 25 rats/sex/dose Another group of 10 rats/sex/dose for blood sampling purposes only. 5 rats/sex/dose for recovery period (week 19) *Guideline value for classification (90 day study):* *STOT RE 1 ≤ 10 mg/kg bw/day (90 day study)* *STOT RE 2 ≤ 100 mg/kg bw/day*	Purity: 99.1-99.6% Oral (diet) Doses of 0, 40, 400 and 4000 ppm equivalent to 0, 2.65/2.91, 26.2/29.3 and 265/292 mg/kg bw/day for ♂/♀ Parameters observed: mortality, clinical signs, bodyweight and food consumption, haematology, clinical chemistry, organ weights, urynalisis, macropathology and histopathology (additional liver histology examination)	Clinical signs: hair loss, swollen eyes and soiling around nose and eyes in both sexes and not related to test substance administration. **4000 ppm** (265♂/292♀ mg/kg bw/day) Bodyweight and food consumption: ▪ (↓) bw in ♀ [week 9 (7% ndr), 12 (8% ndr) and 13 (8% ndr)]. ▪ (↓) bw gain in ♂/♀ [week 2 (14%/19%)] and in ♀ [week 9 (57%) and 12 (78%)]. (↓n.s.) total bodyweight gain on weeks 1-13 [♂/♀ (8% ncdr/17%)]. ▪ (↓) food conversion in ♂ [week 2 (10%)] and in ♀ [week 5 (25% ndr)]. Haematology ▪ (↓) Hb in ♂/♀ [week 8 (3% ndr/4% ncdr) and week 12 (4% ndr/7%)] and in ♀ [week 4 (3% ndr)]. ▪ (↓) RBC in ♀ [week 4 (4% ndr)]. ▪ (↓) PCV in ♂ [week 8 (4%)] and in ♀ [week 4 (4% ncdr)]. ▪ (↓) MCH in ♂/♀ [week 12 (4%/6%)]. ▪ (↓) MHCH in ♀ [week 12 (5%)]. ▪ (↑) platelets in ♂ [week 12 (13% ncdr)] and in ♀ [week 4 (19% ncdr) and week 19 (28%)]. Clinical chemistry: ▪ (↑) cholesterol in ♂/♀ [week 4 (24%/60%), week 8 (29%/51%) and week 12 (44%/102%)]. ▪ (↑) total proteins in ♂/♀ [week 4 (7%/8%)] and in ♂ [week 8 (6%) and week 12 (9%)]. ▪ (↑) albumin in ♂ [week 8 (10% ncdr) and week 12 (13%)] and in ♀ [week 4 (6%)]. ▪ (↑) globulins in ♂/♀ [week 4 (11%/10%)], in ♂ [week 12 (4% ncdr)] and in ♀ [week 8 (5% ndr)]. ▪ (↓) albumin/globulin ratio in ♂ [week 4 (7% ndr)] and (↑) in ♂/♀ [week 8 (10% ndr/7% ncdr)] and ♂ [(week 12 (10% ndr)]. ▪ (↑) calcium in ♂/♀ [week 4 (4% ndr/7%)] and in ♂ [week 8 (4% ndr)]. ▪ (↓) phosphate in ♂ [week 12 (9%)] and (↑) in ♀ [week 4 (23% ndr) and week 12 (10% ndr)]. ▪ (↓) sodium in ♀ week 8 (1% ndr) and week 12 (9.6% ndr)] and (↑) in ♀ [week 4 (7%)]. ▪ (↑) potassium in ♀ [week 4 (17% ndr)]. ▪ (↑) urea in ♂ [week 4 (9%) and 8 (13% ncdr)]. ▪ (↓) creatinine in ♂/♀ [week 4 (5% ncdr/11% ncdr) and 8 (6% ncdr/17%)]. ▪ (↑) glucose in ♂ [week 8 (15%)]. ▪ (↓) AST in ♂ [week 12 (20% ndr)] and in ♀ [week 8 (22%)]. ▪ (↓) ALT in ♂/♀ [week 8 (15%/32%)]. ▪ (↓) AP in ♂/♀ [week 4 (25% ndr/23% ndr)] and in ♀ [week 8 (24% ndr)]. ▪ (↑) LDH in ♂ [week 4 (38% ndr)] and in (↓) in ♀ [week 8 (35% ncdr)]. Organ weights: ▪ Liver: (↑) abs wt in ♂/♀ [week 13 (47%/41%)] and (↑) rel wt in ♂/♀ [week 13 (56%/52%)]. (↑) rel wt ♂ [week 19 (recovery) (20% ndr)]. ▪ Heart: (↑) rel wt in ♂ [week 13 (7%) and week 19 (16%)]. ▪ Kidneys: (↑) abs wt in ♂ [week 13 (11%) and week 19 (16% ndr)]. (↑) rel wt in ♂/♀ [week 13 (18% /13%) and week 19 (20%/10% ndr)]. ▪ Spleen: (↑) abs wt in ♀ [week 13 (17% ndr)] and (↑) rel wt in ♂/♀ [week 13 (13% ndr/ 24%)]. ▪ Testes: (↑) rel wt [week 13 (8%)]. Histopathology: Liver ▪ Centrilobular hepatocyte enlargement: 20/20♂and 20/20♀ at terminal kill (week13) appeared to be reversible after recovery (week 19). **400 ppm** (26.2♂/29.3♀ mg/kg bw/day) Haematology: ▪ (↓) Hb in ♂/♀ [week 12 (4% ndr/5%). ▪ (↓) MCH in ♀ [week 12 (5%)].	**Anonymous 74** **(1981)** Anonymous 75 (1983) (Additional examination of the liver histology) **(AS)** **B.6.3.2.1.1-02**

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results [Effects statistically significant and dose-related unless stated otherwise as not significant (n.s.) or not dose-related (ndr) or not clearly dose-related (ncdr)]	Reference
		<u>Clinical chemistry:</u> ▪ (↑) cholesterol in ♂/♀ [week 12 (18%/50%)] and in ♀ [week 4 (34%) and 8 (23%)]. ▪ (↑) total proteins in ♂ [week 4 (6%), week 8 (5%) and week 12 (3%)] ▪ (↑) albumin in ♂ [week 8 (10% ncdr) and week 12 (6%)]. ▪ (↑) globulins in ♂ [week 4 (5%)] and in ♀ [week 8 (5% ndr)]. ▪ (↑) albumin/globulin ratio in ♂ [week 8(10% ndr)]. ▪ (↑) calcium in ♂/♀ [week 4 (6% ndr/6%)] and in ♂ [week 8 (6% ndr)]. ▪ (↑) phosphate in ♀ [week 4 (31% ndr)]. ▪ (↓) sodium in ♀ [week 12 (6% ndr)] and (↑) in ♀ [week 4 (5%)]. ▪ (↑) potassium in ♀ [week 4 (19% ndr)]. ▪ (↑) urea in ♂ [week 8 (9% ncdr)]. ▪ (↑) glucose in ♂ [week 8 (13%)]. ▪ (↓) AST in ♀ [week 8 (19%)]. ▪ (↑) LDH in ♂ [week 4 (60% ndr)] and (↓) in ♀ [week 8 (33% ncdr)]. <u>Organ weights:</u> ▪ Liver: (↑) absolute weight in ♂/♀ [week 13 (11%/13%)] and (↑) relative weight in ♂/♀ [week 13 (13%/9%)]. ▪ Kidneys: (↑) relative weight in ♂ [week 13 (7% ncdr)]. ▪ Spleen: (↑) absolute weight in ♀ [week 13 (19% ndr)]. <u>Histopathology:</u> ▪ Centrilobular hepatocyte enlargement: 13/20♂and 0/20♀ at terminal kill (week13) appeared to be reversible after recovery (week 19). **40 ppm** (2.65♂/2.91♀ mg/kg bw/day) <u>Clinical chemistry:</u> ▪ (↑) total proteins in ♂ [week 8 (4%)]. ▪ (↑) albumin in ♂ [week 8 (8% ncdr)]. ▪ (↑) albumin/globulin ratio in ♂ [week 8(10% ndr)]. ▪ (↑) calcium in ♀ [week 4 (5%)]. ▪ (↑) phosphate in ♀ [week 4 (15% ndr)]. ▪ (↓) sodium in ♀ [week 12 (3% ndr)]. <u>Histopathology:</u> Liver ▪ Localized hepatocyte enlargement in 7/20♂ at terminal kill (week13) appeared to be reversible after recovery (week 19). After re-evaluation of histology the lesion was considered an artefact since no significant changes with respect to controls were observed. **NOAEL**: 2.65♂/2.91♀ mg/kg bw/day **LOAEL**: 26.2♂/29.3♀ mg/kg bw/day	
Oral 13-week neurotoxicity study in rats Method: OECD 424 GLP: Yes Rat strain: Crl:CD® (SD)IGS BR 10 rats/sex/dose **Study acceptable** *Guideline value for classification (90 day study):* *STOT RE 1 ≤ 10 mg/kg bw/day*	Purity: 99.7% Oral (diet) Doses of 0, 250, 1750, 12250 ppm equivalent to 0, 18.5/24.8, 131/168, 930.6/1222 mg/kg bw/day for ♂/♀ Parameters observed: mortality, clinical signs, bodyweight and food consumption, ophthalmoscopy,	**12250 ppm** (930.6♂/1222♀ mg/kg bw/day) <u>Clinical signs:</u> pink staining of various parts of the body including the muzzle, head, forelimbs, tail or whole body surface. Bedding (week 2 onwards) and faeces (week 1 onwards) were also stained pink. Pink is the colour of the test material. <u>Bodyweight and food consumption</u>: ▪ (↓) bw in ♀ [week 9 (7%), week 11 (7%), week 12 (8%) and week 13 (9%)]. ▪ (↓) bw in ♀ during FOB [week 13 (9%)]. ▪ (↓) bw gain in ♀ [week 0-13 (19%)]. <u>Motor activity</u> *(10 beams were set at 2 height levels in the cage (5 low and 5 high) to detect cage floor and rearing activity respectively)* ▪ (↓) high beam score in ♀ on week 4 [42 min (88%) and 48 min (70%)] and on week 8 [min 6 (31%), min 24 (73%) and min 30 (68%)]. ▪ (↓) total high beam score in ♀ on week 8 (42%). ▪ (↓) low beam score in ♀ on week 4 [42 min (72%)] and on week 8 [min 6 (26%), min 30 (57%), min 36 (73%] and (↑) in ♂ [week 8 (min 12 (25%)]. ▪ (↓) total low beam score in ♀ on week 8 (35%) and (↑) in ♂ on week 8 (24%).	**Anonymous 76** **(2006)** **(AS)** **B.6.7.1**

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results [Effects statistically significant and dose-related unless stated otherwise as not significant (n.s.) or not dose-related (ndr) or not clearly dose-related (ncdr)]	Reference
STOT RE 2 ≤ 100 mg/kg bw/day	functional observation battery (FOB), motor activity, mfacroscopic pathology, neurohistopathology, brain weights	Macropathology: ▪ Pink contents predominantly in the stomach, duodenum, caecum, colon, ileum and rectum (♂10/10 and ♀7/10). **1750 ppm** (131♂/168♀ mg/kg bw/day) Motor activity: ▪ (↓) low beam score in ♀ on week 4 [42 min (51%)] and on week 8 [30 min (51%)] and (↑) in ♂ on week 8 [min 12 (52%)]. ▪ (↑) total low beam score in ♂ on week 8 (56%). **NOAEL general toxicity**: >930.8♂ and 168♀ mg/kg bw/day **LOAEL**: not achieved♂ and 1222♀ mg/kg bw/day **NOAEL neurotoxicity:** >930.8♂ and >1222♀ mg/kg bw/day	
Oral 90-day dietary study in mice Method: OECD 408 GLP: No (prior to GLP enforcement) Mice strain: CD-1 20 mice/sex/dose (main group) treated during 90 days and two additional supplementary groups of 10 mice/sex/dose used for clinical chemistry on weeks 4 and 12 respectively **Study acceptable** Deficiencies: functional observational battery not performed. Weight of thymus, and uterus not determined. Reticulocyte count was not measured. *Guideline value for classification (90 day study):* *STOT RE 1 ≤ 10 mg/kg bw/day* *STOT RE 2 ≤ 100 mg/kg bw/day*	Purity: not stated Oral (diet) Doses of 0, 200, 1000 and 5000 equivalent to 0, 30.3/35.2, 151.4/176.5 and 757.1/884.9 mg/kg bw/day for ♂/♀ Parameters observed: mortality, clinical signs, bodyweight, water and food consumption, haematology, clinical chemistry, organ weights, urinalysis, macropathology and histopathology	Mortality: Five mice died: 3 in treatment group (2 males and 1 female at 200 ppm) and 2 in supplementary group (1 female at control and 1 female at 5000 ppm). No deaths were associated with treatment Clinical signs: Swelling of the penis associated with bleeding, soiling and inflammation, and scarring of the penis and the tissues at the base of the penis occured with no dose-relationship at all dose levels including controls and were not considered related to treatment. In females, apart from injury to the eye due to orbital sinus bleeding at 5000 ppm, no abnormalities were detected. **5000 ppm** (757.1♂/884.9♀ mg/kg bw/day) Haematology: ▪ (↓) Hb in ♂ [day 29 (3% ncdr) not seen on day 85]. ▪ (↑) platelets in ♀ [day 36 (24% ndr) not seen on day 92]. Clinical chemistry: ▪ (↑) triglycerides in ♂ [day 86 (152%)] and in ♀ [day 37 (34% ndr) not seen on day 93]. ▪ (↑) proteins in ♀ [day 37 (15% ndr) not seen on day 93]. ▪ (↑) albumin/globulin ratio in ♂ [day 30 (10%) not seen on day 93]. ▪ (↓) calcium in ♂ [day 86 (5%)] and (↑) in ♀ [day 93 (12%)]. ▪ (↑) phosphate in ♂ [day 86 (15%)] and in ♀ [day 37 (13% ndr) and 93 (22%)]. ▪ (↓) sodium in ♀ [day 37 (2% ndr) not seen on day 93]. ▪ (↑) potassium in ♂ [day 86 (9% ndr)] ▪ (↑) urea in ♂ [day 86 (14%)] and in ♀ [day 37 (17%) not seen on day 93]. ▪ (↓) creatinine in ♀ [day 37 (10% ndr) and 93 (9% ndr)]. Organ weights (end of treatment period): ▪ Liver: (↑) abs wt in ♂ (21%) and (↑) rel wt in ♂/♀ (16%/9%) ▪ Thyroid: (↑) abs wt in ♀ (22%) and (↑) rel wt in ♀ (28%). Histopathology: Liver: Centrilobular hepatocyte enlargement: 5/20♂. **1000 ppm** (151.4♂/176.5♀ mg/kg bw/day) Clinical chemistry: ▪ (↑) triglycerides in ♂ [day 86 (33%)]. ▪ (↑) proteins in ♀ [day 37 (22% ndr) not seen on day 93]. ▪ (↑) calcium in in ♀ [day 93 (8%)]. ▪ (↑) phosphate in ♀ [day 37 (14% ndr) and 93 (19%)]. ▪ (↓) sodium in ♀ [day 37 (2% ndr) not seen on day 93]. ▪ (↑) urea in ♀ [day 37 (14%) not seen on day 93]. Organ weights (end of treatment period): ▪ Liver: (↑) rel wt in ♂/♀ (6%/7%). ▪ Thyroid: (↑) abs wt in ♀ (19%) and (↑) rel wt in ♀ (23%). **NOAEL**: 151.4♂/>884.9♀ mg/kg bw/day **LOAEL**: 757.1♂/-♀ mg/kg bw/day	**Anonymous 77** **(1982)** **(AS)** **B.6.3.2.2.1**

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results [Effects statistically significant and dose-related unless stated otherwise as not significant (n.s.) or not dose-related (ndr) or not clearly dose-related (ncdr)]	Reference
Oral 13-week dietary study in dog Method: broadly comparable to OECD 409 GLP: No (prior to GLP enforcement) Dog strain: Beagle 4 dog/sex/dose **Study acceptable** Deficiencies: required 4 animals/sex/group were not achieved at termination sacrifice because two animals (1 from the high dose and 1 from the mid dose groups) were killed *in extremis* due to polyarteritis. *Guideline value for classification (90 day study):* *STOT RE 1 ≤ 10 mg/kg bw/day* *STOT RE 2 ≤ 100 mg/kg bw/day*	Purity: 99.7%-99.9% Oral (diet) Doses of 0, 3200, 8000 and 20000 ppm equivalent to 0, 80, 200 and 500 mg/kg bw/day Parameters observed: mortality, clinical signs, bodyweight and food consumption, haematology and coagulation, electrocardiography, clinical chemistry, ophthalmology, urinalysis, organ weights, macropathology and histopathology and microscopic examination of all tissues.	Mortality: 1 ♂at 20000 ppm and 1 ♀at 3200 ppm were killed moribund on week 6 probably due to polyarteritis developed during the study despite treatment with antibiotics. Clinical signs: Pink coloured faeces in all treated groups. Histopathology: Polyarteritis was observed as a spontaneous entity in 4♂ (1 control, 1 at 3200 ppm, 1 at 8000 ppm and 1 at 20000 ppm) and 2♀ (1 at 3200 ppm and 1 at 8000 ppm). The lesion, which varied in extent and severity, was observed in thymic, pulmonary, meningeal, intestinal and cardiac vessels as well as those associated with the sciatic nerve. In certain animals, the lesion was sufficiently advanced to give rise to clinical symptoms and was considered to be directly responsible for the moribund condition of 4 dogs. The aetiology of the condition remains uncertain, but there is no evidence to indicate that the condition was in any way related to or exacerbated by treatment with clofentezine. **20000 ppm** (500 mg/kg bw/day) Haematology: ▪ (↓) RBC in ♂ [pretest (12%) and day 29 (15% ndr)]. ▪ (↑) retics in ♀ [day 57 (>100%)]. ▪ (↑) platelets in ♂ [day 85 (65% ndr)]. ▪ (↑) WBC in ♂ [day 29 (60% ndr)]. ▪ (↓) neutrophiles in ♂ [day 85 (44% ndr)]. Clinical chemistry: ▪ (↑) triglycerides in ♂/♀ [day 58 (36%/37%)]. ▪ (↑) AP in ♀ [day 30 (46% ndr) and day 86 (53% ndr)]. Organ weights (end of treatment period): ▪ Liver: (↑n.s.) abs wt and rel wt in ♂ (34% and 13% both ndr) and (↑) abs wt and rel wt in ♀ (24% and 31%). **8000 ppm** (200 mg/kg bw/day) Haematology: ▪ (↓) neutrophiles in ♂ [day 85 (50% ndr)]. Clinical chemistry: ▪ (↑) AP in ♀ [day 30 (38% ndr) and day 86 (63% ndr)]. Organ weights (end of treatment period): ▪ Liver: (↑n.s.) abs wt and rel wt in ♂ (17% and 20% both ndr) and (↑) abs wt and rel wt in ♀ (21% and 26%). **3200 ppm** (80 mg/kg bw/day) Clinical chemistry: ▪ (↑) AP in ♀ [day 30 (94% ndr) and day 86 (44% ndr)]. Organ weights (end of treatment period): ▪ Liver: (↑n.s) abs wt and rel wt in ♀ (13% and 12%) and in ♂ (↑) abs wt (31% ndr) and (↑n.s) rel wt (20% ndr). **NOAEL**: not achieved. **LOAEL**: <80 mg/kg bw/day in ♂/♀.	**Anonymous 78** **(1981)** **(AS)** **B.6.3.2.3.1**
Oral 1-year dietary study in dog Method: comparable to OECD 452 GLP: No (prior to GLP enforcement) Dog strain: Beagle	Purity: 98.2% Oral (diet) Doses of 0, 50, 1000 and 20000 ppm equivalent to 0, 1.75/1.70, 33.2/38.8, 682.6/719.1 mg/kg bw/day for ♂/♀ Parameters	Mortality: No observed. Clinical signs: Reddish-pink coloration of faeces, hair and external skin at 20000 ppm due to the colour of the test material. **20000 ppm** (682.6♂/719.1♀ mg/kg bw/day) Bodyweight and food consumption: ▪ (↓) bw gain in ♂/♀ after 4 weeks (19%/22%). Haematology: ▪ (↓) Hb in ♂ [week 12 (9% ncdr)].	**Anonymous 79** **(1984)** **(AS)** **B.6.3.3.3.1**

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results [Effects statistically significant and dose-related unless stated otherwise as not significant (n.s.) or not dose-related (ndr) or not clearly dose-related (ncdr)]	Reference
6 dog/sex/dose **Study acceptable** *Guideline value for classification (1-year study):* *STOT RE 1 ≤ 2.5 mg/kg bw/day* *STOT RE 2 ≤ 25 mg/kg bw/day*	observed: mortality, clinical signs, bodyweight and food consumption, ophthalmoscopy haematology, electrocardiography and blood pressure, clinical chemistry, organ weights, urinalysis, macropathology and histopathology and microscopic examination of tissues.	▪ (↓) RBC in ♂ [week 4 (12% ncdr)]. ▪ (↓) PCV in ♂ [week 4 (7%), 8 (10%), 12 (15%) and 51 (10%)] and in ♀ [week 12 (16%)]. ▪ (↑) platelets in ♂/♀ [week 8 (45%/25%)] and in ♀ [week 12 (18%)]. ▪ (↑) MCHC (Mean corpuscular haemoglobin concentration) in ♂ [week 8 (7% ndr) and week 12 (6%)] and in ♀ [week 12 (10%)]. ▪ (↑) MCV in ♂ [week 4 (4% ndr)] and (↓) in ♂ [week 12 (14%)] and in ♀ [week 8 (6% ndr) and week 12 (15%)]. ▪ (↑) MCH in ♂ [week 4 (6% ndr)] and (↓) in ♀ [week 12 (8% ndr)]. <u>Clinical chemistry:</u> ▪ (↓) glucose in ♂/♀ [week 4 (10%/10%), week 12 (7%/10%) and week 26 (7%/9%)]. ▪ (↑) cholesterol in ♂/♀ [week 4 (34%/25%), week 8 (45%/45%), week 26 (32%/37%) and week 51 (46%/33% ncdr)] and in ♂ [week 12 (36%)]. ▪ (↑) triglycerides in ♂/♀ [week 4 (46%/54%) and week 12 (18% ndr/20% ndr)] and in ♂ [week 8 (21%) and week 26 (15% ndr)]. ▪ (↑) AP in ♂ [week 8 (36%) and week 12 (35%)]. <u>Organ weights (end of treatment period):</u> ▪ Liver: (↑) abs wt in ♂/♀ (22%/29%) and (↑n.s.) rel wt in ♂/♀ (12%/28%). ▪ Adrenals: (↑) abs wt in ♂ (19%) and (↑n.s.) rel wt in ♂ (9.6%). ▪ Thyroid: (↑) abs wt in ♀ (22% ncdr) and (↑n.s.) rel wt in ♀ (23%). <u>Histopathology:</u> ▪ Periportal hepatocytes with cytoplasmic eosinophilia in ♂ (minimal in 4/6) and ♀ (minimal in 4/6 and slight in 1/6). **1000 ppm** (33.2♂/38.81♀ mg/kg bw/day) <u>Haematology:</u> ▪ (↓) RBC in ♂ [week 4 (12% ncdr)]. ▪ (↓) PCV in ♂ [week 8 (8%)]. ▪ (↑) platelets in ♂/♀ [week 8 (18%/21%)]. ▪ (↑) MCHC in ♂ [week 8 (8% ndr). ▪ (↑) MCV in ♂ [week 4 (10% ndr)] and (↓) in ♂ [week 12 (8%)]. ▪ (↑) MCH in ♂ [week 4 (10% ndr)] and (↓) in ♀ [week 12 (8% ndr)]. <u>Clinical chemistry:</u> ▪ (↓) glucose in ♂/♀ [week 12 (4%/6%)] and in ♀ [week 4 (6%) and week 26 (8%)]. ▪ (↑) cholesterol in ♂ [week 26 (18%) and 51 (27%)] in ♀ [week 51 (32% ncdr)]. ▪ (↑) triglycerides in ♂/♀ [week 4 (27%/31%) and week 12 (21% ndr/19% ndr)] and in ♂ [week 26 (23% ndr)]. <u>Organ weights (end of treatment period):</u> ▪ Liver: (↑) absolute weight in ♀ (21%) and (↑n.s.) relative weight in ♀ (20%). <u>Histopathology:</u> ▪ Periportal hepatocytes with cytoplasmic eosinophilia in ♀ (slight in 2/6). <u>**50 ppm** (1.75♂/1.70♀ mg/kg bw/day)</u> <u>Haematology</u> ▪ (↓) RBC in ♂ [week 4 (8% ncdr)]. ▪ (↑) platelets in ♂ [week 8 (19%)]. ▪ (↑) MCV in ♂ [week 4 (4% ndr)]. ▪ (↑) MCH in ♂ [week 4 (6% ndr)]. <u>Clinical chemistry:</u> ▪ (↓) glucose in ♀ [week 12 (5%)]. **NOAEL**: 1.75♂/1.70♀ mg/kg bw/day. **LOAEL**: 33.2♂/38.8♀ mg/kg bw/day.	

Other studies relevant for STOT RE

Other long-term exposure studies, such as on carcinogenicity and reproductive toxicity, can also provide evidence of specific target organ toxicity that could be used in the assessment of classification.

Chapter 10.9: 2-year long term toxicity study in rats and 18-month long term toxicity study in mice.

Chapter 10.10: multigeneration study in rats, teratology study in rats and teratology study in rabbits.

These studies are properly summarized in the corresponding chapters. Effects observed in carcinogenicity and reproductive studies are included in the following section 10.12.1.

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

Studies in rats:

In a 17-day oral range finding study in rats (B.6.3.1.1.1) adverse effects in liver were observed at the highest tested dose level of 1280 mg/kg bw/day with cytoplasmic and nuclear staining intensity of hepatocytes in male livers (5/5 vs 0/9 in the control group) and significant increases in the absolute and relative weight of liver of both sexes. Cholesterol was significantly increased in females but dose-dependency was not clear. Effects at lower doses were focused on liver absolute and relative weights increases from 20 mg/kg bw/day in females and from 80 mg/kg bw/day in males. However, the toxicological significance of these variations is doubtful considering the magnitude of the increases (below 10% in many cases), the lack of a clear dose-relationship and the absence of histopathological findings associated to these increases.

1280 mg/kg bw/day is clearly above the extrapolated cut-off value for a 17-day study for STOT RE 2 (529.4 mg/kg bw/day). Consequently, effects at this dose cannot be regarded for STOT RE classification.

In a 21-day oral range finding study in rats (B.6.3.1.1.2) target organs were spleen at 30000 ppm (2768/2652 mg/kg bw/day) and liver from 20000 ppm (1880/1763 mg/kg bw/day) in males. Spleen histopathology in males revealed pale spleen and spleen pitted with a nodule at 2768 mg/kg bw/day. Small spleen in males at 914 mg/kg bw/day and 1880 mg/kg bw/day was not seen at the top dose level and consequently it is not considered toxicologically relevant. Discoloration/pallor liver was seen from 1880 mg/kg bw/day in males. No effects were seen in females. It has to be noted that organ weigths are not available.

The lowest tested dose level in this study (914/862 mg/kg bw/day) at which no effects were observed is clearly above the cut-off value for STOT RE 2 classification for a 21-day dose-repeated study (extrapolated value to 428.6 mg/kg bw/day). Consequently mentioned effects in liver and spleen at upper doses are not regarded for STOT RE classification.

In a 90-day oral dietary toxicity study in rats (B.6.3.2.1.1-01) target organs were liver and thyroid with effects from 3000 ppm (202/221 mg/kg bw/day).

The main effect in liver was the high incidence of centrilobular hepatocyte enlargement in both sexes from 3000 ppm. This histopathological finding was accompanied by other significant and dose-dependent effects in liver such as increased absolute and relative weight and increases in plasma cholesterol in both sexes and triglycerides in females. The significant increase in the level of triglycerides was observed in males from 9000 ppm (602/662 mg/kg bw/day). Additionally, at the highest dose level of 27000 ppm (1892/1992 mg/kg bw/day) it was also observed liver dark in both

sexes (not clearly dose-dependent in males) and incidence of liver enlarged and accentuation of hepatic architecture in males.

In thyroid effects from 202/221 mg/kg bw/day were colloid depletion, follicular cell size and central resting follicles in both sexes.

It has to be noted that 202/221 mg/kg bw/day was the lowest tested dose level and clearly above the cut-off value for STOT RE 2 classifications for a 90-day dose-repeated study (100 mg/kg bw/day). Consequently effects are not regarded for STOT RE classification.

In a 90-day oral short-term toxicity study in rats (B.6.3.2.1.1-02) the target organ was the liver with effects from 400 ppm (26.2/29.3 mg/kg bw/day).

Centrilobular hepatocyte enlargement was observed at the end of treatment period amongst male rats from 400 ppm and female rats dosed at 4000 ppm (265/292 mg/kg bw/day). Histologically, the cytoplasm of affected hepatocytes was increased in area and electron microscopy demonstrated an association with proliferation of smooth endoplasmic reticulum. These changes appeared to be reversible after 6 weeks of treatment. The absolute and relative weight liver weight was increased in both sexes from 400 ppm. It showed recovery (week 19) with the exception of a non-dose related increase in males at 4000 ppm. Clinical chemistry showed significant and dose-dependent increases in the plasma cholesterol level in both sexes from 400 ppm. The extent of the hypercholesterolemia increased during the latter half of the treatment period, particularly amongst females. The cholesterol levels of all treatment groups were comparable to those of controls after 4 and 6 weeks of treatment withdrawal showing a complete reversibility of the effect. The increased cholesterol and the significant increases in total protein and albumin and/or globulin may be associative.

These effects observed from 400 ppm (26.2/29.3 mg/kg bw/day) in liver are within the range concentration to classify a substance as STOT RE 2 (> 10 mg/kg bw/day and ≤100 mg/kg bw/day).

In a 13-week oral neurotoxicity toxicity study in rats (B.6.7.1) there were no effects showing target organ toxicity up to the highest tested dose level of 12250 ppm (930.6 mg/kg bw/day for males and 1222 mg/kg bw/day for females) clearly above the value for a 90-day study for STOT RE 2 (100 mg/kg bw/day). The only effects potentially associated to neurotoxicity were ramdom variations of motor activity observed in both sexes, mainly at 12250 ppm, regarded as unrelated to treatment.

It has to be noted that 930.6/1222 mg/kg bw/day is clearly above the cut-off value for STOT RE 2 classifications for a 90-day dose-repeated study (100 mg/kg bw/day).

In a 2-year long-term toxicity and carcinogenicity study in rats (see section 10.9) target organs were liver and thyroid at 400 ppm equivalent to 17.3 and 22.1 mg/kg bw/day for males and females respectively

Effects in male thyroid were manifested at 400 ppm in males by increased statistically significant and dose-dependent free thyroxine (T4) in month 27 and dose-trend significant agglomeration of colloid at interim sacrifice, terminal sacrifice and for the total no. of animals that was also pairwise significant for terminal sacrifices.

Effect in male liver at 400 ppm were clear regarding histopathology with centrilobular hepatocyte vacuolation, centrilobular hepatocyte enlargement, focal cyst degeneration of hepatocytes, fat deposits in non-specific distribution and focal hepatocyte necrosis. These effects that were accompanied by increased absolute liver weight in month 27 and relative liver weight in month 12 and 27.

Dose of 17.3 mg/kg bw/day at which effects in male liver and thyroid were observed is above the extrapolated cut-off value for a 2-year study for STOT RE 2 cut-off value (12.5 mg/kg bw/day).

In a multigeneration study in rats (B.6.6.1.1) it was observed at 400 ppm (equivalent to 36.1 mg/kg bw/day) in F1 males an increase in the relative liver weight (16%) associated with histopathology (increased incidence of minimal centrilobular hepatocyte enlargement).

F1 males were treated continuously to give F2A and F2B generations in a total period of 33 weeks. The extrapolated dose range for STOT RE 2 is C>3.9 mg/kg bw/day and C≤ 39 mg/kg bw/day. However, it has to be noted that F1 males come from F1A generation, exposed to the substance during lactation and gestation of F0 parental females. Accordingly, the extrapolated range for STOT RE 2 can be overestimated.

Although the dose of 36.1 mg/kg bw/day, at which liver damage was seen, is slightly below the extrapolated cut-off value for STOT RE 2 (39 mg/kg bw/day), this cut-off value is deemed overestimated.

In a teratology study in rats (B.6.6.2.1) there was an increase in the relative liver weight, when corrected for the uterine contents, associated with histopathology changes (staining and enlargement of centrilobular hepatocytes) at 3200 mg/kg bw/day. However, this effect was observed at a very high dose level clearly above the extrapolated value for STOT RE 2 considering a 2-week treatment (600 mg/kg bw/day).

Studies in mice:

In a 42-day oral palatability study in mice (B.6.3.1.2.1) liver was the only organ examined and considered the target organ in males from 5000 ppm (766/912 mg/kg bw/day). Liver histopathology revealed centrilobular hepatocytomegaly in males from 766 mg/kg bw/day. Areas of necrosis in liver were also seen in males at 79.5 and 5149 mg/kg bw/day but regarding the absence of effects at 766 mg/kg bw/day it is only considered of toxicological relevance the incidence at 5149 mg/kg bw/day. These findings in males were accompanied by increases in the absolute and relative weight from 766 mg/kg bw/day. It has to be noted that no effects were seen in females.

The lowest dose of 766 mg/kg bw/day at which liver toxicity was observed in males is clearly above the extrapolated cut-off value for a 42-day study for STOT RE 2 (214.3 mg/kg bw/day). Consequently effects are not regarded for STOT RE classification.

In a 90-day oral short-term toxicity study in mice (B.6.3.2.2.1) liver was the target organ. At the highest dose level of 5000 ppm (757.1/884.9 mg/kg bw/day) centrilobular hepatocyte enlargement was observed in males. This effect was accompanied at this dose level by significant increase in the relative weight of liver of both sexes (but >10% only in males), increase absolute liver weight in males and increase in the level of triglycerides in both sexes. At the immediate lower dose level of 1000 ppm (151.4/176.5 mg/kg bw/day) the effects in liver diminished and were not regarded as adverse with increases in triglycerides levels in males and in the relative weight of liver (lower than 10%) in both sexes.

It has to be noted that effects on thyroid observed from 1000 ppm in females were considered not relevant according to the data available on the mechanism of action (MoA) on thyroid tumours in rodents available in section 10.9 (Carcinogenicity). Clofentecine caused effects in thyroid through a known rodent-specific mechanism not relevant to humans *via* liver enzyme induction increasing thyroid hormome metabolism. The relative weight thyroid increase in female in this mice study could be due to this known MoA not relevant for humans. Besides, histopathology did not reveal findings on thyroid.

757.1 mg/kg bw/day corresponding to the lowest dose level at which clear liver effects were observed in males is above the cut-off value for STOT RE 2 classifications for a 90-day dose-repeated study (100 mg/kg bw/day). Consequently effects are not regarded for STOT RE classification.

In a 2-year carcinogenicity study in mice (B.6.5.2) liver was the target organ from 500 ppm equivalent to 50.7 and 56.9 mg/kg bw/day for males and females respectively.

Increased incidence of foci/areas of altered hepatocytes (eosinophilic) was noted from 500 ppm in females above historical controls. This effect could be related with the slightly increased absolute liver weight in females (18%) at 5000 ppm (543.4/557.1 mg/kg bw/day) not clearly dose-dependant. However this significant liver weight variation was not seen at the lower dose level of 500 ppm.

Effects in liver at 56.9 mg/kg bw/day in females are of doubtful toxicological relevance. Besides they are above the extrapolated cut-off value for STOT RE 2 classification for a 2-year carcinogeicity study (12.5 mg/kg bw/day). Consequently effects are not regarded for STOT RE classification.

Studies in dogs:

In a 17-day oral range-finding study in dogs (B.6.3.1.3.1) there were no effects showing target organ toxicity up to the highest tested dose level of 2000 mg/kg bw/day. This dose is clearly above the extrapolated cut-off value for a 17-day study for STOT RE 2 (529.4 mg/kg bw/day).

In a 28-day oral palatability study in dogs (B.6.3.1.3.2) there were no effects showing target organ toxicity up to the highest tested dose level of 1000 mg/kg bw/day. This dose is clearly above the extrapolated cut-off value for a 28-day study for STOT RE 2 (300 mg/kg bw/day).

In a 28-day oral range-finding study in dogs (B.6.3.1.3.3) liver was the target organ from 100 mg/kg bw/day with increases in the absolute and the relative weight of this organ in both sexes. However it has to be noted that control group and statistics were not available. The comparison was performed with the lowest tested dose level of 10 mg/kg bw/day. Besides, dose-relationship of this effect was only clear in females and the effect was not accompanied by changes in clinical chemistry (cholesterol o triglycerides). The toxicological relevance of this finding is doubtful.

100 mg/kg bw/day is in the range for classification for STOT RE 2 considering the extrapolated values for a 28-day toxicity study (30 mg/kg bw/day ≥ and ≤ 300 mg/kg bw/day).

In a 13-week oral short-term toxicity study in dog (B.6.3.2.3.1) liver was the target organ from 3200 ppm (80 mg/kg bw/day) with increase in the relative and absolute weight of this organ in both sexes. However, the level of statistical significance was only achieved in females from 8000 ppm (200 mg/kg bw/day) for both relative and absolute weight increases and in males for absolute weight occasionally at 3200 ppm but not at higher doses. Besides, dose-dependency was only clear in females. Clinical chemistry showed incrases in triglycerides on day 58 in both sexes at 20000 ppm (500 mg/kg bw/day) but not at the end of the study. Alkalyne phosphatase (AP) increases in females from 3200 ppm on day 30 or 86 were not dose-dependent. Taking into account the absence of histopatholgical findings and the exigous variations in clinical chemistry, the adversity of the liver weight variations is unconclusive below the highest tested dose level of 20000 ppm. The MSCA consideres that effects in liver weights at 3200 ppm and 8000 ppm are by themselves not relevant for STOT RE.

80 mg/kg bw/day is in the range for classification for STOT RE 2 considering the extrapolated values for a 90-day toxicity study (10 mg/kg bw/day ≥ and ≤ 100 mg/kg bw/day). However, effects in liver at this dose level are not regarded as adverse.

In an oral 1-year toxicity study in dog (B.6.3.3.3.1) liver was the target organ with clear adverse effects at 20000 ppm (682.6/719.1 mg/kg bw/day) manifested by periportal hepatocytes with cytoplasmic eosinophilia in both sexes accompanied by increases of absolute and relative weight of liver and increases in the cholesterol and triglycerides levels. Periportal hepatocytes with cytoplasmic eosinophilia was also observed at 1000 ppm (33.2/38.8 mg/kg bw/day) in females with less incidence and severity. Increases of liver absolute and relative weight in females was also observed at this dose level.

It has to be noted that dose of 33.2/38.8 mg/kg bw/day corresponding to the lowest tested dose level at which toxicity was seen is above the cut-off value for STOT RE 2 classifications for a 1-year toxicity study (25 mg/kg bw/day). Consequently effects are not regarded for STOT RE classification.

Studies in rabbits:

In a teratology study in rabbits (B.6.6.2.2) no effects showing target organ toxicity were seen up to the highest tested dose level of 3000 mg/kg bw/day. This dose is clearly above the extrapolated cut-off value for a 21-day treatment for STOT RE 2 (approximately 450 mg/kg bw/day).

10.12.2 Comparison with the CLP criteria

Classification for repeated dose toxicity depends on the type of effects and the dose at which the effects are observed. The CLP criteria state that STOT RE is assigned on the basis of findings of ‘significant’ or ‘severe’ toxicity. In this context ‘significant’ means changes which clearly indicate functional disturbance or morphological changes which are toxicologically relevant. ‘Severe’ effects are generally more profound or serious than ‘significant’ effects and are of a considerably adverse nature which significantly impact on health.

Table 45: Summary table of relevant effect for STOT RE classification

Dose levels and duration of exposure	Effest relevant for STOT RE [Effects statistically significantly and dose-related unless stated otherwise as not significant (n.s.) or not dose-related (ndr)/ncdr (not clearly dose-related)]	Reference
Oral 17-day range-finding study in rats Doses: 0, 5, 20, 80, 320 and 1280 mg/kg bw/day daily for 17 days	**STOT RE 2 (≤ 529.4 mg/kg bw/day)** 320 mg/kg bw/day ▪ Liver: (↑) absolute weight in ♀ (16%) and (↑) relative weight in ♂/♀ (7% n.s./10% ncdr). 80 mg/kg bw/day Organ weights: ▪ Liver: (↑n.s.) absolute weight in ♀ (14%) and (↑) relative weight in ♂/♀ (9% ncdr/12% ncdr). **MSCA opinion:** Liver absolute and relative weight increases were not accompanied by histopathological findings or clinical chemistry. The relative weight increases in both sexes are not clearly dose-related along dose levels and below 10% in males. **STOT RE 1 (≤ 52.9 mg/kg bw/day):** 20 mg/kg bw/day Organ weights: ▪ Liver: (↑) absolute (14%) and relative (9% ncdr) weight in ♀. **MSCA opinion**: liver absolute and relative weight increases only occurred in females and were not accompanied by histopathological findings or clinical chemistry. The relative weight increase is not clear dose-related along all dose levels and below 10%.	**Anonymous 68 (1980)** **(AS) B.6.3.1.1.1**

Dose levels and duration of exposure	Effest relevant for STOT RE [Effects statistically significantly and dose-related unless stated otherwise as not significant (n.s.) or not dose-related (ndr)/ncdr (not clearly dose-related)]	Reference
Oral 90-day dietary study in rat Doses of 0, 40, 400 and 4000 equivalent to 0, 2.65/2.91, 26.2/29.3 and 265/292 mg/kg bw/day for ♂/♀	**STOT RE 2 (≤ 100 mg/kg bw/day):** 400 ppm (26.2♂/29.3♀ mg/kg bw/day) Clinical chemistry: ▪ (↑) cholesterol in ♂/♀ [week 12 (18%/50%)] and in ♀ [week 4 (34%) and 8 (23%)]. Organ weights: ▪ Liver: (↑) absolute weight in ♂/♀ [week 13 (11%/13%)] and (↑) relative weight in ♂/♀ [week 13 (13%/9%)]. Histopathology: ▪ Centrilobular hepatocyte enlargement: 13/20♂ (reversible) **MSCA opinion**: effects relevant for STOT RE. Besides, it has to be noted that there are no data available for the interval from this dose level (26.2♂/29.3♀ mg/kg bw/day) up to the cut-off value for STOT RE (100 mg/kg bw/day)	**Anonymous 74 (1981)** **Anonymous 75 (1983) (Additional examination of the liver histology)** **(AS) B.6.3.2.1.1-02**
Oral 28-day range-finding study in dog Doses of 200, 2000 and 20000 ppm equivalent to 10, 100 and 1000 mg/kg bw/day (no control group)	**STOT RE 2 (≤ 300 mg/kg bw/day):** 2000 ppm (100 mg/kg bw/day) ▪ Increase in ♂/♀ in the liver absolute (38%/7%) and relative weight (34%/21%) with respect to the lowest dose but only clearly dose-related in ♀. **MSCA opinion**: no statistics performed and no control group available. The changes were not accompanied by clinical chemistry changes or histopathological findings. The toxicological relevance is doubtful.	**Anonymous 73** **(1983)** **(AS) B.6.3.1.3.3**
Oral 13-week dietary study in dog Doses of 0, 3200, 8000 and 20000 ppm equivalent to 0, 80, 200 and 500 mg/kg bw/day	**STOT RE 2 (≤ 100 mg/kg bw/day and > 10 mg/kg bw/day):** 3200 ppm (80 mg/kg bw/day) Clinical chemistry: ▪ (↑) AP in ♀ [day 30 (94% ndr) and day 86 (44% ndr)]. Organ weights (end of treatment period): ▪ Liver: in ♂ (↑) abs wt (31% ndr) and (↑n.s) rel wt (20% ndr) and in ♀ (↑n.s) abs wt and rel wt (13% and 12%). **MSCA opinion:** increases in AP are not dose-related. Increases in the absolute and relative weights of liver were above 10% but not significant and dose-dependent in females. Besides, these effects were not accompanied by histopathological findings.	**Anonymous 78** **(1981)** **(AS) B.6.3.2.3.1**
Multigeneration study in rats F1 treatement for 33 weeks Doses: $F_{1\ males}$→ F2A and F2B: 36.1 mg/kg bw/day	**STOT RE 2 (≤ 39 mg/kg bw/day and > 3.9 mg/kg bw/day):** Organ weights: ▪ ↑ Relative liver weight (16%) in ♂. Histopathology ▪ Increased incidence of minimal centrilobular hepatocyte enlargement in ♂ (4/10 *vs* 0/10 in controls). **MSCA opinion:** effects are of doubtful toxicological relevance. Extrapolated value for STOT RE 2 of 39 mg/kg bw/day considering a treatment of 33 weeks is overestimated since F1 males come from F1A generation, previously exposed to test substance during gestation and lactation of F0 parental females. Besides, it has to be taken into account that the severity of centrilobular hepatocyte enlargement was minimal.	**Anonymous 61** **(1984)** **(AS) B.6.6.1.1**

10.12.3 Conclusion on classification and labelling for STOT RE

The main target organ was liver according to the results of the available studies. However, the only effects deemed relevant were found in the oral 90-day dietary study in rat with centrilobular hepatocyte enlargement in males and increses in the abosulte and relative weights of liver and in the level of cholesterol at 26.2/29.3 mg/kg bw/day in both sexes. Effects were below guidance value for STOT RE 2 (100 mg/kg bw/day) classification. Besides, it has to be noted that no more doses were tested from 26.2/29.3 mg/kg bw/day to the limit dose of 100 mg/kg bw/day since the following tested dose level in the study was 265/292 mg/kg bw/day. It could be acceptable that the severity of the effects in liver can increase from 26.2/29.3 mg/kg bw/day onwards. Taking into account the observed effects in liver at this dose level and the lack of data at higher doses below cut-off value for STOT

RE 2, the MSCA considers that there is some uncertaninty on the potential incidence of clofentezine causing adverse effects in liver. However, the weight of the evidence based on the whole available information on all studies in several species indicate that clofentecine does not cause liver toxicity at dose levels below guidance values for STOT RE classification. Consequently, STOT RE classification is not proposed.

10.13 Aspiration hazard

Regarding the available data for the toxicity of clofentezine included in this dossier besides the physicochemical properties of the active substance it does not seem to pose an aspiration toxicity hazard to humans. There are no data in humans indicating evidence of this toxicity and clofentezine is a solid organic substance but not a hydrocarbon.

The MSCA is of the opinion, with the current data available on clofentezine, that classification due to aspiration hazard is not required.

11 EVALUATION OF ENVIRONMENTAL HAZARDS

Clofentezine is an acaricidal active substance considered under Directive 91/414/EEC (subsequently Regulation 1107/2009) for representative use as a foliar spray as a mite growth regulator on pome fruits, grapes, strawberries and ornamentals. Available environmental fate and ecotoxicology studies have been considered and summarised in the original RAR and the renewal of approval dossier.

The key information pertinent to determining the environmental hazard classification for clofentezine is presented below. Unless otherwise stated, these studies were conducted in accordance with GLP and the validity criteria of the representative test guideline, if applicable. Full robust summaries of these studies are presented in Annex 1 to this dossier.

11.1 Rapid degradability of organic substances

Clofentezine is considered not readily biodegradable. It is hydrolytically stable at pH 4, but degrades under neutral and alkaline conditions to primary degradates. Degradation was also observed in natural surface water to primary degradates, with low mineralisation (max. 10.8%). In natural water/sediment systems, clofentezine degraded to primary metabolites and carbon dioxide (max. 42.6%). Degradation also occurred under photolytic conditions to primary degradates, with no mineralisation. Volatilisation of clofentezine was found to be minimal from soil and leaf surfaces.

Table 46: Summary of relevant information on rapid degradability

Method	Results	Remarks	Reference
Ready biodegradation OECD guideline 301B (revised 1992)	Clofentezine: 12% CO_2 after 28 days ”	Results indicate Clofentezine is not readily biodegradable	Clarke, N. (2001)
Hydrolisis Kinetics of hydrolysis of NC 21314 (Clofentezine) under acid, neutral and acidic conditions	Clofentezine: DT_{50} (22ºC) 248.8 at pH 5 34.4 at pH 7 4.3 at pH 9 DT_{50} (38ºC) 49.8 at pH 5 5.1 at pH 7	Clofentezine could no have been fully dissolved. See Peer Review and conclusion obtained after PRAPeR Expert Meeting 62 (13-15 January 2009). Due to methodological concerns the study is considered supplementary information	Kelly, (1985a)
Characterization of hydrolysis products of clofentezinee EPA Guidelines Series 161-1m 1982 and BBA Guidelines (Merkblatt No. 55 part I)	Major hydrolysis product: AE C593600 (45.1% AR at pH9). This metabolite was further hydrolized to 2-chlorobenzonitrile (AE F023666) and 2-chlorobenzonitril (AE F092117) which were both present at <10% AR in all buffer solution.	The acceptability of this study was discussed at PRAPer expert meeting 62, point 4.3. Conclusions of the study was finally accepted by EFSA. The conclusions of the study were finally accepted and by EFSA and published in EFSA scientific report (200), but the study as considered supplementary information.	Smith and Kelly, (1985)

Method	Results	Remarks	Reference
Hydrolysis at three different pH values OECD Guideline 111 (1982)	DT_{50} (25ºC and 35ºC) <1.1 days at pH 7 Hydrolysis products: 2-Chlrobenzoic acid which rapidly hydrolized to 2-Chlorobenzonitrile and 2-chlorobenzamide. Clofentezine may be considered as hydrolytically stable under environmentally relevant acidic conditions and hydrolytically unstable under alkaline conditions. Under neutral conditions hydrolysis is very rapid.	As the study only tested hydrolysis at pH 7, and only two temperatures were tested for pH 7, the study was considered supplementary information.	Van der Gauuw, (2001)
Hydrolysis as a function of pH. OECD guideline 111 (April 2004)	Clofentezine: DT_{50} (h) pH 4: stable. pH 7: 833, 517 and 34.2 hours at 20, 25 and 50ºC. pH 9: 58.8, 46.2 and 0.592 hours at 20, 25 and 50ºC Metabolites: DT_{50} (h) and DT_{90} (h AE C593600: 12.1 – 3,480, and 3,480 - >10,000 2-CBZ: >10,000 2´CBA: >10,000	Kinetic evaluation study by Spickermann (2016). Data analysed with CAKE version 3.2. Clofentezine is hydrolytically stable at pH 4 and hydrolytically unstable at pH 9.	Göcer, M. (2016) Spickermann, G. (2016)
Aerobic mineralisation. OECD guideline 309 (November 2004)	Clofentezine: DT_{50}: 5.6 to 7.2 days (at concentrations 4µg/L and 41.3 4µg/L respectively) DT_{00}: 18.5 to 24 days at concentrations 4µg/L and 41.3 4µg/L respectively). Max. 10.8% mineralisation after 30 days (low concentration).	In aerobic aquatic environment, clofentezine degrades rapidly to AE C593600, 2-CBA, 2-CBZ, carbon dioxide and low levels of unknown metabolites	Ilieva, D. (2016)

Method	Results	Remarks	Reference
Aerobic aquatic metabolism in water/sediment systems. OECD guideline 308 (April 2002)	DT_{50} (Taunton River and Weweantic River respectively): 16.5 and 37 days (total system) 3.73 and 3.21 days (water) 34.3 and 36 days (sediment). DT_{50} (modelling endpoints): 23 and 37 days (total system) 7.7 and 11.5 days (water) 34.3 and 36 days (sediment). Max. mineralisation: 46.2% Metabolites: DT_{50} 2-CBA: 13 days (Weweantic River)	Kinetic evaluation study by Spickermann (2016). Modelling endpoints were calculated and metabolites identified.	Turk, R. (2014) Spickermann,G. (2016)
Photodegradation in water. JMAFF test guidelines section 2-6-2 (November 2004) and SETAC section 10 (March 1995)	DT_{50} (pH 5 buffer) : 5.88 days (irradiated), 11.35 days (dark). DT_{50} (natural water): 9.56 days (irradiated), 22.32 days (dark). No mineralisation observed. Quantum yield was determined to be 7.97 x 10^{-5} molecules degraded/photon. DT_{50} values of 3.54 days (pH 5 buffer) and 5.99 days (natural water) calculated for Europe based on mean maximum summer sunlight at latitudes 30, 40 and 50ºN.	Study performed to GLP. Not performed to OECD guidelines, but considered reliable. No mineralisation observed. Photoegradation metabolistes were identified.	Brice, A. (2007)
Photolysis Calculation of the real half-lives aqueous photolysis using GCSOLAR	Clofentezine: DT_{50}: 2.67-5.63 days across spring, autumn and summer at 30 to 50ºN. Clofentezine was found to degrade under photolytic conditions	Not GLP – calculation only	Dean, G. (2007)

Method	Results	Remarks	Reference
Photodegradation of [14C]-Clofentezine in water under sunlight conditions.	DT_{50} < 7d (compared to the hydrolysis half-life in dark controls of >31 d). Major photolysis product was 2-chlorobenzonitrile (74.6% AR after 31 d). Other metabolites detected: 2-chlorobenzaldehyde, 2 chlorobenzamide and 2-chlorobenzoic acid, all present at <10% AR throughout and at similar levels in both light and dark controls. Total recovery of radioactivity from the photolysis samples was 95.0 ± 4.2%.	After discussion of this study at the PRAPER Expert Meeting 65 (13-15 January 2009), it was concluded that photolysis was unlikely to be a significant route of dissipation in most natural surface waters. However, the study was considered supplementary information and a data gap was identified.	Kelly1985b
Volatility from plant surfaces. BBA guidelines (part IV, 6-1, 1990)	Volatile losses over the 24 hr period were 1.1% - 1.8% AR from plant surfaces and 0.8 – 1.7 AR from the soil surface.	Clofentezine was not volatile from leaf or soil surfaces.	Van der Gaauw, A. (2001)

11.1.1 Ready biodegradability

Author(s); year: N. Clarke, 2001. **PREVIOUSLY evaluated in DAR 2005.**
Title: Clofentezine: assessment of ready biodegradability: CO_2 evolution test.
Guidelines: OECD 301B
GLP: Yes (certified laboratory)

Summary:

A study to address the data requirement for ready biodegradability (Clarke, 2001) was included in the submission for Annex I inclusion under Directive 91/414/EEC and was deemed acceptable following evaluation and peer review at EU level (2009). The study followed the OECD guideline 301B (revised 1992) and was conducted according to GLP. A summary is provided below, with a robust summary provided in Annex I to this dossier.

A sample of activated sewage sludge was collected from a sewage treatment works which had a predominantly domestic sewage and an inoculum cultured medium was prepared (final concentration of 30 mg suspended solid) in accordance with OECD test guidelines. Two vessels were treated with clofentezine to give a nominal concentration of 10 mg carbon/L. Five additional vessels were also prepared; two reference material (sodium benzoate) control vessels, two blank control vessels containing only inoculated cultured medium and a single toxicity control vessel containing both the clofentezine and sodium benzoate. Vessels were sealed and CO_2-free air was bubbled through the solution, which was stirred continuously by a magnetic stirrer. All vessels were connected to two traps containing sodium hydroxide to trap CO_2.

The test system was incubated at 21°C in the dark. Trap analysis for evolved CO_2 was performed on the first trap at regular intervals (17 sample intervals in total) for up to 29 days after treatment. The second trap was analysed at 0 and 29 DAT.

Findings:

Under the conditions of the test, clofentezine attained 12% degradation after 28 days, and therefore cannot be considered as readily biodegradable according to OECD criteria.

11.1.2 BOD_5/COD

No data available.

11.1.3 Hydrolysis

Three studies to address the data requirement of hydrolytic degradation (Kelly, 1985a, R-12520 (CA 7.2.1.1/01); Smith and Kelly, 1985, R-12522- (CA 7.2.1.1/02) and Van der Gaauw, 2001, R-13318 (CA 7.2.1.1/03)) were included in the submission for Annex I inclusion under Directive 91/414/EEC and were deemed acceptable following evaluation and peer review at EU level (2009).

Author(s): Kelly, 1985a. **DAR 2005.** Reference: B.8.2.1.1
Title: The kinetics of the hydrolysis of NC 21314 under acid, neutral and basic conditions.
Guidelines:
GLP: No

Summary:

The kinetics of hydrolysis of NC 21314 was studied in aqueous solution under acid, neutral and basic conditions at two temperatures and two concentrations. A first order rate of hydrolysis was observed under all conditions of pH, temperature and concentration studied.

In this study, the water solubility of clofentezine was determined by adding clofentezine dissolved in acetone to buffer and shaking in water bath for 16 hours at 22ºC. The solution was centrifuged and filtered. The total radioactivity in the filtrate was measured and taken to be the actual solubility of clofenezine (0.029 mg/L). The hydrolysis study procceded using concentrations of 48% and 88% of this value, 0.014 and 0.026 mg/L respectively.

Later the water solubility was determined in a much more rigorous way, with a shorter equilibration time, lower initial concentration and the filtrate being analysed chromatographically (Smith and Kelly 1985, Annex II A 2.6/0.1, DAR Page9). For this, the water solubility at pH5 was determined to be 2,52 µg/L and < 2 µg/L at pH 7 and 9.

According to the peer review and the conclusion obtained after PRAPeR Expert Meeting 62 (13-15 January 2009) in the point No 4.3, the clofentezine could not have been fully dissolved. Hence, the study was no longer considered realiable. Irrespective of this conclusion, it appears that the authors were able to determine rates of hydrolysis at different pH values and temperatures. However, characterization of the hydrolysis products from this study was investigated in a further hydrolysis study (Van der Gaauw, 2001, (KCA 7.2.1.1/03)) and *comparable results were obtained at pH 7".* Part of these results (pH 7 conditions) were used as complementary information of the subsequent studies (KCA 7.2.1.1/02 and KCA 7.2.1.1/03) and published in EFSA Scientific Report (2009), 269, 1-113.

Findings:

The hydrolysis rate did not vary significantly with concentration. Half lives at 22ºC were 248.8, 34.4, and 4.3 days at pH 5, 7 and 9 respectively, and 49.8 and 5.1 days at pH 5 and 7 (38ºC) respectively.

This study is no longer considered reliable (high concentrations exceeded 50% solubility and low mass balance, 49%). However, the characterization of the hydrolysis products from this study was

investigated in a further hydrolysis study (Smith and Kelly, 1985), which is considered acceptable, but is not required for the risk assessment.

The study is considered supplementary information.

Author(s): S. Smith, I.D. Kelly, 1985. **DAR 2005.** Reference: B.8.2.1.1
Title: Characterisation of the hydrolysis products of clofentezine in aqueous solution under acid, neutral and basic conditions.
Guidelines: US EPA pesticide assessment guidelines subdivision N (Oct 1982)
GLP: No

Summary:

The hydrolytic stability of clofentezine was studied according to EPA guidelines (Pesticide Assessment Guidelines, Subdivision N, Series 161-1, 1982) and BBA guidelines (Merkblatt No. 55, part 1). The study predated the requirement for GLP (1993), however it was adequately reported and was considered acceptable.

The measured concentrations of clofentezine were used to calculate the first order DT_{50} values. The characterisation of hydrolysis products was reported in a separate study following HPLC and confirmatory HPLC/GC-MS (Smith and Kelly, 1985). The major hydrolysis product was AE C593600, which accounted for a maximum of 45.1% AR at pH 9.18 and 22 ± 1°C. This metabolite was further hydrolysed to 2-chlorobenzonitrile (AE F023666) and 2-chlorobenzamide (AE F092117) which were both present at <10% AR in all buffer solutions. Total recovery was >98%AR in all samples.

Findings:

This study was previously submitted to the original DAR. Due to the low solubility of clofentezine, there were discrepancies in the acceptability of this study. It was commented by EFSA in the No 4(29) of the Reporting Table (rev.1-2 03.01.2008) that a clarification of the methodology used is needed since precipitation of the substance may have been the cause of the disappearance of the substance, rather than its degration. The RMS proposed to ask the Notifier in a point for clarification. Acceptance was discussed in the PRAPeR expert meeting 62 (15/01/2009) in the point No 4.3. However, after proving similar results were obtained in the subsequent study (Van der Gaauw, 2001, KCA 7.2.1.1/03), it was concluded that the information provided by the applicant was reasonable. These conclusions were finally accepted by EFSA and published in EFSA Scientific Report (2009), 269, 1-113. **This study is considered as supplementary information.**

In addition to the two studies just presented, another study conducted at below the water solubility, 2,52 µg/L (van der Gaauw, 2001, Annex II A 2.91/03, DAR Pages 13 and 322) in compliance with OECD 111 and GLP, was also conducted and evaluated by RMS.

Author(s): Van der Gaauw, 2001. DAR 2005. Reference: B.8.2.1.1
Title: [14C]-Clofentezine: hydrolysis at three different pH values.
Guidelines: OECD 111
GLP: Yes

Summary:

In this study, the hydrolysis of clofentezine was studied at a concentration of clofentezine below the water solubility, 2,52 µg/L, and was also evaluated by RMS.

The results from this study were completely in line with those from the Kelly 1985a study, with rates at pH7 in the range of 0.2 days at 38ºC to 1.4 days at 22ºC. Thus, on this occasion, the solubilisation of clofentezine does not appear to have influenced the kinetics of the rate of degradation.

Findings:

The study concluded that Clofentezine may be considered as hydrolytically stable under environmentally relevant acidic conditions and hydrolytically unstable under alkaline conditions (half life < 1 day at 25ºC). Under more neutral conditions, its hydrolysis is still very rapid.

This study is no longer considered appropriate since no main test was performed for pH 9 and only two temperatures were tested for pH 7, even though a DT_{50} of 2.4 hours was seen at 50°C. Previous conclusions have been summarized in this monograph.

The study is considered as supplementary information.

A new study (Göcer, 2016) was submitted for the EU review on the hydrolytic degradation of clofentezine. The study followed the OECD guideline 111 (April 2004) and was conducted to GLP. A kinetics assessment of these data (Spickermann, 2016f, R-34979b (CA 7.2.1.1/05) was performed in accordance with FOCUS (2006) guidance on the data generated from this study and submitted for the EU review. Summaries of both studies are presented below, with robust summaries presented in Annex I of this dossier.

Author(s): M. Göcer (2016). **Submitted for the purpose of renewal.** Reference: B.8.2.1.1
Title: [^{14}C]-Clofentezine hydrolysis as a function of pH**.**
Guidelines: OECD No. 111; 'Hydrolysis as a Function of pH'; Adopted 13 April 2004
GLP: Yes (certified laboratory)

Summary:

This study was provided by the applicant in order to comply with the new data requirements according to OECD 111.

The hydrolytic behaviour of [^{14}C]-clofentezine was studied at pH 4, 7 and 9 in aqueous solution at different temperatures. A Tier 1 preliminary test was performed at each pH and 50°C.

[^{14}C]-Clofentezine was stable at pH4, accounting for 99.4% AR after 168 hours. [^{14}C]-Clofentezine degraded rapidly at pH 7, accounting for 19.5% AR after 168 hours. [^{14}C]-Clofentezine degraded rapidly at pH 9, accounting for 3.1% AR after 5 hours.

A Tier 2 test was performed at pH 7 and 9 at 20, 25 and 50°C.

The metabolites AE C593600 (maximum occurrence with 93.8% AR at pH 9 and 25°C and after 504 hours), 2-CBZ (maximum occurrence with 27.5 % AR at pH 9 and 50°C and after 504 hours) and 2-CBA (maximum occurrence with 50.6 % at pH 9 and 50°C and after 99 hours) were each observed. The metabolite 2-CBN was not detected.

The DT_{50} values for the pH 7 samples were 828, 526 and 37.7 hours at 20, 25 and 50°C, respectively. The DT_{50} values for the pH 9 samples were 81.4, 62.8 and 0.574 hours at 20, 25 and 50°C, respectively. The DT_{50} values have been superseded by a new full FOCUS kinetics assessment of these data (Spickerman 2016).

Findings:

The DT_{50} values for the pH 7 samples were 828, 526 and 37.7 hours at 20, 25 and 50°C, respectively. The DT_{50} values for the pH 9 samples were 81.4, 62.8 and 0.574 hours at 20, 25 and 50°C, respectively. The DT_{50} values have been superseded by a new full FOCUS kinetics assessment of these data (Spickerman 2016).

This study was accepted by the RMS. Taking into account previous considerations from EFSA in the initial approval of the substance, the concentrations used in this study both in Tier 1 (0.5 mg/L) and in Tier 2 (0.3 mg/L) made its acceptability doubtful, according to the RMS, since the solubility of the substance is 2.52 ug/L. However, the high transformation of clofentezine into the products even when a complete hydrolysis is reached in the study period, and the low percentage of AR in non-resolved residues tend to indicate that loss of clofentezine is due to hydrolysis and not via precipitation. Thus, the study was considered valid.

Additionally, it should be noted that the mass balance of AR was around 90%. However, just three points data were under the lower limit of 90% of mass balance. Therefore, data were considered valid.

The kinetics of this study have been assessed according to FOCUS degradation kinetics guidance (2006). Results of DT50 values are obtained from the kinetic evaluation in the subsequent study (Spickermann, 2016f, KCA 7.2.1.1/05).

Author(s): G. Spickermann (2016f). **Submitted for the purpose of renewal.** Reference: B.8.2.1.1
Title: Kinetic evaluation of the decline of clofentezine and its metabolites observed in a hydrolysis study.
Guidelines: FOCUS (2006) EC Document Sanco/10058/2005 version 2.0.
GLP: No

Summary:

The hydrolytic degradation behaviour of clofentezine and its metabolites, AE C593600, 2-CBZ and 2-CBA at pH 4, 7 and 9 in water and 20°C, 25°C and 50°C, was investigated by kinetic analysis.

The data were analysed using the CAKE version 3.2[9] (2016) package according to guidance provided by FOCUS (2006). DT_{50} and DT_{90} values were calculated for comparison with relevant study triggers and persistence criteria.

The FOCUS (2006) flowcharts for calculating persistence/trigger and modelling endpoints have been followed. Each soil has been considered following the steps in the flowchart.

Findings:

The DT_{50} and DT_{90} values of clofentezine were shown to be in the range of 0.592 to 833 hours and 74.7 to 3,420 hours, respectively.

The DT_{50} and DT_{90} values of AE C593600 were shown to be in the range of 12.1 to >10,000 hours and 3,480 to >10,000 hours, respectively.

The DT_{50} and DT_{90} values of 2-CBZ were both shown to be >10,000 hours.

The DT_{50} values of 2-CBA were shown to be in the range of 2,300 to >10,000 hours and the DT_{90} value was >10,000 hours.

9 Fits generated by CAKE version 3.1; reports generated by CAKE version 3.2

11.1.4 Other convincing scientific evidence

No data available.

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

No data available.

11.1.4.2 Inherent and enhanced ready biodegradability tests

No data available.

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

Aerobic mineralisation

A new study (Ilieva, D. (2016) was submitted for the EU review on the aerobic mineralisation of clofentezine in surface water. The study followed OCED guideline 309 (November 2004) and was conducted to GLP. A summary is provided below, with a robust summary provided in Annex I of this dossier.

Author(s): D. Ilieva, 2016
Title: Aerobic mineralisation of [tetrazine-^{14}C(U)]-clofentezine in surface water. **NEW STUDY. Submitted with the purpose of renewal.**
Guidelines: OECD 309
GLP: Yes (certified laboratory)

Summary:

The degradation of clofentezine under aerobic conditions was investigated in one natural German surface water in the dark.

[Tetrazine-^{14}C]-clofentezine was applied to the surface water to give nominal test concentrations of 4.0 and 41.3 µg/L. The samples were incubated in the dark at 20 ± 2°C under constant bubbling of air through the water. Traps for organic volatiles and carbon dioxide were used.

Six samples were treated with [ring-U-^{14}C]-sodium benzoate at a concentration of 8.5 µg/L and incubated under test conditions for99 up to 7 days to verify biological activity in the test system. Four further samples were treated with [Tetrazine-^{14}C]-clofentezine to give a concentration of 41.3 µg/L and incubated under sterile conditions at 20 ± 2°C and in the dark for up to 2 days. Additionally, two blank controls were treated with the highest level of solvent dosed and used to verify pH and dissolved oxygen concentration of the test systems during the incubation period.

The mass balance in the water system with the low concentration ranged from 96.3% to 103.8% AR throughout the entire study. The mass balance in the water system with the high concentration ranged from 90.0 to 104.4% AR.

Findings:

CO_2 and organic volatiles were observed the low concentration test system in amounts up to 10.8% and 4.6% AR, respectively. In the high concentration test system CO_2 and organic volatiles were observed at 3.6 and 3.1% AR, respectively.

In conclusion, [^{14}C]-clofentezine degraded rapidly (DT_{50} of 5.6 ti 7.2 days in the 4.0 and 41.3 µg/L samples, respectively) to AE 593600, C-CBA, 2-CBZ, carbon dioxide and low levels of unknown metabolites.

Water/sediment

Regarding water/sediment system, two studies a laboratory study (Leake and Arnold, 1983c, R-12696 and a kinetic evaluation study (Jene, 2001, R-13301) were included in the submission for Annex I inclusion under Directive 91/414/EEC and were deemed acceptable following evaluation and peer review at EU level (2009). However, after a deeper revision, these studies are not accepted due to show low recovery of mass balance (Leake and Arnold, 1983c) and DT_{50} of clay loam water/sediment system cannot be obtained by a SFO kinetic model because do not show an acceptable fit (Jene, 2001), respectively.

In addition, two new studies (Turk, 2014, R-23966a and Spickermann, 2016g, R-23966b) were submitted for the EU reivew on the degradation of clofentezine in water/sediment systems. The study followed the OECD guideline 308 (April 2002) and was conducted to GLP. A kinetics assessment Spickerman, G (2016) was performed in accordance with FOCUS (2006) guidance on the data generated from this study. Results obtained by Turk, 2014 provide information about acidic water bodies of water/sediment systems. Additionally, these studies provide a worst case data. Therefore, these results were proposed as new endpoints (Tables 2.8.2-04). The major metabolite formed was 2-chlorobenzoic acid (2-CBA).

Summaries of both studies are presented below, with robust summaries presented in Annex 1 of this dossier.

Author(s): R. Turk, 2014
Title: [14C]-Clofentezine – Aerobic aquatic sediment metabolism. New study. Submitted with the purpose of renewal. Reference: B.8.2.2.3Report No: R-23966a
Guidelines: OECD Guideline 308. OPPTS Guideline 835.4300
GLP: Yes (certified laboratory)

Summary:

The degradation of clofentezine under aerobic conditions was investigated in two water sediment systems – Taunton River (sandy loam) and Weweantic River (sand). [Tetrazine-U-^{14}C] clofentezine was applied to give an nominal concentration of 0.3 mg/L.

The major degradation product for the total system was 2-CBA, with a maximum level at 26.7% AR at 58 DAT. A minor metabolite, AE C593600, was seen to occur, with maximum levels of 3.9% AR, and several minor unknowns were observed, none of which reached >5% AR at two consecutive time points.

Findings:

The DT_{50} values of clofentezine in aerobic water and the total system were 7.6 and 27 days respectively for the Taunton River system, and 15 and 28 days respectively for the Weweantic River system (assuming first order kinetics). The DT_{90} values of clofentezine in aerobic water and the total system were 25 and 89 days respectively for the Taunton River system, and 49 and 94 days respectively for the Weweantic River system (assuming first order kinetics). The DT_{50} and DT_{90} values have been superseded by a new full FOCUS kinetics assessment of these data (Spickerman, 2016).

Author(s): G. Spickermann, 2016g. **NEW STUDY. Submitted with the purpose of renewal.** Reference: B.8.2.2.4
Title: Kinetic evaluation of the decline of clofentezine observed in two aquatic sediment systems.
Guidelines: FOCUS (2006) EC Document Sanco/10058/2005 version 2.0
GLP: No

This study consisted in the kinetic evaluation of the data obtained in the study by Turk (2014), which was considered appropriate for calculation of both persistence and modelling endpoints.

The data from these studies were analysed using the CAKE v3.2[10] (2015) software package according to guidance provided by FOCUS (2006) based on level P-1 and M-1 kinetics (single compartment kinetics). DT_{50} and DT_{90} values were calculated for comparison with relevant study triggers and persistence criteria and separate DT_{50} values were calculated for use as modelling endpoints.

Findings:

The clofentezine persistence DT_{50} values in the total system ranged from 16.5 to 37 days and DT_{90} values ranged from 76.4 to 123 days. Modelling total system DT_{50} values ranged from 23 to 37 days, with a geometric mean of 29.2 days.

The clofentezine persistence DT_{50} values in the surface water ranged from 3.21 to 3.73 days and DT_{90} values ranged from 25.4 to 38.1 days. Modelling surface water DT_{50} values ranged from 7.7 to 11.5 days, with a geometric mean of 9.4 days.

The clofentezine persistence DT_{50} values in the sediment ranged from 34.3 to 36 days and DT_{90} values ranged from 114 to 120 days. Modelling surface water DT_{50} values also ranged from 34.3 to 36 days, with a geometric mean of 35.1 days.

The 2-CBA persistence DT_{50} value in the total Weweantic River system was 13 days and the DT_{90} value was 43.2 days. The modelling total system DT_{50} value was also 13 days.

A kinetics assessment was not performed for the water or sediment in the Weweantic River system or the Taunton River system as only low levels of 2-CBA at limited sampling intervals were observed.

Results from this kinetic evaluation indicate that clofentezine cannot be considered rapidly biodegradable according to these criteria.

11.1.4.4 Photochemical degradation

There are four studies available on photochemical degradation. Three studies were included in the submission of the revised dossier (March 2009).

The study by Brice, A (2007) was included in the submission of a revised dossier (March 2009) but was not included in the peer review at EU level (2009). The study followed JMAFF test guidelines (Section 2-6-2, November 2000) and SETAC procedures for assessing the environmental fate and ecotoxicity of pesticides (section 10, March 1995) and was conducted according to GLP. A second study (Dean, 2007) was also submitted in the EU review, and was a calculation based on the result of the first study. Summaries of both studies are provided below, with robust summaries provided in Annex I to this dossier. The third study (Kelly 1985b) was was considered as supplementary information.

10 Fits generated by CAKE version 3.1; reports generated by CAKE version 3.2

Author(s): A. Brice, 2007. **PREVIOUSLY SUBMITTED**
Reference: B.8.2.1.2
Title: [^{14}C]-Clofentezine: Photodegradation and quantum yield in water.
Guidelines: JMAFF test guidelines section 2-6-2 (24 November 2000)
SETAC procedures for assessing the environmental fate and ecotoxicity of pesticides, section 10 (March 1995).
GLP: Yes (certified laboratory)

Summary:

The photodegradation and quantum yield of clofentezine was investigated in aqueous buffered solution at pH 5 and natural water. [^{14}C]-Clofentezine was applied at a nominal application rate of 2 µg/L to test vessels (buffer and natural water) under sterile conditions and vessels were incubated for up to 15 DAT under continuous irradiation at 25 ± 2ºC. Control vessels were likewise treated and were incubated under continuous darkness for up to 15 DAT at 25 ± 2ºC.

Findings:

The DT50 values for clofentezine were determined to be 5.88 and 11.35 in the pH 5 buffer for irradiated and dark samples, respectively. The DT90 values for clofentezine were determined to be 19.54 and 37.71 in the pH 5 buffer for irradiated and dark samples, respectively.

The DT50 and DT90 values of clofentezine for Europe were estimated as 3.54 and 11.75 days, respectively, at 25°C.

This study has not been previously evaluated until now. The study has been carried out following the Guideline JMAFF. It has been provided by the applicant in order to comply with the data gap 4.4 opened on 13-15 January 2009 in the PRAPeR Expert Meeting 62 which was also considered a formal data gap in the published EFSA Scientific Report (2009), 269, 25-113.

The results obtained in this study have been proposed as new endpoints. Photolytic degradation of active substance and metabolites above 10%: DT50 = 5.88 days; Quantum yield of direct phototransformation in water at ∑>290 nm = 7.97x10-5 molecules degrades/photon.

Estimated DT50 values at 30ºN, 40ºN and 50ºN are proposed from the calculations obtained in the subsequent study (Dean, 2007, KCA 7.2.1.2/03).

Regarding metabolites, clofentezine degraded under direct photolytic conditions, in the pH 5 buffer to major metabolites 2-CBN and 2 CBZ, minor metabolite 2-CBA, other unknowns and no degradation to carbon dioxide occurred. In the absence of light, in the pH5 buffer, clofentezine degraded to major metabolites 2-CBN and AE C593600, minor metabolites 2-CBZ and 2-CBA and other unknowns. In natural water under light and dark conditions, clofentezine degraded to major metabolite 2-CBN, minor metabolites 2-CBZ, 2-CBA, AE C593600 and other unknows.

In buffer solutions, an unknown metabolite was found >10% ARin the irradiated and dark samples but it was not detected in natural waters, so it was not considered later.

Author(s): G. Dean, 2007. NEW STUDY. Submitted for the purpose of renewal. Reference: B.8.2.1.2
Title: Clofentezine (Apollo 50 SC) – Calculation of the real half-lives of aqueous photolysis using GCSOLAR.
Guidelines: None
GLP: No

Summary:

The GCSOLAR program was used to determine the real half-lives at three latitudes (30º N, 40º N and 50º N), and at three seasons (spring, summer and autumn).

Findings:

Clofentezine was found to degrade under photolytic conditions with a DT_{50} value of 3.54 days.

The DT_{50} values were found to be in the range of 2.67 to 5.63 days across the latitudes and seasons.

Author(s): Kelly, 1985b. **PREVIOUSLY SUBMITTED (new summary)**
Reference: B.8.2.1.2
Title: The photodegradation of [^{14}C]-Clofentezine in water under sunlight conditions.
Guidelines: None
GLP: No

Summary:

The aqueous photolysis of clofentezine was studied, not in accordance with any guideline or to GLP. However the study predated the requirement for GLP (1993), was adequately reported and was considered acceptable.

Clofentezine was observed to undergo relatively rapid photolysis, with the first order DT_{50} reported to be < 7d (compared to the hydrolysis half-life in dark controls of >31 d). The major photolysis product was 2-chlorobenzonitrile which accounted for 74.6% AR after 31 d. Other metabolites detected included 2-chlorobenzaldehyde, 2 chlorobenzamide and 2-chlorobenzoic acid, all present at <10% AR throughout and at similar levels in both light and dark controls. Total recovery of radioactivity from the photolysis samples was 95.0 ± 4.2%.

Findings:

According to EFSA Scientific Report (2009) 269, 26-113, this study was not considered reliable and the experts´meeting identified a formal data gap for a new photolysis study in water. However this data was was not considered essential to finalize the EU assessment. After the PRAPer Expert Meeting 62 (13-15 January 2009), it was concluded that photolysis was unlikely to be a significant route of dissipation in most natural surface waters, and it was considered that an additional photolysis study under controlled conditions would have added value at that point. However, the RMS proposed that if further assessment was required a full evaluation of photolysis with a new study should be performed to ensure that the assessment is based on the most appropriate information.

The study was considered as supplementary information.

Author: S. Heinicke (2016).
Title: [^{14}C]-Clofentezine phototransformation of [^{14}C]-Clofentezine – soil photolysis. ADAMA Irvita NV, Unpublished report No.: R-34977 ADAMA Irvita NV, Unpublished report No.: R-34977

Guideline: SETAC 1995 Procedures for assessing the environmental fate and ecotoxicity of pesticides

Summary:

The objective of the study was to determine the phototransformation of [^{14}C]-Clofentezine in soil-soil photoloysis. The photodegradation rate of [^{14}C]-Clofentezine was studied in one European soil with continuous irradiation that simulate natural sunlight conditions at 20 °C.

The study follow the Test Guideline "SETAC 1995 Procedures for assessing the environmental fate and ecotoxicity of pesticides" and in a broad manner "OECD Draft Documment: Phototransformation of chemicals on soil surfaces", the report have some deviations from the guides and there is some uncertainty associated to this data and it is not possible to conclude.

Clofentezine degraded steadily in the irradiated samples with 64.5% AR remaining at 333 HAT. One major metabolite, 2-CBN, was observed in the irradiated samples, accounting for a maximum values of 17.4% AR. All other metabolites in the irradiated samples accounted for < 3.0% AR. No notable degradation of clofentezine was observed in the dark control samples with 94.9% AR remaining at 333 HAT. All metabolites in the dark controls were present at < 2.0% AR.

Findings:

Clofentezine photodegraded steadily on a soil surface to 2-CBN, minor metabolites, bound residues and carbon dioxide.

The DT50 and DT90 values have been superseded by a new full FOCUS kinetics assessment of these data (Spickermann (2016a, R-34977b, CA 7.1.1.3/03).

This study consists of the kinetic evaluation of the decline of clofentezine and its metabolite 2-CBN (2-chlorobenzonitrile) observed in a soil photolysis study. ADAMA Irvita NV, Unpublished report No.: R-34977b

Author: G. Spickermann (2016a). NEW STUDY.
Title: Kinetic evaluation of the decline of clofentezine and its metabolite 2-CBN (2-chlorobenzonitrile) observed in a soil photolysis study. ADAMA Irvita NV, Unpublished report No.: R-34977b
Guidelines: FOCUS (2006)
GLP: NA

Summary:

The degradation behaviour of clofentezine and its metabolite, 2-CBN, on soil surfaces with continuous irradiation to simulate natural sunlight has been investigated in one study. The data from this study have been used to determine the irradiated soil half-life of clofentezine and 2-CBN.

Findings:

The clofentezine persistence/trigger DT50 and DT90 values were 1,210 and >10,000 hours, respectively.

The 2-CBN persistence/trigger DT50 and DT90 values were 1,380 and 4,590 hours, respectively.

The RMS reviewed the study and indicated that DT50 values were higher than the duration of the experiment. The last data point had to be removed due to the low AR in the experiment. After removing the last data point the last experiment days was 168 hours equivalent to 15 dyas of sunlight.

Metabolite CBN (2-chlorobenzonitrile) did not reach an equilibrium plateau and its concentration increased in last measure.

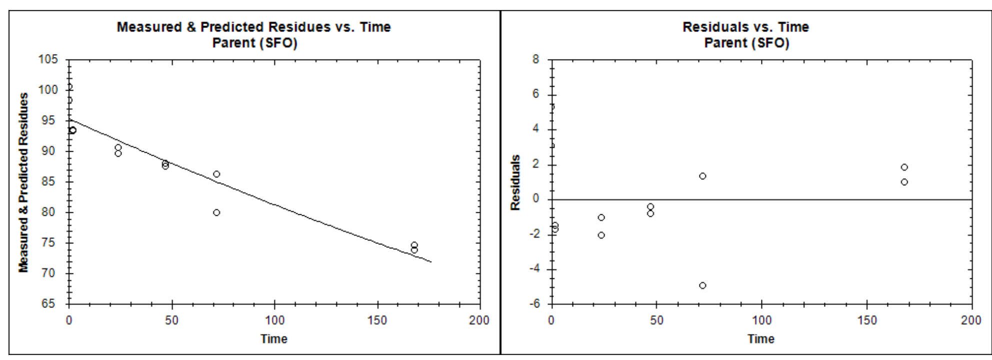
SFO was the kinetic calculation choosen by the RMS.

The statistics of persistence/trigger endpoints for clofentezine are presented below

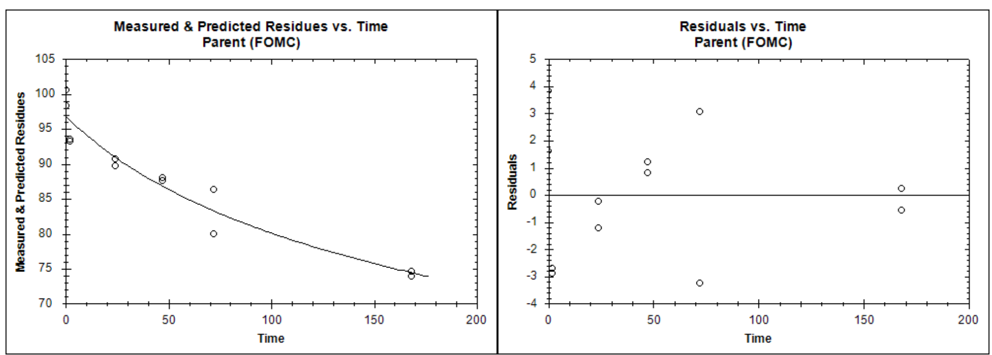
The statistics of persistence/trigger endpoints for clofentezine are presented below

Soil	Kinetic model	DT50 (d)	DT90 (d)	Chi^2 (%)	M (0)	Parameter	CI Lower	CI Upper	Prob <t
2.4 Klaumann 2016a	**SFO**	**18.1**	**>1000**	**1.95**	**95.31**	**$1.597e^{-3}$**	**$1.247e^{-3}$**	**0.002**	**$2.22e^{-6}$**
	FOMC	>1000	>1000	1.669	96.76	0.2205 73.53	-0.012 -50.697	0.453 198.057	0.0481 0.1384
	DFOP	19.2	>1000	0.642	99.5	0.0013 1.1206 0.9342	0.0010 -1.244 0.9004	0.002 3.485 0.968	$9.13e^{-6}$ 0.19 $7.42e^{-12}$
	HS	19.2	>1000	0.6242	99.5	$3.163e^{-2}$ $1.359e^{-3}$ 2.247	$1.301e^{-2}$ $1.064e^{-3}$ $9.378e^{-1}$	0.050 0.002 3.556	0.0052 $9.13e^{-6}$ 0.00494

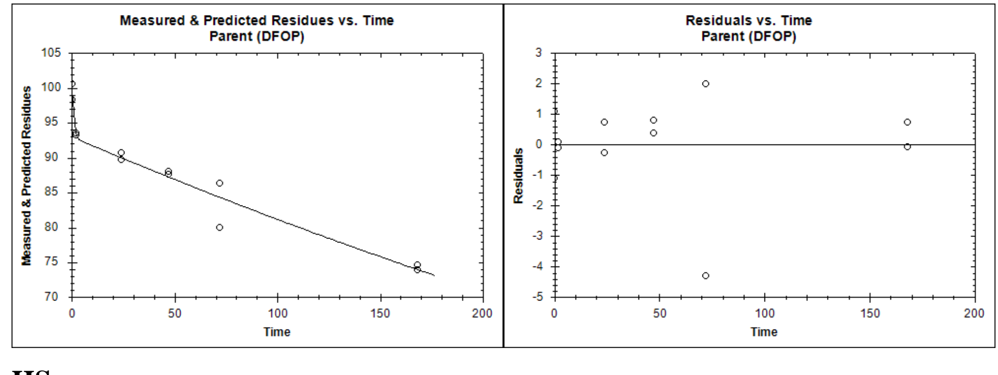
SFO



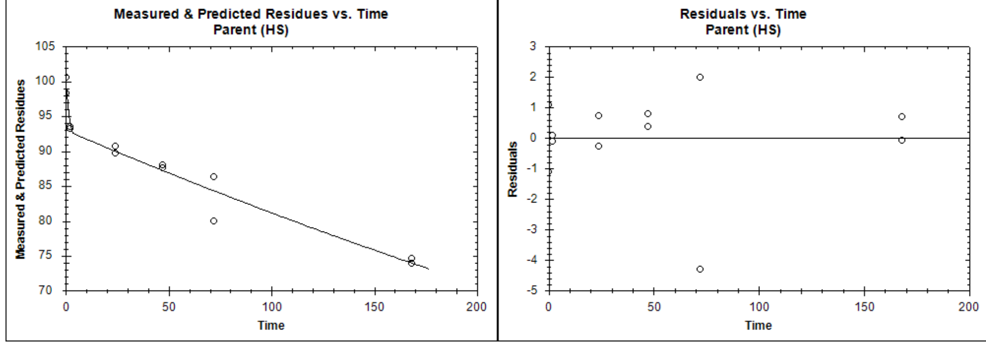
FOMC



DFOP



HS



The degradation behaviour of clofentezine and its metabolite, 2-CBN, on soil surfaces with continuous irradiation to simulate natural sunlight has been investigated in one study. The data from this study have been used to determine the irradiated soil half-life of clofentezine and 2-CBN.

The clofentezine persistence/trigger DT50 and DT90 values were 1,210 and >10,000 hours, respectively.

The 2-CBN persistence/trigger DT50 and DT90 values were 1,380 and 4,590 hours, respectively.

11.2 Environmental transformation of metals or inorganic metals compounds

Not applicable.

11.2.1 Summary of data/information on environmental transformation

Not applicable.

11.3 Environmental fate and other relevant information

11.3.1 Adsorption and desorption in soil

Adsorption/desorption *Adsorption/desorption properties of clofentezine in four European soils* *OECD 106 Test Guideline and GLP.*	Freundlich adsorptions coefficients corrected for organic carbon content ranged from 3753.7 cm^3/g to 17318.6 cm^3/g.	Adsorption coefficient values indicate chemical is slight to inmobile class. Adsorption partically irreversible.	Traub, M. (2016)

Two studies to address the data requirement for the adsorption desorption of clofentezine (Leake, 1989, R-12710, CA 7.1.3.1/01) and Mackenzie, 1999, R-13254, CA 7.1.3.1/02) were included in the submission and peer review at EU level (2009). Both studies are considered supportive information.

A new study (Traub, 2016, R-34978, CA 7.1.3.1.1/03) has been performed to provide experimental values for clofentezine and is summarized in this document.

Auithor: M. Traub (2016). NEW STUDY
Title. [Tetrazine-^{14}C(U)]-clofentezine determination of adsorption/desorption behaviour in four soils. ADAMA Irvita NV, Unpublished report No.: R-34978
Guidelines: OECD Guidelines 106 (1981)
GLP: Yes (certified laboratory)

Summary:

The objective of this study was to determine the adsorption/desorption properties of [tetrazine-^{14}C(U)]-clofentezine in four different European soils (LUFA 2.2, 2.3, 6S and Bulgarian soil BU). The study follows the Test Guideline “OECD 106” and GLP.

The adsorption test was performed at five test concentrations between 3.24 and 317 ng/mL in 0.01 M calcium chloride solution and soil mixtures (soil:solution ratio of 1:100). The samples were shaken for 24 hours. After which the supernatants were analysed by LSC. The Freundlich adsorption coefficients were calculated (KF, KFOC and 1/n).

Findings:

The adsorption KFOC values ranged from 3754 to 17319 mL/g and the 1/n values ranged from 0.7914 to 0.9739.

11.3.2 Volatilisation

Author(s): A. van der Gaauw (2001a)
Title Investigation of the volatilisation of [^{14}C]-clofentezine from soil and plant leaf surfaces. **PREVIOUSLY evaluated in DAR 2005.** Reference: B. 8.3.2/01
Report No: R-13319
Guidelines: BBA Guidelines (Part IV, 6-1, 1990)
GLP: Yes (certified laboratory)

Summary:

One study on the volatilisation properties of clofentezine [van der Gaauw, A. 2001] was included in the submission for Annex I inclusion under Directive 91/414/EEC and was deemed acceptable following evaluation and peer review at EU level (2009). A conclusion is provided below, with a robust summary provided in Annex I of this dossier.

To estimate the rate of volatility, the amounts of radiolabelled test item recovered at different sampling times were related to the initial applied amount. The mean mass balances from the soil samples ranged from 99.6% to 106.1% AR. The mean mass balances from the leaf samples ranged from 94.1% to 99.3% AR.

Findings:

Over the 24 h period volatile losses were reported to be 1.1% to 1.8% AR from plant surfaces, and 0.8% to 1.7% AR from the soil surface. This was within the analytical precision of the radioactivity measurements (± 2% AR), and therefore it was concluded that clofentezine was not volatile from leaf or soil surfaces.

11.4 Bioaccumulation

Table 47: Summary of relevant information on bioaccumulation

Method	Results	Remarks	Reference
Partition coefficient *n*-octanol/water; Clofentezine (purity 99.9%); OECD 107	log P_{OW} = 4.09 at 25°C	log P_{OW} = 4.09 indicates a potential for bioaccumulation. The study is accepted.	Bright, A.A.S &Stalker, A.M. 1990
Partition coefficient *n*-octanol/water; Clofentezine (purity 99.7%); OECD 117	log P_{OW} = 4.1 at 40°C (at pH 2.0, 7.0 and 9.0)	The study concluded that the partition coefficient is not affected by pH The study is accepted.	Mühlberger, B (2001)
Biconcentration test in bluegill sunfish. US EPA Guideline 560/B82-002 and EG10 E57; purity 99.2% (technical) and 99.6 % (radiochemical)	Clofentezine whole fish BCF: 248. Depuration whole fish (after 7 days): 93%	Due to several methodological concerns, the study was not accepted.	Anonymous (1987) B.9.2.2/04-05 Anonymous (1988) B.9.2.2/04-05

11.4.1 Estimated bioaccumulation

As experimental data are available, estimations of bioaccumulation potential are not required.

11.4.2 Measured partition coefficient and bioaccumulation test data

Author(s): Bright A.A.S and A.M.Stalker (1990)
Title: Clofentezine determination of the partition coefficient between octanol and water at 25ºC.
Guidelines: Method A.8 of the European Community (1984)1 based on the OECD Test Guideline 107 (1981).

Summary:

Clofentezine determination of the partition coefficient between octanol and water at 25ºC. The study followed the OECD Test Guideines 107 (1981).

Findings:

The partition coefficient (P) of clofentezine between- n-octanol and water at 25ºC was determined by a shake-flask method over a range of concentrations and found to be P=12411, $\log_{10} P = 4.09$

Author(s): Mühlberger B. 2001
Title: AE B084866 Partition coefficient N-Octanol/Water (HPLC Method).
Guidelines: OECD 117

Summary:

The partition coefficient (N-octanol/water) of AE B08486 was determined according to OECD 107 guideline and EEC-guideline 92/69/EWG a 8, HPLC-method. Therefore 9 neutral calibration substances were injected into a HPLC-system under the same analytical conditions (column temperature 40ºC) as the test substance. The eluent was adjusted on three different pH values (pH 2,7,9) to check pH dependence of the partition coefficient. A calibration curve was created by using the measure retention times (log K-values) and the known log Pow values of the calibration substances for linear regression.

The measured log K-value of the test substance was within the calibrated log Log K-range.

Findings:

The partition coefficient (N-octanol/water) and Log Pow for the three pH 2, 7 and 9 was 12589 and 4.1 respectively. Thus, it can be concluded that the partition coefficient was not affectd by pH and was 4.1.

Author(s): B.2.2/04-05; B.2.2/04-05. In DAR (2005). Annex II reference IIA 8.2.3
Title: Determination of the accumulation and elimination of [^{14}C]-Clofentezine in Bluegill sunfish (*Lepomis macrochirus*); Clofentezine: Bioconcentration of clofentezine in bluegill sunfish
Guidelines: US EPA 560/B-82-002, EG10, E57 (1982)
GLP: Yes

Summary:

This study amied to determine the accumulation and elimination of [14C]-Clofentezine in bluegill sunfish (*Lepomis macrochirus*).

Bluegill sunfish were exposed in a flow through test system to a nominal concentration of 0.03 mg/L of [^{14}C]-Clofentezine for a period of 14 days followed by a 7 day period of depuration in fresh water. The overall (whole body) BCF was 248x, with small amounts (3% or less) of clofentezine detected in all tissue types, equivalent to a concentration of approximately 0.2 µg/g in the whole fish. The majority was present in the viscera. After 7 days depuration, 93% of the accumulated residues had been eliminated from the whole body of the fish.

Findings:

A summary of the bioaccumulation of clofentezine in bluegill sunfish is presented in the table below.

Table 48: Bioaccumulation of clofentezine in bluegill sunfish

Fraction	Concentration of [14C] residues (mg/L)	BCF	Depuration (%)		
			1 day	3 days	7 days
Viscera	75.7	2294	68	94	96
Edible tissue	1.3	39	86	91	93
Non-edible tissue	2.4	73	64	73	78
Whole fish	8.2	248	70	90	83

The overall (whole body) BCF was 248x, with small amounts (3% or less) of clofentezine detected in all tissue types, equivalent to a concentration of approximately 0.2 µg/g in the whole fish. The majority was present in the viscera. After 7 days depuration, 93% of the accumulated residues had been eliminated from the whole body of the fish.

This study was not accepted by the RMS, who identified several concerns. First, a dynamic test system was used instead of the flow-through test recommended in OECD 305. As clofentezine is rapidly hydrolysed (<14 days), it could not be demonstrated that the total radioactivity measured in the water was the parent compound. Additional data was considered necessary to prove that concentration of the parent compound was mainained during the uptake phase.

Additionally, no data were available to calculate the lipid-normalised, kinetic bioconcentration factor (BCF_{KL}) and the Lipid-normalised growth corrected bio concentration factor (BCF_{GL}) according the requirements of the current guidelines (CD TG 305), and no justification was presented.

The mean measured concentration of clofentezine all over the time of the study was determined to be 0.033 mg/L. This concentration exceeds the water solubility of clofetezine (2.52 µg/L) and hence, in accordance to OECD TG 305, one of the validity criteria of the test is not met.

The fact that the water concentration of clofentezine is higher than its limit of solubility indicates that the bioavaible fraction of clofentezine by fish in water during the uptake phase is unknown. However, the BCF can be estimated only if constant dissolved exposure level is maintained during the uptake phase.

The limit of solubility of clofentezine (2.52 µg/L) could be considered as the maximum dissolved exposure concentration in water during the study. Based on this assumption, the BCF could be estimated using the residue level reached on fish at steady-state resulting on BCF = 3216 (8.2 mg as/Kg/2.52 µg/L)

In the opinion of the RMS, this estimation of BCF entail uncertainties as well as the value of BCF reported by B.9.2.2/04-05. However, the BCF = 3216 could be used as an unrealistic but worst-case approach for assessing the seodncary poisoning in birds and mammals eating fish.

The RMS considered that a new study should be submitted by applicant as confirmatory data, and requested a new bioconcentration study in fish in order to confirm the bioaccumulation potential of clofentezine.

The Log K_{OW} of clofentezine is 4.09, this is greater than the trigger value of 4 in the CLP Regulation and so indicates a potential for bioaccumulation. With regards to the bioaccumulation potential, the

study on the bluegill sunfish (Lepomis macrochirus) resulted in a bioconcentration factor (BCF) of 248. However, during the review, the RMS identified several concerns. First, a dynamic test was used insteadof the flow-through test recommended in OECD 305, and the number of volume replacement through each test chamber per day is not stated. Additionally, clofentezine is rapidly degraded by hydrolysis (<14 days), and is not likely to be found in a stable concentration over the exposure period. Although the level of radioactivy was stable throughout the test, this may have been adscrbibable to both the parent and metabolites formed. The RMS considered that additional data was needed to prove that the parent compound concentration in the test chamber is maintained during the uptake phase. Secondly, the mean measured concentration of clofentezine during the duration of the study was determined to be 0.033 mg/L. This concentration exceeds the water solubility of clofentezine (2.52 µg/L). Therefore, the experiment does not meet one of the validity criteria OECD 305 test guideline, which requires that the concentration of the test substance is below its limit of solubility in water. The BCF can be estimatd only if constant dissolved exposure level is maintained during the uptake phase.

According to the RMS, the BCF could be estimated using the solubility of clofentezine (2.52 µg/L) as the maximum dissolved exposure concentration in water during the study, and the residue level reached on fish at steady-state resulting on BCF =3216 (8.2 mg as/Kg / 2.52 µg/L). Both this estimation of the BCF and that reported by B.9.2.2/04-05 entails uncertainty. The BCF = 3216 culd be used as unrealistic but worst-case approach for assessing secondary poisoning in birds and mammals eating fish. Nevertheless this value is not robust enough and the RMS requested a new study to be submitted as confirmatory data.

In the absence of a reliable bioaccumulation study, the information of the octanol/water partition coefficient should be taken into account to evaluate the substance´s bioaccumulation potential. Already at the DAR and addenda in 2005, as well as at the EFSA Conclusion in 2009, the log Kow value of 4.09 was accepted. This log Kow can be considered to reflect the bioaccumulation potential of clofentezine.

11.5 Acute aquatic hazard

Table 49: Summary of relevant information on acute an chrohnic aquatic toxicity of clofentezine and its metabolites

Method	Species	Test substance (purity)	Results[a]	Remarks	Reference
Acute toxicity of Clofentezine					
Acute toxicity to fish The study was in line with OECD 203 Guideline	Rainbow trout (Oncorhyncus mykiss)	Clofentezine (98.6%) [^{14}C]-clofentezine, purity not stated.	96 hour LC_{50} >0.0146 mg/L, based on mean measured concentrations.	Since the toxicity endpoint was above the water solubility, the result cannot be considered reliable. Therefore the study is considered supplementary information. Supplementary	**Anonoymous (1986) B.9.2.1/01** 1986)

Method	Species	Test substance (purity)	Results[a]	Remarks	Reference
Acute toxicity of Clofentezine					
Acute toxicity to fish	Bluegill sunfish (*Lepomis macrochirus)*	Clofentezine, (99.8%)	96 hour LC_{50} >0.25 mg/L, based on mean measured concentrations.	Since the toxicity endpoint was above the water solubility, the result cannot be considered reliable. Therefore the study is considered supplementary information.	**Anonoymous (1981) B.9.2.1/02**
Acute toxicity to aquatic invertebrates Guideline: OECD 202 I and US EPA EG1 31: 5007-5009	Water flea (*Daphnia magna)*	Clofentezine, (99.8%)	RMS: 48 hour EC_{50} >0.001123 mg/L EC_{50} >0.00084 mg/L, based on mean measured concentrations.	Since the toxicity endpoint was above the water solubility, the result cannot be considered reliable. Therefore the study is considered supplementary information.	Barrett, K.Ñ &Arnold D.J. (1988)
Acute toxicity to aquatic invertebrates Guideline US EPA 660/3-75-009(1975) and US EPA draft (1978)	Water flea (Daphnia magna)	Clofentezine, (99.0%)	RMS: 48 hour EC50 >0.00004 µg/L	Since the toxicity endpoint was above the water solubility, the result cannot be considered reliable. Therefore the study is considered supplementary information.	Lines, D (1981)
Acute toxicity to algae	Green algae (*Scenedesmus pannonicus)*	Clofentezine, (purity not stated)	120h EC_{50} >0.32 mg/L, based on nominal	Supplementary	Oldersma, H. Hanstveit, A. O. &

Method	Species	Test substance (purity)	Results[a]	Remarks	Reference
Acute toxicity of Clofentezine					
Guideline Dutch draft NEN 6506			concentrations.		Pullens, M.A.H.L (1983)

Method	Species	Test substance (purity)	Results[a]	Remarks	Reference
Acute toxicity of metabolites					
Acute toxicity to fish Guidelines: OECD 203	Rainbow trout (Oncorhyncus mykiss)	2-Chlorobenzoic acid (2-CBA)	96 hour LC_{50}>100 mg/L	Accepted	**Anonoymous (2010a) B.9.2.1/07**
Acute toxicity to fish Guidelines: OECD 203	Rainbow trout (Oncorhyncus mykiss)	2-Chlorobenzonitrile	96 hour LC_{50}>22 mg/L	Accepted	**Anonoymous (2001a) B.9.2.1/06**
Acute toxicity to fish Guidelines: OECD 203 and OECD 126 (2010)	Rainbow trout (Oncorhyncus mykiss)	AE C593600	96 hour LC_{50} >5.30 mg /L based on nominal concentration >2.81 mg/L based on measured concentration	Accepted	**Anonoymous (2001a) B.9.2.1/08**
Acute toxicity to fish Guidelines: OECD 203 and OECD 126 (2010)	Rainbow trout (Oncorhyncus mykiss)	AE F092117	96 hour LC_{50} >100 mg/L based on nominal concentrations	Accepted	**Anonoymous (2001a) B.9.2.1/09**
Acute toxicity to aquatic invertebrades Guidelines: OECD 202	Water flea (Daphnia magna)	2-Chlorobenzoic acid (2-CBA)	48 hour LC_{50}>100 mg/L	Accepted	Kuhl, R., Deierling T. (2010b)

Method	Species	Test substance (purity)	Results[a]	Remarks	Reference
Acute toxicity of metabolites					
Acute toxicity to aquatic invertebrates Guidelines: OECD 202	Water flea (Daphnia magna)	2-Chlorobenzonitrile	48 hour EC_{50}=13 mg/L	Accepted	Wetton, P.M., Mullee, D.M. (2001B)
Acute toxicity to aquatic invertebrades Guidelines: OECD 202	Water flea (Daphnia magna)	AE C593600	48 hour LC_{50} >1.54 mg/L	Accepted	Eser, S. (2015a)
Acute toxicity to aquatic invertebrades Guidelines: OECD 202	Water flea (Daphnia magna)	2-Chlorobenzamide	48 hour LC_{50}>100 mg/L	Accepted	Dogerloh, M. (2003)
Toxicity to algae	Algae (Pseudokirchneriella subcapitata)	Metabolite 2-CBA	72 hour ErC_{50} = 47 mg/L NOEC for growth and yield = 6.25mg/L	Accepted penging on recalculated endpoints	Mead, C. Mulee, C.M (2001)
Toxicity to Algae	Algae (Pseudokirchneriella subcapitata)	Metabolite 2-CBA	72 hour ErC_{50}>100 mg/L 72 hr NOEC for growth and yield = 1 mg/L	Accepted	Kuhl, R., Deierling T. (2010c)
Toxicity to algae	Algae (Pseudokirchneriella subcapitata)	Metabolite AE C593600	The E_rC_{50} >5.30 mg/L (nominal)	Accepted	Dabrunz, A. (2015a).

Method	Species	Test substance (purity)	Results[a]	Remarks	Reference
Acute toxicity of metabolites					
Toxicity to algae	Algae (Pseudokirchneriella subcapitata)	Metabolite AE F092117	The E_rC_{50} >100 mg/L	Accepted	Dabrunz, A. (2015b).
Clofentezine has a low water solubility (0.00252 mg/L) therefore these endpoints resulting from studies with the technical material were determined above the limit of solubility of the substance. No effects were reported in these studies at the highest test concentration possible. Studies were also conducted with the formulated product, MCW-8927 (Apollo 50 SC), to allow for higher concentrations to be tested. However in accordance with the CLH requirements only data available with the active substance should be considered for the classification. It is therefore noted that these effect concentrations with clofentezine are very conservative as no effects were observed and the concentrations which could be tested were limited by the low solubility of the substance.					

11.5.1 Acute (short-term) toxicity to fish

Two studies have been submitted on the acute toxicity of clofentezine to fish. One was carried out with rainbow trout (*Oncorhynchus mykiss*) (B.9.2.1/01), and one with bluegill sunfish (*Lepomis macrochirus)* (B.9.2.2/02). In both study reports the guideline was not stated, and only the study with bluegill sunfish was performed according to GLP standards. In both studies no effects were reported at the highest concentrations tested and so the LC_{50} values were considered to be greater than this concentration. For the bluegill sunfish the maximum concentration tested was 0.25 mg/L and for the rainbow trout the highest concentration was 0.0146 mg/L, based on mean measured concentrations.

Author(s): B.9.2.1/01. In DAR (2005). Annex II reference: IIA. 8.2.1
Title: Determination of the acute toxicity of [^{14}C]-Clofentezine to rainbow trout (*Salmo gairdneri*) using a dynamic test system
Guidelines: Not stated
GLP: No

Summary:

The 96 hour LC_{50} of [^{14}C]-Clofentezine to rainbow trout (*Salmo gairdneri*) was assessed under continuous flow conditions. The test was conducted in 25 L volume glass vessels containing 15 L of the test solution with a flow through rate of approximately 4.2 L/hour. Due to the extremely low solubility of clofentezine in water, the compound was firstly absorbed to pumice which was then used, via a saturation column, to supply a constant level of dissolved [^{14}C]-labelled clofentezine to the fish. A mean measured concentration of 14.6 µg clofentezine/L was determined in the test vessels throughout the exposure period.

Findings:

No mortalities were recorded during the exposure period in either treatment or control vessels. The 96 hour LC_{50} of clofentezine to rainbow trout is therefore greater than the maximum concentration used (14.6 µg/L), and therefore greater than its maximum solubility in water. Study did not follow GLP.

The study was considered supplementary

Author(s): B.9.2.1/02). In DAR (2005). Annex II reference: IIA. 8.2.1
Title: Determination of the acute toxicity of NC21314 to bluegill sunfish (*Lepomis macrochirus*)
Guidelines: Not stated
GLP: Yes

Summary:

The acute toxicity of clofentezine to bluegill sunfish was determined in freshwater at 22ºC using a continuous flow-through system. The study was considered to be valid in accordance with current requirements as the validity criteria were met: no mortality occurred in the freshwater control (although 20 % mortality was reported in the solvent control) and dissolved oxygen levels. The mean measured values of clofentezine were 80 – 83 % of the nominal concentrations. Therefore it is acceptable to calculate effect concentrations based on nominal concentrations.

The test was conducted with two measured concentrations of clofentezine suspended in water, 0.25 and 0.12 mg/L. The suspension was aided by first dissolving clofentezine in acetone/Tween 80.

Only two mortalities occurred in the twenty fish at 0.30 mg/L and 96 hours, and no deaths occurred at 0.15 mg/L clofentezine at this time. Four deaths occurred at 96 hours in the solvent control but no deaths occurred in the freshwater control at this time. As no significant effects on mortality were shown at either test concentration, the 96 hour LC_{50} value is considered to be above the highest concentration tested of 0.25 mg/L (concentration in suspension), and it is therefore higher than clofentezine maximum solubility in water. LC_{10} and LC_{20} values could not be calculated as there was no dose response shown.

Findings:

The 96 hour LC_{50} for clofentezine to bluegill sunfish was >0.25 mg/L (concentration in suspension) and is therefore greater than its maximum solubility.

Only two mortalities occurred in the twenty fish at 0.30 mg/L and 96 hours, and no deaths occurred at 0.15 mg/L clofentezine at this time. Four deaths occurred at 96 hours in the solvent control but no deaths occurred in the freshwater control at this time. As no significant effects on mortality were shown at either test concentration, the 96 hour LC_{50} value is considered to be above the highest concentration tested of 0.25 mg/L (concentration in suspension), and it is therefore higher than clofentezine maximum solubility in water. LC_{10} and LC_{20} values could not be calculated as there was no dose response shown

Studies have also been undertaken to assess the toxicity of clofentezine metabolites.

METABOLITES

Author(s); year: B.9.2.1/07 NEW STUDY.
Title: Acute Toxicity of 2-chlorobenzoic acid to rainbow trout (*Oncorhynchus mykiss*) in a 96-hour static limit-test
Guidelines: OECD 203 (1992); Commission Regulation (EC) No 440/2008 Annex, Part C, C1 (2008)
GLP: Yes
Test substance: 2-chlorobenzoic acid
Summary:

A 96-hour static acute toxicity limit-test was performed in order to evaluate the influence of 2-chlorobenzoic acid on the mortality and sublethal symptoms of *Oncorhynchus mykiss*. A group of seven rainbow trout (*Oncorhynchus mykiss*) was exposed to 2-chlorobenzoic acid at the nominal concentration of 100 mg test item/L. There was also a control group with seven rainbow trout. The acute toxicity to unfed juvenile rainbow trout was determined in an aerated static system of 12 L

glass aquaria with 10 L of test medium under 16 hours light and 8 hours dark (with 30 min dawn/dusk period per day) for 96 hours. The temperature (measured daily) was 13 – 15 °C throughout the entire study. Dissolved oxygen concentration (measured in each vessel every 24 hours) was 91 to 98% of the air saturation value. The pH of the test media (measured in each vessel every 24 hours) was 7.7 to 8.0. The samples of the test medium were analysed via HPLC-method at test start and after 96 hours. The test fish were observed after approximately 2, 24, 48, 72 and 96 hours test duration for sublethal effects and mortality. Dead fish were removed at least once daily and discarded. The NOEC, the LOEC and the LC_0 were determined directly from the raw data.

Findings:

In the control and the only test concentration of 100 mg test item/L, all fish survived until the end of the experiment and showed no sub lethal effects during the exposure time. The observed mortality results after 2, 24, 48, 72 and 96 hours are presented in the table below.

Based on the test results, the LC_{50} of 2-chlorobenzoic acid for rainbow trout (*Oncorhynchus mykiss*) at 96 hours after application was determined to be higher than 100 mg test item/L based on nominal concentrations. The LC_{10} and LC_{20} values are also considered to be >100 mg/L. The no observed effect concentration (NOEC) was determined to be ≥ 100 mg test item/L and the LOEC was determined to be > 100 mg test item/L, both values also based on nominal concentrations.

Author(s); year: B.9.2.1/06. In DAR (2005). Annex II reference IIA 8.2.1
Title: 2-Chlorobenzonitrile: Acute toxicity to rainbow trout (*Oncorhynchus mykiss*)
Guidelines: 92/69/EEC C.1 = OECD 203 (1992)
GLP: Yes
Test substance: 2-chlorobenzonitrile

Summary:

The acute toxicity of 2-chlorobenzonitrile to rainbow trout was determined in fresh water using a semi-static system. The test was conducted with 10 / 18 / 32 / 56 / 100 mg/L of 2-chlorobenzonitrile, with concentrations according to a preliminary range-finding test. The mortality and sub-lethal effects were determined 3 and 6 h after exposure, then daily. Final mortality rate was reached within 24 hours exposure. The validity criteria were met for the study as no mortality occurred in the control, dissolved oxygen was > 60 % and measured concentrations of the test substance were >80 % of nominal (97 – 104 %).

Findings:

The 96 hour LC_{50} for 2-chlorobenzonitrile to rainbow trout was 22 mg/L. The NOEC was 10 mg/L. No sub-lethal effects were observed at 18 mg/L or below.

The dose response was limited as 100 % mortality occurred in the three highest concentrations 32, 56 and 100 mg/L with 14 % mortality at 18 mg/L. LC_{10} and LC_{20} values could therefore not be estimated.

Author(s); year: (B.9.2.1/08). NEW STUDY.
Title: Metabolite AE C593600: Toxicity to the Rainbow Trout *Oncorhynchus mykiss* under laboratory conditions (Acute toxicity test- Semi Static)
Guidelines: OECD 203 (1992) and OECD 126 (2010)
GLP: Yes
Test substance: Hydrazide-Hydrazone

Summary:

The toxicity of the metabolite AE C593600 to *Oncorhynchus mykiss* was assessed in a 96 hour semi static acute test, in accordance with OECD 203 (1992) and OECD 126 (2010). The test was conducted as a semi static limit test at the solubility threshold of the test item, 5.30 mg/L. A control and solvent control were tested in parallel. Analytical samples were taken from fresh and aged test solutions to confirm the test item concentration throughout the exposure period. Aged measured concentrations fell below 80 % of the nominal the toxicological endpoints were assessed using nominal and measured concentrations, based on the geometric mean of fresh and aged samples. No significant effects were reported in the treatment concentration and so the LC_{50} was determined to be >5.30 mg/L based on nominal concentrations and >2.81 mg/L based on measured concentrations. The corresponding NOEC was determined to be 5.30 mg/L based on nominal and 2.81 mg/L based on measured concentrations.

Findings

The study was conducted as a limit test at the solubility (5.30 mg/L) of metabolite AE C593600. No mortality was observed at the test concentration at limit of solubility and so the LC_{50} (96 h) was determined to be > 5.30 mg/L based on nominal concentration and >2.81 mg test item/L based on measured concentrations. The corresponding NOEC (96 h) is 5.30 mg/L (nominal) and 2.81 mg/L (measured).

Author(s); year: B.9.2.1/09. NEW STUDY.
Title: Metabolite AE F092117: Toxicity to the rainbow trout *Oncorhynchus mykiss* under laboratory conditions (acute toxicity test – static)
Guidelines: OECD 203 (1992) and OECD 126 (2010)
GLP: Yes
Test substance: 2-chlorobenzamide

Summary:

This study was conducted to address the risk from clofentezine metabolites to aquatic organisms applicant submitted this ecotoxicological data on 2-AE F092117. A 96-hour static acute toxicity limit-test was performed in order to determine the effects of metabolite AE F092117 (2-chlorobenzamide), on the mortality and sublethal symptoms of *Oncorhynchus mykiss*. Juvenile rainbow trout were exposed to aqueous test media containing the test item at nominal concentration of 100 mg/L under defined conditions. One control with untreated test medium containing sever fish were also tested. A limit test was performed with a threshold concentration of 100 mg test item/L The concentration of the test item was analysed at t = 0 and 96h, and since measured concentrations of metabolite AE F092117 in the test solution were between 80 % and 120 % of nominal, the toxicological endpoints were evaluated using nominal test item concentrations. The acute toxicity to unfed juvenile rainbow trout was determined in an aerated static system of 25 L glass aquaria with 15 L of test medium under 16 hours light and 8 hours dark for 96 hours. Assessments on effects and mortality after 0, 4, 24, 48, 72 and 96 hours were conducted. Temperature, pH-value and % oxygen saturation of the test solutions were measured after 0, 24, 48, 72 and 96 hours. Hardness of the test water was measured at the start of the test. Analytical samples were analysed in the control and 100 mg/L at the test start and at the end of the test.

Findings:

No mortality or sublethal effects were observed during the test, in the control and at the threshold concentration of 100 mg/L.

Based on the test results, the LC_{50} (96 h) of the test item was determined to be > 100 mg test item/L (nominal). As no mortality occurred the LC_{10} and LC_{20} values are also taken to be>100 mg/L. The corresponding NOEC (mortality) (96 h) was 100 mg/L (nominal).

No sublethal effects were observed in either the control or at 100 mg/L (nominal). The absence of mortality at the threshold concentration indicates that the fish is not the most sensitive group of test organism and that the LC_{50} is greater than the limit test concentration.

The study is considered acceptable and suitable for risk assessment purposes and the proposed endpoint is 96h- LC_{50}> 100 mg AE F092117/L.

11.5.2 Acute (short-term) toxicity to aquatic invertebrates

Two studies have been submitted on the acute toxicity of clofentezine to *Daphnia magna*. The study by Barrett & Arnold (1988) was undertaken following the Guideline OECD 202 I and US EPA EG1 31: 5007-5009, and it was performed according to GLP standards. The other study, by Lines (1981), followed US EPA 660/3-75-009(1975) and US EPA draft (1978), but was not performed according to GLP. In both studies effects were not reported at the highest concentrations tested of 0.00084 mg/L and 0.08 mg/L and so the EC_{50} values can be considered to be greater than the concentrations tested.

Author(s): Barrett, K.L., Arnold, D.J. (1988a)
Title: Determination of the acute toxicity of Clofentezine technical to *Daphnia magna*.
Guidelines: OECD 202 I, US EPA EG1 31: 5007-5009 - static conditions
GLP: Yes

This study evaluated the acute toxicity of clofentezine to the freshwater crustacean Dapnhia magna. The study followed OECD 202 Guidelines. The test was conducted under static conditions, at a temperature of 20ºC ± 1ºC. Due to the low solubility of the test compound in water, daphnia were exposed to only a single concentration (1.45 µg/L) representing the maximum solubility attainable under the test conditions with the use of 0.5 ml/L acetone/Tween 80 (50/50 v/v) solvent concentration. Observations were made over a 48 hour period.

No toxic effects were observed at the concentration tested and the number of daphnids immobilised in the treatment solution was slightly greater than that recorded in the controls but did not reach an EC_{50} value. After 48 hours the amount of clofentezine present was reduced to 0.00084 mg/L, representing 57.93% of the original concentration.

The EC_{50} value is therefore greater than the maximum solubility of the compound in water and concluded to be >0.00084 mg/L.

Author(s): Lines, D. (1981) in DAR (2005). Annex II reference IIA 8.2.4.1
Title: Determination of the acute toxicity of technical NC21314 to the water flea, *Daphnia magna.*
Guidelines: US EPA 660/3-75-009 (1975), US EPA Guideline draft (1978) – static conditions
GLP: No

The study evaluated the acute toxicity of clofentezine technical to the freshwater crustacea *Daphnia magna.* Toxicity was assessed over a 48 hour period under static conditions, at a temperature of 20ºC ± 1ºC. The nominal concentration was 100 mg clofentezine/L.

The measured concentration of clofentezine was between 0.01 and 0.14 mg/L, with a mean measured concentration of 0.08 mg/L after 48 hours (test termination). No toxic effects were observed at the concentration tested and the number of daphnids immobilised in the treatment solution was similar to that recorded in the controls but did not reach an EC_{50} value.

The EC_{50} value is therefore greater than the maximum solubility of the compound in water and concluded to be >0.08 mg/L.

METABOLITES

Author(s); year: Kuhl, R. Deierling, T. (2010b). CONFIRMATORY DATA.
Title: Acute Toxicity of 2-chlorobenzoic acid to *Daphnia magna* in a Static 48-hour immobilisation limit-test
Guidelines: OECD 202 (2004) equivalent to the Commission Regulation (EC) No 440/2008, C.2. (2008)
GLP: yes
Test substance: 2-chlorobenzoic acid

Summary:

This study was provided as confirmatory data to address the risk of clofentenzine metabolites to aquatic organisms. A 48-hour static acute toxicity limit-test was performed in order to evaluate the effect of the test item 2-chlorobenzoic acid on the mobility of *Daphnia magna*. Young daphnids (< 24 hours old) were exposed to the test item added to test water at the concentration of nominal 100 mg test item/L. The purpose of the analytical part of this study was to verify the concentration of the test item in the test water. After 48 hours of exposure no immobilisation of the test animals was observed in the control and in the only test concentration of nominal 100 mg/L. The study was considered acceptable by the RMS and suitable for risk assessment.

Findings:

After 48 hours of exposure no immobilisation of the test animals was observed in the control and in the only test concentration of nominal 100 mg/L. The 48-hour NOEC was determined to be ≥ 100 mg test item/L. The 48-hour EC_{50} value was determined to be > 100 mg 2-chlorobenzoic acid/L

Author(s); year: Wetton, P.M., Mullee, D.M. (2001b). In DAR (2005). Annex II reference IIA 8.2.4.1
Title: 2-Chlorobenzonitrile: Acute toxicity to *Daphnia magna*
Guidelines: 92/69/EEC C.2 = OECD 202 (1984) – static conditions
GLP: yes
Test substance: 2-chlorobenzonitrile

Summary:

The toxicity of 2-chlorobenzonitrile to the freshwater crustacean *Daphnia magna* was assessed over a 48 hour exposure period under static conditions at concentrations of 1.0 / 1.8 / 3.2 / 5.6 / 10 / 18 / 32 / 56 / 100 mg/L based on a range-finding study. The test was conducted according to OECD 202. However, only two replicates per tested concentration were used, instead of the four recommended in OECD 202. Nine tested substance concentrations were used. The measured concentrations ranged from 103-112% of nominal value. The EC_{50} of 2-chlorobenzonitrile to *Daphnia magna* after 48 h was calculated as 13 mg/L (95% conf. interval 12 – 15 mg/L). No abnormal behaviour of the daphnids was detected.

Findings:

The EC_{50} of 2-chlorobenzonitrile to *Daphnia magna* after 48 h was 13 mg/L.

Author(s); year: Eser, S. (2015a). NEW STUDY.
Title: Metabolite AE C593600: Toxicity to the water flea *Daphnia magna* Straus under laboratory conditions (Acute immobilisation test –static)
Guidelines: OECD 202 (2004)
GLP: Yes
Test substance: Hydrazide-Hydrazone

Summary:

This study was provided as confirmatory data to address the risk of clofentenzine metabolites to aquatic organisms. The toxicity of the metabolite AE C593600 to *Daphnia magna* was assessed in a 48 hour acute immobilisation test, in accordance with OECD 202 (2004). The test was conducted as a static limit test at the solubility limit of the test item, 5.30 mg/L. A control and solvent control were tested in parallel. Analytical samples were taken at the start of the stud and at 48 hours from aged test solutions to confirm the test item concentration throughout the exposure period. Aged measured concentrations fell below 80 % of the nominal the toxicological endpoints were assessed using nominal and measured concentrations, based on the geometric mean of fresh and aged samples. No significant effects were reported in the treatment concentration and so the EC50 was determined to be >5.30 mg/L based on nominal concentrations and >1.54 mg/L based on measured concentrations.

Findings:

The study was conducted as a limit test at the solubility (5.30 mg/L) of metabolite AE C593600. According to the results of the test, the EC_{50} (48 h) was determined to be > 5.30 mg/L based on nominal concentration and > 1.54 mg test item/L based on measured concentrations. The corresponding NOEC (48 h) is 5.30 mg/L (nominal) and 1.54 mg/L (actual).

Author(s); year: Dorgerloh M. (2003). CONFIRMATORY DATA.
Title: Acute Toxicity of SIR 8514-2-chlorobenzamide (tech.) to water fleas (*Daphnia magna*)
Guidelines: OECD 202 (1984, 2000); OPPTS 850.1010 (1996); EEC Directive 92/69/EWG, C.2 (1992
GLP: yes
Test substance: 2-chlorobenzamide

Summary:

This study was provided as confirmatory data to address the risk of clofentenzine metabolites to aquatic organisms. A 48-hour static acute toxicity-test was performed in order to evaluate the effect of the test item 2-chlorobenzamide on the mobility of water fleas, *Daphnia magna*. Thirty young daphnids (< 24 hours old) were exposed to the test item added to test water at the concentration of nominal concentrations of 0, 10, 18, 32, 56 and 100 mg pure metabolite/L. The purpose of the analytical part of this study was to verify the concentration of the test item in the test water. The only concentration showing immobilisation (27 %) after 48 hours was 100 mg metabolite/L. Abnormal behaviour or appearance of symptoms were observable in concentrations higher than 32 mg pure metabolite/L after 48h. The 48-hour EC_{50} value was determined to be > 100 mg test item/L.

Summary:

Based on nominal concentrations, the EC_{50} (24 and 48 hours) for SIR 8514-2-chloro benzamide (tech.) was higher than 100 mg pure metabolite/L (95%-confidence limits not calculable). The EC_{10} and EC_{20} values at 48 hours can be estimated to be 56 mg/L.

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

A study has been submitted on the toxicity of clofentezine to green algae (*Scenedesmus subspicatus*). This study was undertaken following the Dutch draft NEN 6506 guideline, as with the fish and Daphnia magna studies, no effects were reported at the highest concentration tested and so the EC_{50} value estimated to be >0.32 mg/L.

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

No data are available.

11.6 Long-term aquatic hazard

Table 50: Summary of relevant information on chronic aquatic toxicity

Method	Species	Test material	Results	Remarks	Reference
Chronic toxicity					
Long term chronic toxicity to fish	Rainbow trout (*Oncorhynchus mykiss)*	Clofentezine, (99.5%)	NOEC = >0.007 mg/L, based on mean measured concentrations.	Due to methodological concerns, the study was considered supplementary.	Anonoymous (1993) B.9.2.2/01

Method	Species	Test material	Results	Remarks	Reference
Reproductive toxicity to Daphnia Guideline OECD 202 II (1984) and US EPA 540/9-86-141	Water flea (*Daphnia magna)*	Clofentezine, (99.8%) [^{14}C]-clofentezine, purity 99.21%.	NOEC = >0.025 mg/L, [^{14}C] – Clofentezine	Not considered in the scope of the renewal.	Barber, I. & Latimore, a.E. (1992).
Lifecycle toxicity test Guideline OPPTS 850.1350	Saltwater mysid shrimp (*Americamysis bahia)*	Clofentezine, (98.29%)	28 day NOEC= >0.0269 mg/L, based on mean measured concentrations. Endpoint accepted is: 28d- NOEC Mean total young per f0-female = 0.0033 mg a.s./L	Accepted	Aufderhide, J. (2009). Aufderhide, J. (2016).
Effects on growth of green algae Dutch draft standard method NEN 6506	Algae (Scenedesmus pannonicus)	NC 21314 Technical clofentezine	120 hour - EC_{50} >0.32 mg/L	No endpoint was stated (claimed to be greater than the water solubility of clofentezine). No endpoints for growth rate are available from this study.	Oldersma, H, Hanstveit, A.O., Pullens, M.A.H.L (1983) [114]
Effects on growth of green algae Guidelines: OECD 201 (1984) modified by EG-8 and ES-5	Algae (Selenastrum capricornotum)	Clofentezine, (99.8%) [^{14}C]-clofentezine, purity 99.21%.	72 hour - EC_{50} not reported 72-hour NOEC ≥ 40 mg a.s/L	Accepted RMS calculated endpoints: 92h-EbC_{50} > 34 mg a.s./L 92h-ErC_{50} > 34 mg a.s./L	Hanstveit, A.O. (1987)
Chronic Toxicity of metabolites to Algae					
Toxicity to algae	Algae (Pseudokirchneriella subcapitata)	Metabolite 2-CBA	72 hr NOEC for growth and yield = 6.25mg/L	Accepted pending on recalculated endpoints	Mead, C. Mulee, C.M (2001)

Method	Species	Test material	Results	Remarks	Reference
Toxicity to Algae	Algae (Pseudokirchneriella subcapitata)	Metabolite 2-CBA	72 hr NOEC for growth and yield = 1 mg/L	Accepted	Kuhl, R., Deierling T. (2010c)
Toxicity to algae	Algae (Pseudokirchneriella subcapitata)	Metabolite AE C593600	72 hr NOEC for growth and yield = 5,30 mg/L (nominal)	Accepted	Dabrunz, A. (2015a).
Toxicity to algae	Algae (Pseudokirchneriella subcapitata)	Metabolite AE F092117	72 hr NOEC > 100 mg/L (nominal) for growth rate and yield.	Accepted	Dabrunz, A. (2015b).
Clofentezine has a low water solubility (0.0025–0.034 mg/L) therefore these endpoints resulting from studies with the technical material were determined above the limit of solubility of the substance. No effects were reported in these studies at the highest test concentration possible. Studies were also conducted with the formulated product, MCW-8927 (Apollo 50 SC), to allow for higher concentrations to be tested. However in accordance with the CLH requirements only data available with the active substance should be considered for the classification. It is therefore noted that these effect concentrations with clofentezine are very conservative as no effects were observed and the concentrations which could be tested were limited by the low solubility of the substance.					

11.6.1 Chronic toxicity to fish

Author(s): B.9.2.2/01. In DAR (2005). Annex II reference IIA 8.2.2.
Title: The toxicity of Clofentezine technical to early life stages of the rainbow trout *Oncorhynchus mykiss*, in a flow through system
Guidelines: According to US EPA directives, guideline not stated. Method is equivalent to, and partially exceeds, the requirements of OECD 210 (1992)
GLP: Yes

Summary

The study evaluated the toxicity of Clofentezine technical to early life stages of the rainbow trout Oncorhynchus mykiss, in a flow through system. Rainbow gametes were exposed to a mean measured concentration of 0.007 mg/L clofentezine technical. This study was carried out according to GLP standard but no guideline was stated in the study report.

The study was conducted to according to US EPA. It was highlighted in the report that the study had been conducted at the request of the US EPA who had ‘expressed concern that there may be long term reproductive effects on fish based on the ovicidal action of clofentezine to mites.’ Applicant stated that the applied Method is equivalent to, and partially exceeds, the requirements of OECD 210. However, several technical protocol deviations were reported; particularly temperature deviated from the specified value, feeding regime, and length determination of fish.

Due to the low solubility of clofentezine in water, a single concentration was tested. The compound was firstly absorbed to pumice stone that was then used, via a saturation column, to supply dissolved clofentezine to the embryos. Mean measured clofentezine for 97 days was 0.007 mg/L.

Findings:

Hatchability and survival of embryos were not significantly affected at 0.007 mg/L, as well as length, dry weight or wet weight.

In summary, clofentezine technical had no chronic toxicity to early life stages of the rainbow trout at the maximum solubility obtained under study conditions (i.e. 0.007 mg/L) over a 97 day continuous exposure period, so the NOEC can be estimated to be >0.007 mg/L over a 97-day continuous exposure period.

With only one concentration being assessed, the NOEC (No Observed Effect Concentration), LOEC (Lowest Observed Effect Concentration), and MATC (Maximum Acceptable Toxicant Concentration) are not calculable. The LC_{50} for the 97 day study period was greater than the maximum solubility of the compound under the study conditions. Therefore, it can be concluded that clofentezine technical had no chronic toxicity to early life stages of the rainbow trout at the maximum solubility obtained under study conditions (*i.e.* 0.007 mg/L) over a 97 day continuous exposure period.

97d – NOEC = 0.007 mg a.s/L

RMS showed particular concern regarding the differences in the water temperature through the test. The range was 8.9-12.1ºC, exceeding the range recommended in OECD 210 for Oncorhynchus mykiss (10±1.5ºC).

In a flow-through test substance concentrations should be measured three times during the first week but only once is reported in the study. Moreover, during the 4th and 5th week of exposure the measured concentration was below 60% of nominal.

Clofentezine main degradants are much less acutely toxic than the parent substance and therefore they are not considered further in relation to the classification.

RMS considered this study only as supplementary data (RAR of September 2017). Thus it will not be taken into consideration for the classifications proposal.

The study was accepted as supplementary data

11.6.2 Chronic toxicity to aquatic invertebrates

Two studies have been submitted on the chronic toxicity of clofentezine to aquatic invertebrates. One of these was undertaken with *Daphnia magna* Barber & Lattimore, A.E. 1992) following the OECD 202 II (1984) and US EPA 540/9-86-141 guidelines. The other one was performed with saltwater mysid shrimps (*Americamysis bahia)* according to OPPTS 850.1350 (Aufdeerhide 2009, 2016). Both studies were conducted according to GLP standards. Again no significant effects were shown at the highest concentrations tested of 0.025 and 0.0269 mg/L and so the NOEC are considered to be greater than this concentration range.

Author(s): Barber, I., Lattimore, A.E. (1992). In DAR (2005). Annex II reference IIA 8.2.5
Title: Determination of the effects of [^{14}C]-Clofentezine on the life cycle of *Daphnia magna*
Guidelines:

GLP: yes
Summary:

The study evaluated the effects of [^{14}C]-Clofentezine on the life cycle of *Daphnia magna*, First instar daphnids (less than 24 hours old) were exposed to the single concentration of [^{14}C]-Clofentezine, 25 µg/L, which represented the maximum sustainable concentration under the test conditions, for 21

days. Due to the extremely low solubility of clofentezine in water, the compound was first absorbed to pumice stone that was then used, via a saturation column, to supply dissolved clofentezine to the test chambers.

Findings:

The results indicated that [^{14}C]-Clofentezine had no effect on survival, growth or reproduction of *Daphnia magna* at the maximum solubility under the conditions of this test, therefore the NOEC was concluded to be 0.025 mg a.s./L.

The study was already evaluated in DAR (2005) and the endpoint included in the aquatic risk assessment, although an open point regarding the relevance of this NOEC was included in the Reporting Table rev. 1-2 (03.01.2008).

In Addendum 2 (2008), the RMS in response to Open point 5.5 identified in Evaluation Table (rev. 0-0, 03.01.2008), stated that in this study the compound was absorved on to pumice in order to supply clofentezine to the test chamber and the effect of this on the overall NOEC was unknown. *As a result of this study, the NOEC was 0.025 mg a.s./L (this was the only concentration tested) or ten times the clofentezine water solubility".*

During the peer review, this point was revised and discussed in the PRAPeR Expert 63 meeting (2009). Finally it was concluded that this endpoint will not be considered for risk assessment purposes.

Author(s): Aufderheide, J. (2009; 2016). NEW STUDY.
Title: Clofentezine: Life-cycle toxicity test of the saltwater mysid, *Americamysis bahia*, conducted under flow-through conditions
Guidelines: OPPTS 850.1350
Deviations: None
GLP: Yes

Summary:

The aim of this study was to assess the effects of clofentezine on survival, growth, and reproduction of saltwater mysid shrimp, *Americamysis bahia*. The study followed OPPTS 850.1350 Guidelines.

This is a new study. A life-cycle toxicity test under flow-through conditions with the test organism *Americamysis bahia* exposed to technical clofentezine was conducted.

Five different test substance concentrations were tested, using DMF as solvent to enhance clofentezine solubility. The test was conducted according to the OPPTS 850.1350 and met all the validity criteria. The applicant reported that the negative control (dilution water control) and vehicle control response data were not statistically different for any biological parameter evaluated.

Findings:

The number of young per female mysid was the only biological parameter that resulted in a statistically significant difference when compared to the negative control data at the concentrations of 6.65 and 26.9 µg clofentezine/L (mean measured concentrations). Therefore, the NOEC value determined for mean number of total young produced per female was 3.30 µg clofentezine/L.

The applicant submitted an additional statistical analysis (the Williams' trend test) using the vehicle control data instead of dilution water control (in accordance with the OECD Number 54 Guidance document). Based on the results from this new test, no statistically significant reduction in the reproductive data for any of the treatment levels tested were determined. Subsequently, the NOEC value for mean total number of young per F_0-female mysid resulted in 26.9 µg/L.

However, an apparent dose-response cannot be undoubtedly ruled out, and in this case, the RMS proposes to maintain the NOEC=3.30 µg/L as a conservative approach.

In the opinion of RMS, this endpoint is clearly conservative as no statistical significant effects were observed at any treatment level when they are compared to vehicle control which is a most realistic approach than the comparation with water control.

The endpoing accepted was 28d- NOEC Mean total young per f0-female = 0.0033 mg a.s./L*, which is considered a conservative approach.

Studies have also been undertaken to assess the toxicity of clofentezine metabolites.

11.6.3 Chronic toxicity to algae or other aquatic plants

Author(s): Oldersma, H, Hanstveit, A.O., Pullens, M.A.H.L. (1983). In DAR (2005), Annex II, reference: IIA. 8.2.6
Title: The effect of the product NC21314 technical on the growth of the green alga *Scenedesmus pannonicus*.
Guidelines: Dutch draft Standard method NEN 6506
GLP: Yes

Summary:

The effect of clofentezine on algal growth was investigaded with the green algal species *Scenedesmus pannonicus*, was investiaged following the Guideline Dutch draft Standard method NECE 6506, GLP.. The 120-hour EC_{50} was > 0.32 mg/L, indicating that clofentezine has low toxicity to green algae at its limit of water solubility. A full summary of this study is provided in the Annex.

Findings:

The results showed that clofentezine in concentrations up to its solubility limit in water did not impair the growth of the alga *Scenedesmus pannonicus* under the conditions of the test. In concentrations exceeding that limit, however, it had a slight effect on growth yield. The 120-hour EC_{50} value was > 0.32 mg/L, indicating that clofentezine has low toxicity to green algae at its limit of water solubility.

This study was already evaluated during Annex I inclusion of Clofentezine. However the EC_{50} endpoint was not stated (claimed to be greater that the water solubility of clofentezine) and it was not used for risk assessment purposes in DAR (2005).

Comparison of the growth curves of algal suspension exposed to the test substance with those algal controls (solvent controls with DMSO, no negative controls were used) the NOEC was estimated to be 0.32 mg/L (n), although no statical treatment of data were reported. When actual concentrations were measured, less than 10% of nominal were found at the end of the test. Therefore, applicant reported that the results showed that clofentezine in concentrations up to its solubility limit in water did not impair the growth of the alga *Scenedesmus pannonicus* under the conditions of the test.

The endpoint was not stated (claimed to be greater than the water solubility of clofentenzie). No endpoints for growth rate are available from this study.

The study was considered supplementary information.

Author(s): Hanstveit, A.O. (1987). In DAR (2005).Annex III reference IIIA 10.2.1
Title: The effects of Apollo 50 SC on the growth of the alga *Selenastrum capricornutum*
Guidelines: OECD 201 (1984) modified by EG-8 and ES-5
Deviations: The centrifugation step before chemical analysis was replaced by filtering followed by sonication of the re-suspended pellet
GLP: yes

Summary:

This study was already evaluated during Annex I inclusion of Clofentezine and it was accepted byMMSS in DAR (2005). Endpoint was considered for risk assessment purposes during the peer review.

The effects of Apollo 50 SC (purity 50% w/w) on the growth of the alga Selenastrum Capricornotum was studied in a static system (duration 92 hours). The study was conducted according to OECD 201 (1984) and meets the requirements of current OECD 201 version (2006, 2011).

Findings:

No significant effects were observed at the test concentration of 40 mg a.s/L. The EC_{10}, EC_{20} and EC_{50} values can therefore be considere to be > 40 mg a.s./L and the NOEC to be 40 mg/L. Overall recovery rates for the active substances were approx.. 70% at test start and 30 – 40% at test end (92 h).

The determination of the a.s. content was very difficult since the dispersed particles had similar size as the algae and therefore co-centrifuged. The centrifugation step before chemical analysis was hence replaced by filtering followed by sonication of the re-suspended pellet. A determination of the a.s. content was very difficult since the dispersed particles had similar size to the alge and therefore mixed ended in the pellet by the centrifugation. A determination carried out with the lowest concentration tested showed some active ingredient in the algal pellet. Analysis of the test substance in the test medium (only at the start and at the end of the test) indicated that concentration ranged from 46-87.5% of nominal at the start, and from 34 to 63% after 92 h. Applicant suggested that the active ingredient was hydrolysed based on the analysis of the test samples by HPLC.

The algae cell counts showed a stimulating effect at the highest concentrations tested of Apollo 50 SC (3.2 mg7L of Apollo 50 SC). This growth can be considered due to compunds containing nitrogen which are released upon hydrolysis of Apollo 50 SC and enhance growth.

The study was accepted and the endpoint was 92h-ErC_{50}>34 mg a.s./L

METABOLITES

The toxicity of metabolites to algae was evaluated in three studies.

Author(s): Mead, C., Mullee, D.M. (2001). Accepted In DAR (2005). Annex II reference: IIA. 8.2.6
Title: 2-Chlorobenzonitrile: Algal inhibition test
Guidelines: 92/69/EEC C.3 = OECD 201 (1984)
GLP: yes

Summary:

The study investigated the effecto of metaboline 2-chlorobenzonitrile on algal growth following guidelines 92/69/EEC C.3 = OECD 201 (1984).

Pseudokirchneriella subcapitata was exposed to an aqueous solution of the test material 2-chlorobenzonitrile at concentrations of 6.25, 12.5, 25, 50 and 100 mg/L (three replicate flasks per concentration) for 72 hours, under constant illumination and shaking at a temperature of 24 ±

1°C.Exposure of *Pseudokirchneriella subcapitata* to the 2-chlorobenzonitrile gave an E_bC_{50} (72 h) value of 16 mg/L and an E_rC_{50} (0 -72 h) value of 47 mg/L. The No Observed Effect Concentration (NOEC) was 6.25 mg/L.

Data from growth and biomass of the test organism showed a clear effect of the test material over the 72-h exposure period. Accordingly, applicant calculated the E_rC_{50} and E_bC_{50} values based on nominal test concentrations (47 and 16 mg/L, respectively).

However, whilst at 0 h the measured test concentrations ranged from 93% to 95% of nominal, after 72 h the measured concentration were found to be 59-62% of nominal. There is the possibility that the substance had adsorbed to algal cells. Applicant stated that this decline in the measured test concentrations might be due to adsorption of the substance to algal cells. However, in a pre-study recovery analyses they concluded that the presence of algal cells had no significant effect on the recovery of the test material. Even though those pre-study analyses were performed immediately after addition of the test substance to algal culture, and so a long term adsorption to algal cells after 72h cannot be ruled out, there is uncertainty associated to these data. Given this uncertainty, the RMS proposed to recalculate the ErC50 and EbC50 values considering the geometric mean measured concentrations over the 72 h exposure period.

Findings:

Exposure of *Pseudokirchneriella subcapitata* to the 2-chlorobenzonitrile gave an E_bC_{50} (72 h) value of 16 mg/L and an E_rC_{50} (0 -72 h) value of 47 mg/L. The No Observed Effect Concentration (NOEC) was 6.25 mg/L.

The study was accepted pending on recalculated endpoints.

Author(s): Kuhl, R., Deierling, T. (2010c). NEW STUDY.
Title: Toxicity of 2-chlorobenzoic acid to *Pseudokirchneriella subcapitata* in an algal growth inhibition test
Guidelines: OECD 201 (March 2006)
GLP: yes
Test substance: 2-chlorobenzoic acid

Summary:

This study evaluated the toxicity of 2-chlorobenzoic acid to *Pseudokirchneriella subcapitata* in an algal growth inhibition test, under static conditions. EC_{10} and EC_{50} concentrations after 72 hours were derived. The algae were exposed to five nominal test concentrations; 1.0, 3.2. 10, 32 and 100.0 mg test item/L.

The study followed the Guideline OECD 201 (March 2006). The validity criteria for the study were met as the cell density in the control cultures increased exponentially by a factor of at least 16 within the 72-hour test period (being 147), the co-efficient of variation of daily growth rates in the control cultures did not exceed 35% (being 28.7) and the co-efficient of variation of average growth between control and replicate cultures did not exceed 7% (being 0.4%).

Findings:

The 72-hour NOE_rC and NOE_yC (no observed effect concentration) for the parameters growth rate and yield were determined to be 1.0 mg test item/L and the associated 72-hour LOE_rC and LOE_yC (lowest observed effect concentration) for the parameter growth rate and yield to be 3.2 mg test item/L based on nominal test concentrations. The 72-hour E_rC_{50} value was calculated to be > 100 mg test item/L for the parameter growth rate, and the 72-hour E_yC_{50} value was calculated to be >100 mg test item/L for the parameter yield.

The study was accepted with a 72-h E_rC_{50} > 100 mg 2-chlorobenzoic acid/L

Author(s): Dabrunz, A. (2015a). NEW STUDY.
Title: Metabolite AE C593600: Toxicity to single cell green alga *Pseudokirchneriella subcapitata* Hindák under laboratory conditions
Guidelines: OECD 201 (2011)
GLP: yes

Summary:

This study evaluated the toxicity of metaboliate AE C593600 to single cell green alga *Pseudokirchneriella subcapitata* Hindák under laboratory conditions.

The toxicity of the metabolite AE C593600 to the green algae *Pseudokirchneriella subcapitata* was assessed in a 72 hour static test system. The test was performed with five test concentrations; 0.331, 0.663, 1.33, 2.65 and 5.30 mg/L, and a control and solvent control. Six replicates were performed for the control and solvent control with three replicates used per test item concentration. After 24, 48 and 72 hours, the cell growth was determined by fluorescence detection. The mean value of the cell concentration was plotted against time to produce growth curves for each concentration. The measured concentration of Metabolite AE C593600 at 72 hours fell below 80 % of the nominal concentration. Therefore toxicological endpoints were evaluated using the nominal and the actual concentrations (based on the geometric mean of each measured concentration) of the test item.

Findings:

There were no significant effects on growth rate or yield during the 72 hour exposure. The E_rC_{50} and E_yC_{50} values were therefore considered to be greater than the highest concentration tested; >5.30 mg/L based on nominal concentrations and 3.13 mg/L based on measured concentrations for growth rate (ErC_{50}) and for yield (EyC_{50}).The corresponding NOEC was 5.30 mg/L based on nominal and 3.13 mg/L based on measured concentrations for growth rate and yield.

Author(s): Dabrunz, A. (2015b). NEW STUDY.
Title: Metabolite AE F092117: toxicity to the single cell green alga *Pseudokirchneriella subcapitata* Hindák under laboratory conditions
Guidelines: OECD 201 (2011)
GLP: yes

Summary:

This study evaluated the effect of metabolite AE F092117 on the single cell green alga *Pseudokirchneriella subcapitata* Hindák under laboratory conditions

The aim of the study was the assessment of the effects of metabolite AE F092117 on the growth of the freshwater green algae *Pseudokirchneriella subcapitata* under static conditions and to derive the $EC_{10,}$ EC_{20} and EC_{50} concentrations after 72 hours. The algae were exposed to five nominal test concentrations; 6.25, 12.5, 25.0, 50.0 and 100.0 mg test item/L.

Findings:

The EC_{50}-value of the test item was >100 mg/L (nominal) for growth rate (E_rC_{50}) and yield (E_yC_{50}). Effects around 10% were measurable for yield but the statistical evaluation of EC_{10} was not determined since the effect was only 13% in the highest test concentration and statistically not significant. The NOEC was determined to be 100 mg/L (nominal) for growth rate and yield.

11.6.4 *Lemna* sp. growth inhibition test

No data are available

11.6.5 Chronic toxicity to other aquatic organisms

No data are available.

11.7 Comparison with the CLP criteria

Table 51: Comparison with criteria for environmental hazards

Endpoint	CLP classification criteria	Clofentezine data	Conclusions
Water solubility	-	0.0025 mg/L	Poorly soluble
Rapid degradability	Demostrated rapid/not rapid degradation	Not readily biodegradable and not rapidly degradable	Not rapidly degradable
Short-term toxicity	LC_{50}/EC_{50} value	None of the studies were considered reliable for providing acute toxicity endpoints for classification. .	The toxicity endpoints derived from the different acute toxicity studies were above the water solubility and therefore the studies could not provide adequate data for classification purposes.
Long-term toxicity	NOEC value	Invertebrates' NOEC=0.0033 mg/L. No adequate data for fish nor for algae.	One chronic toxicity data available
Bioaccumulation potential	BCF ≥ 500, or if absent, log Kow ≥ 4	Experimental BCF value considered not valid; and Log Kow = 4.09	Bioaccumulative potential

The Log K_{OW} of clofentezine is 4.09, this is greater than the trigger value of 4 in the CLP Regulation and so indicates a potential for bioaccumulation. With regards to the bioaccumulation potential, the study on the bluegill sunfish (Lepomis macrochirus) resulted in a bioconcentration factor (BCF) of 248. However, during the review, the RMS identified several concerns. First, a dynamic test was used insteadof the flow-through test recommended in OECD 305, and the number of volume replacement through each test chamber per day is not stated. Additionally, clofentezine is rapidly degraded by hydrolysis (<14 days), and is not likely to be found in a stable concentration over the exposure period. Although the level of radioactivy was stable throughout the test, this may have been adscrbibable to both the parent and metabolites formed. The RMS considered that additional data was needed to prove that the parent compound concentration in the test chamber is maintained during the uptake phase. Secondly, the mean measured concentration of clofentezine during the duration of the study was determined to be 0.033 mg/L. This concentration exceeds the water solubility of clofentezine (2.52 µg/L). Therefore, the experiment does not meet one of the validity criteria OECD 305 test guideline, which requires that the concentration of the test substance is below its limit of solubility in water. The BCF can be estimatd only if constant dissolved exposure level is maintained during the uptake phase.

According to the RMS, the BCF could be estimated using the solubility of clofentezine (2.52 µg/L) as the maximum dissolved exposure concentration in water during the study, and the residue level reached on fish at steady-state resulting on BCF =3216 (8.2 mg as/Kg / 2.52 µg/L). Both this

estimation of the BCF and that reported by (B.9.2.2/04-05) entails uncertainty. The BCF = 3216 culd be used as unrealistic but worst-case approach for assessing secondary poisoning in birds and mammals eating fish. Nevertheless this value is not robust enough and the RMS requested a new study to be submitted as confirmatory data.

In the absence of a reliable bioaccumulation study, the information of the octanol/water partition coefficient should be taken into account to evaluate the substance´s bioaccumulation potential. Already at the DAR and addenda in 2005, as well as at the EFSA Conclusion in 2009, the log Kow value of 4.09 was accepted. This log Kow can be considered to reflect the bioaccumulation potential of clofentezine.

11.7.1 Acute aquatic hazard

Aquatic toxicity test on a short-term scale have been conducted with fish, invertebrates and algae. After RMS assessment and conclusions, none of the studies were considered appropriate so they could not not provide reliable toxicity endpoints about the substance. These tests were regarded as supplementary information, therefore they are not adequate data for classification purposes.

This substance with a solubility in water of 0.0025 mg/L is clearly considered as poorly soluble. These are frequently difficult to test and the results might show toxicity levels recorded in excess of their water solubility. Regarding the fact that in this case no acute toxicity was recorded at levels up to the substance´s solubility, no acute hazards classification would apply for clofentezine.

Based on all the above explained information, clofentezine would not be classified for aquatic acute hazards according to Regulation EC No 1272/2008.

Conclusion: Not classified

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

Clofentezine is considered not readily biodegradable. It is hydrolytically stable at pH 4, but degrades under neutral and alkaline conditions to primary degradates. Degradation was also observed in natural surface water to primary degradates, with low mineralisation (max. 10.8%). In natural water/sediment systems, clofentezine degraded to primary metabolites and carbon dioxide (max. 42.6%). Degradation also occurred under photolytic conditions to primary degradates, with no mineralisation. Volatilisation of clofentezine was found to be minimal from soil and leaf surfaces.

Long-term toxicity studies have been conducted with fish (Oncorhynchus mykiss) and aquatic invertebrates (*Daphnia magna* and *Americamysis bahia*), as well as test on the effects on algal growth (Scenedesmus pannonicus). As with the acute studies, the concentration which could be tested in these long-term studies was limited by the extrem low solubility of the substance.

After RMS assessment it was concluded that:

- The 97 days fish test could not provide a reliable NOEC value;
- The *Daphnia magna´s* endpoint was not considered for risk assessment;
- The *Americamysis bahia´*s NOEC endpoint for reproduction was proposed by RMS as NOEC=0.0033 mg/L as a conservative approach.
- The algae´s growth effect test was also considered only as supplementary information (as toxicity claimed to be greater than the water solubility) and no endpoints could be derived from it.

As with the acute studies, the concentration which could be tested in these chronic studies was limited by the low solubility of the substance.

In each of the studies the no observed effect concentration (NOEC) were shown to be the maximum concentration tested as no significant effects were observed.

Only the long-term toxicity endpoint for *Americamysis bahia* was considered a valid endpoint (NOEC for mysid reproduction). RMS as well as Co-RMS agreed in considering the existence of an apparent-dose response in the endpoint for total number of young per female, and because of this the NOEC for reproduction should be set to 0.0033 mg/L.

For bioaccumulation potential, there is a bioncentration study of clofentezine in bluegill sunfish (Lepomis macrochirus) (B.9.2.2/04-05), wich was presented and already evaluated during Annex I inclusion of clofentezine. During the review RMS identified several concerns, which lead to a high uncertainty level derived from low solubility, and rapid hydrolysis of the substance. The RMS questioned the stability of the substance concentration during the duration of the test. Co-RMS agreed during the draft RAR with RMS that an additional new study for bioconcentration in fish should be submitted. Taking this into consideration the BCF value derived from this 1987 study is not considered valid and should not be understood as an actual reflection of the substance´s capacity to bioaccumulate in fish.

Once the bioconcentration study has been assessed, the information about the octanol/water partition coefficient (log Kow) should be taken into account to evaluate the substance´s bioaccumulation potential. The log Kow of 4.09 from a Bright and Stalker, 1990 study, was accepted already at the DAR and addenda in 2005, as well as at the EFSA conclusion in 2009. As this value was deemed appropriate and in absence of a reliable BCF, the log Kow can be considered to reflect the bioaccumulation potencial of clofentezine as it is a measure of its lipophilicity.

Finally, regarding the substance degradability, clofentezine was shown to be not readily biodegradable (Clarke, 2001), as only 12% CO_2 evolved after 28 days. As a test substance must

achive 60% biodegradation by the end of the test in order to be considered readly biodegradable, it was concluded that clofentezine is “not readily biodegradable” according to OECD criteria.

Clofentezine is hydrolytically stable at pH 4 at temperatures between 20° and 50° C. However, its hydrolysis is pH and temperature dependent being faster at a higher pH. DT_{50} of the clofentezine hydrolysis at pH 7 and at pH 9 is in the range of 795h – 78.2h (at 20° C) and in the range of 37.7h – 0.6h (at 50° C), respectively.

In an aerobic mineralization study clofentezine degraded with DT_{50} values of 5.6 to 7.2 days to the following metabolites: hydrazide-hydrazone (AE C593600), 2-chlorobenzoic acid (2-CBA), 2-chlorobenzamide (2-CBZ), and to carbon dioxide and low levels of unknown metabolites.

Regarding the available data from the water/sediment system study, an important part of clofentezine dissipated to the sediment phase and degradation occurred in both water and sediment phases. Its degradation occurs to 2-chlorobenzoic acid (2-CBA) as major metabolite, carbon dioxide and sediment bound residues. The maximal mineralization reached in the system was 46.2%.

Aquatic photolytic degradation of clofentezine is an important pathway of degradation resulting in DT_{50} values in the range of 2.67 to 5.63 days across latitudes and seasons. Based on the results obtained, it can be concluded that the degradation of clofentezine in both buffer solution (pH 5) and in natural water river (pH 4-7) was of photolytic nature and aerobic mineralization, whereas hydrolytic degradation plays a secondary role under natural pH and temperature conditions.

Taking into consideration all the information included above, this substance should be considered as **a not rapidly degradable** substance in the aquatic environment.

11.8 CONCLUSIONS ON CLASSIFICATION AND LABELLING FOR ENVIRONMENTAL HAZARDS

Taking into account all the information and the assessment summarized in the previous sections 11.7.1 and 11.7.2, the following classification class and category can be concluded for this active substance:

According to Table 4.1.0 (b) (i), clofentezine meets the CLP Regulation criteria for being classified as **Aquatic chronic 1 with M factor of 10.**

CLP Criteria:

- Aquatic long-term toxicity reflected by a valid endpoint for invertebrates’ reproduction NOEC (28d)=0.0033 mg/L, and
- For the M factor, clofentezine is considered not rapidly degradable substance and its long-term toxicity value is in the range of 0.001 to 0.01 (NOEC = 0.0033 mg/L).

Therefore, the proposal for classificacion for Clofentezine is:

Aquatic Chronic category 1; M=10 ; H410 – Very toxic to aquatic life with long lasting effects

12 EVALUATION OF ADDITIONAL HAZARDS

12.1 Hazardous to the ozone layer

Not applicable.

Table 52: Summary table of data concerning hazardous properties of the substance for the ozone layer

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

13 ADDITIONAL LABELLING

14 REFERENCES

14.1 Physico-chemical properties

Demangel, B. (2015). Appearance of pure clofentezine and clofentezine as manufactured, Defitraces, France. Unpublished report no.: R-36077

Ball, R.W. (1987). The determination of the melting point of clofentezine by differential scanning calometry (DSC). Unpublished report No. R-12517.

Smeykal, H. (2000a). Melting point, melting range, boiling point, boiling range, thermal stability. Clofentezine substance, pure, Axiva GmbH, Germany. Unpublished report no. : R-13823

Smeykal, H. (2000b). Relative Density. Clofentezine substance, pure, Axiva GmbH, Germany. Unpublished report no.: R-13824

Smeykal, H. (2000c). Vapour pressure. Clofentezine substance, pure, Axiva GmbH, Germany. Unpublished report no.: R-13825

Martinez, J. & Rexer, K. (2000a). Determination of the surface tension. Clofentezine substance, pure. Clofentezine substance, pure, Axiva GmbH, Germany. Unpublished report no.: R-13266

Van Meter, D.S. (2010). Clofentezine: Determination of water solubility of a test substance following OPPTS 830.7840, Springborn Smithers Laboratories, MA, USA. Unpublished report no.: R-25419.

Smith, S., Kelly, I.D. (1985a). Solubility of clofentezine in aqueous solution under acid, neutral and basic conditions. Unpublished report No. R-12523.

Bright, A.A.S. & Stalker, A.M. (1990). Clofentezine: Determination of the partition coefficient between N-octanol and water at 25°C, Schering Agrochemicals Ltd, UK. Unpublished report no.: R-12519

Lowes, P.R. & Bright, A.A.S. (1986). Octanol-water partition coefficient, FBC Ltd., UK. Unpublished report no.: R-12524

Mühlberger, B., (2001c). AEB 084866: Partition coefficient N-octanol/water. Unpublished Report No. R-13314

Anding, C. (2007). Flammability and self-ignition temperature of technical clofentezine, Defitraces, France. Unpublished report no.: R-21216

Franke, J. (2002). Clofentezine CF 101. Explosive properties, Siemens Axiva GmbH and Co. KG, Germany. Unpublished report no.: R-15520

Martinez, J. & Rexer, K. (2000b). Determination of the oxidizing properties. Clofentezine substance, technical, Aventis, Germany. Unpublished report no.: R-13267

Mühlberger, B. (2001b). AEB 084866: Solubility in organic solvents at 20°C, Aventis, Germany. Unpublished report no.: R-13315

Bright, A.S.S. (1987c). Solubility of clofentezine in organic solvents at 25°C, Schering Agrochemicals Ltd, UK. Unpublished report no.: R-12514A

Bright, A.S.S. (1988). Solubility of clofentezine in dimethyl sulfoxide at 25°C, Schering Agrochemicals Ltd, UK. Unpublished report no.: R-12513

Heintze, A. (2003b). Dissociation constant of clofentezine. Statement, GAB Biotechnolgie GmbH and IFU Umweltanalytik GmbH, Germany. Unpublished report no.: R-16523

14.2 Toxicology and metabolism

Toxicokinetics

Anonymous 1 (1981a). The excretion and distribution of radiolabelled residues in male and female rats dosed orally at 0.1 mg/kg with NC21314. FBC Ltd., UK. Unpublished report no.: R-12527

Anonymous 2 (1982a). The excretion and distribution of radiolabelled residues in male and female rats dosed orally at 10 mg/kg NC 21314. FBC Ltd., UK, Unpublished report No.: R-12525

Anonymous 3 (1982b). The excretion and distribution of radiolabelled residues in male and female rats dosed orally at 1000 mg/kg NC 21314. FBC Ltd., UK, Unpublished report No.: R-12537

Anonymous 4 (1983). The excretion and distribution of radiolabelled residues in male and female rats dosed intravenously at 0.1 mg/kg with [^{14}C]-NC21314. FBC Ltd., UK, Unpublished report No.: R-12542

Anonymous 5 (2007). Clofentezine (Apollo) biliary excretion in rats. Huntingdon Life Sciences Limited, UK, Unpublished report No. R-21217

Anonymous 6 (1982a) Excretion and residues of [14C]-NC 21314 in male and female mice given a single oral dose of 10 mg/kg. FBC Ltd., UK. Unpublished report No.: R-12534.

Anonymous 7 (1982b). Excretion and residues of [14C]-NC21314 in male and female rabbits following a single oral dose of 10 mg/kg. FBC Ltd., UK. Unpublished report No.: R-12536.

Anonymous 8 (1982c). Excretion and residues of [14C]-NC21314 in male and female dogs following a single oral dose of 10 mg/kg. FBC Ltd., UK. Unpublished report No. R-12532.

Anonymous 9 (1981). Excretion and tissue residues of [14C]-NC21314 in male and female dogs following a single intravenous dose at 0.1 mg/kg. FBC Ltd., UK. Unpublished Report No. R-12531.

Anonymous 10 (1983). Excretion and distribution of radioactivity after oral administration of [14C]-NC21314 to a male and female baboon. FBC Ltd., UK. Unpublished report No. R-12544.

Anonymous 11 (1985). A comparison of the metabolism of clofentezine in rat, mouse, rabbit, calf, dog and baboon. FBC Ltd., UK, Unpublished report No.: R-12550.

Anonymous 12 (1982). The whole-body autoradiography of [^{14}C]-NC 21314 in rats following oral administration at 10 mg/kg body weight. FBC Ltd., UK, Unpublished report No.: R-12538

Anonymous 13 (1991). The clearance of clofentezine residues from the tissues of rats following a single oral dose of 10 or 1000 mg/kg bodyweight. Schering Agrochemicals Ltd., UK, Unpublished report No.: R-12563

Anonymous 14 (1982). Concentration of [^{14}C]-NC 21314 and its metabolites in the plasma of rats dosed orally with NC21314 at the rate of 10 mg/kg bodyweight. FBC Ltd., UK, Unpublished report No.: R-12540

Anonymous 15 (1985a). Concentration of [^{14}C]-NC 21314 and its metabolites in the plasma of rats dosed orally with NC21314 at the rate of 1000 mg/kg bodyweight. FBC Ltd., UK, Unpublished report No.: R-12546

Anonymous 16 (1985). The metabolism of clofentezine in the rat. FBC Ltd., UK, Unpublished report No.: R-12548.

Anonymous 17 (1983). The metabolism of NC21314 in the baboon. FBC Ltd., UK. Unpublished report No. R-12543.

Anonymous 18 (1982c). The excretion and distribution of radiolabelled residues in male and female rats dosed orally at 10 mg/kg with [^{14}C]-NC21314 following 14 days pre-treatment by dosing with unlabelled NC21314 at 10 mg/kg. FBC Ltd., UK, Unpublished report No.: R-12539

Anonymous 19 (1981b). The distribution and level of radiolabelled residues in rats following repeat oral dosing with [^{14}C]-NC 21314 at 20 mg/kg/day. FBC Ltd., UK, Unpublished report No.: R-12529

Anonymous 20 (1981). The distribution of radioactivity in the maternal tissues and foetuses of rats after an oral dose of [^{14}C]-NC21314. FBC Ltd., UK, Unpublished report No.: R-12526

Anonymous 21 (1985b). Concentration of [^{14}C]-clofentezine and its metabolites in the plasma of rats given fourteen daily doses of clofentezine followed by a single dose oral dose of [^{14}C]-clofentezine at 10 mg/kg bodyweight. FBC Ltd., UK, Unpublished report No.: R-12547

Anonymous 22 (1983a). The effect of eight weeks dietary administration of NC21314 at 40 and 27000 mg/kg on the hepatic mixed-function oxidase system of the male rat. FBC Ltd., UK, Unpublished report No.: R-12528

Anonymous 23 (1983b). The effect of a two week withdrawal period on the induction of hepatic microsomal mixed-function oxidases caused by dietary administration of NC21314 at 2700 mg/kg diet. FBC Ltd., UK, Unpublished report No.: R-12533

Anonymous 24 (1986). The effect of clofentezine on the hepatic mixed function oxidase system of the male and female rat following dietary administration at 10, 40 or 400 ppm diet for two weeks. FBC Ltd., UK, Unpublished report No.: R-12553

Anonymous 25 (1984). The effect of eight weeks dietary administration of NC21314 at 27000 and 400mg/kg diet on the hepatic mixed-function oxidase system of the male mouse. FBC Ltd., UK. Unpublished report No. R-12535.

Webster, K. (2016). Clofentezine: Pharmacokinetic data evaluation of existing data. Charles River, UK, Unpublished report No.: R-36258

Acute toxicity

Anonymous 26 (1980). The acute oral toxicity of unformulated NC 21314 to the male and female rat. Fisons Ltd, UK, Unpublished report No.: R-12815

Anonymous 27 (1986a). Technical Clofentezine: Acute oral toxicity in the rat. Schering Agrochemicals Ltd., UK, Unpublished report No.: R-12630

Anonymous 28 (1980a). The acute oral toxicity of unformulated NC 21314 (CR 20099/4) to the mouse. Fisons Ltd, UK, Unpublished report No.: R-12818

Snowdon, P.J. (1980a) Determination of NC 21314 concentrations in aqueous gum tragacanth suspension for an acute oral toxicity study with mice Company Report No. R-12818 Fisons Ltd, UK Not-GLP, Unpublished

Anonymous 29 (1986b). Technical Clofentezine: Acute oral toxicity in the mouse. Schering Agrochemicals Ltd., UK. Unpublished report No.: R-126290

Anonymous 30 (1980). The acute oral toxicity of unformulated NC21314 to the hamster Company Report N0. R-12567 Fisons Ltd, UK GLP, Unpublished.

Snowdon, P.J. (1980b). Determination of NC 21314 concentrations in aqueous gum tragacanth suspension for an acute oral toxicity study with hamsters Company Report No. R-12819 Fisons Ltd, UK Not-GLP, Unpublished.

Anonymous 31 (1981). The acute oral toxicity of technical (pilot plant) NC 21314 to the male and female beagle dog Company Report No. R-12568 FBC Ltd., UK Not-GLP, Unpublished.

Snowdon, P.J. (1981 a). Determination of NC 21314 concentrations in aqueous gum tragacanth suspension for an acute oral toxicity study with dogs. Company Report No. R-12820 FBC Ltd., UK. Not GLP, Unpublished.

Anonymous 32 (1980 b). The acute dermal toxicity of unformulated NC21314 to the male and female rat. Fisons Ltd, UK. Unpublished report No.: R-12822

Snowdon, P.J. (1980c). Determination of NC 21314 concentrations in aqueous gum tragacanth suspension for an acute dermal toxicity study with rats Company Report No. R-12823 Fisons Ltd, UK Not-GLP, Unpublished.

Anonymous 33 (1987) Technical Clofentezine: Acute dermal toxicity in the rat. Schering Agrochemicals Ltd., UK. Unpublished report No.: R-12631

Anonymous 34 (1982). The acute inhalational toxicity of NC21314, 80WP CR 15569, to the rat. Schering Agrochemicals Ltd., UK. Unpublished report no.: R-12606.

Anonymous 35 (2010). Clofentezine technical: Acute inhalation toxicity (nose only) study in the rat. Harlan Laboratories Ltd, UK. Unpublished report no.: R-25420

Anonymous 36 (1980 c). The primary skin irritancy of unformulated NC 21314 (CR 20099/5) to the guinea pig. Fisons Ltd, UK. Unpublished report No.: R-12600

Snowdon, P. J. (1980d). Determination of NC 21314 concentrations in aqueous gum tragacanth suspensions for an acute skin irritancy study with guinea pigs Company Report No. R-12824 Fisons Ltd, UK Not-GLP, Unpublished

Gehrke, H. (2015). In vitro skin irritation: human skin model test with clofentezine technical. BSL Bioservice Scientific Laboratories GmbH, Germany Unpublished report no.: R-29843

Anonymous 37 (1986) Technical Clofentezine: Irritant effects on the rabbit eye. Schering Agrochemicals Ltd., UK. Unpublished report No.: R-12628.

Anonymous 38 (1982) Delayed dermal sensitisation study in the guinea pig. NC21314 technical. Toxicol Laboratories, UK. Unpublished report No.: R-12612

Germ cell mutagenicity

McConville, M. (1980) Ames test for mutagenic activity carried out with technical NC21314 (CR 20099/4). Inveresk Research International, Scotland. Unpublished report No.: R-12564.

Bowles, A.J. (2005) Clofentezine: Reverse mutation assay “Ames test” using Salmonella typhimurium). Safepharm Laboratories Ltd, UK. Unpublished report No.: R-17812.

Bootman, J. & Rees, R. (1982) Technical NC21314: Investigation of mutagenic activity in the TK+/- mouse lymphoma cell mutation system. Life Science e Research, UK. Unpublished report No.: R-12615

Wallner, B. (2015a) In vitro mammalian cell gene mutation (hprt locus) in Chinese hamster V79 cells with clofentezine technical. Eurofins BioPharma Product Testing, Germany. Unpublished report No.: R-34981.

Allen, J.A. Brooker, P.C. & Godfrey, S.R. (1987) Technical clofentezine: Metaphase chromosome analysis of CHO cells cultured in vitro. Huntingdon Research Centre, UK Unpublished report No. R-12635.

Riach, C.G. & McGregor, D.B. (1983) Technical NC21314: Induction of gene conversion and mitotic recombination in yeast. Inveresk Research International, Scotland. Unpublished report No. R-12619.

Inoue, H. & Nakajima, M. (1986) REC-assay with spore method on clofentezine. BioSafety Research Centre, Japan. Unpublished report No. R-12633.

Anonymous 39 (1982). The micronucleus study in mice using technical NC21314. FBC Ltd., UK. Unpublished report No. R-18737.

Anonymous 40 (1987). Technical clofentezine: Mouse micronucleus test. Huntingdon Research Centre, UK. Unpublished report No. R-12636.

Anonympous 41 (1983) Technical NC21314: Dominant lethal mutation assay in male rats. FBC Ltd., UK. Unpublished report No.: R-12617.

Carcinogenicity

Anonymous 42 (1985a). The oncogenicity and chronic toxicity of technical NC21314 (Clofentezine) in the diet to the rat (final report). FBC Ltd., UK. Unpublished report No.: R-12623A

Anonymous 43 (1985-1988). The oncogenicity and chronic toxicity of technical NC21314 (Clofentezine) in the diet to the rat (report addendum number 1 to number 20). FBC Ltd., UK. Unpublished report No.: R-12623A_Addenda.

Anonymous 44 (1988). Discussion Document – Clofentezine: A consideration of the background and significance of thyroid changes in rats. Schering Agrochemicals Ltd., UK. Unpublished report No.: R-12623C_Supplement.

Seamons, M.C. & Crofts, M. (1985) Determination of NC 21314 dietary concentrations in an oncogenicity and chronic toxicity study in the rat Company Report No. R-12623B FBC Ltd., UK GLP, Unpublished.

Anonymous 45 (1985). Technical NC 2131: Oncogenicity in the diet to the mouse (final report). Huntingdon Research Centre, UK. Unpublished report No.: R-12624A.

Cherry, C.P., Cannon, M.W.J. & Gopinath, C. (1985). Photomicrography addendum to histopathology report No. FSB/14 oncogenicity of technical NC 21314 in the diet to the mouse Company Report No. R-12624A_Addendum1 Huntingdon Research Centre, UK GLP, Unpublished.

Crofts, M. (1985). Determination of NC 21314 dietary concentration in a two year feeding study with mice. Company Report No. R-12624B FBC Ltd., UK Not-GLP, Unpublished.

Earl, L. (2016). Historical control data report. Long term mouse studies conducted at Huntingdon Research Centre between March 1980 and July 1983 Company Report No. R-36233 Envigo, UK Not GLP, Unpublished.

Anonymous 46 (1985). The effects of clofentezine on thyroid function. FBC Ltd., UK. Unpublished report no.: R-12552A

Anonymous 47 (1988) The effect of the dietary administration of clofentezine on the biliary excretion of a single intravenous dose of L(125I)-thyroxine. A Schering Agrochemicals Ltd., UK. Unpublished report No.: R-12556

Anonymous 48 (1988) The effect of the dietary administration of clofentezine on the excretion of a single intravenous dose of L-(125I)-thyroxine. Schering Agrochemicals Ltd., UK. Unpublished report No.: R-12558

Anonymous 49 (1983). NC 21314 tolerance study in baboons. Huntingdon Research Centre, UK. Unpublished report No.: R-12618

Anonymous 50 (1982) The effect of dietary administration of NC21314 on plasma cholesterol and triglyceride values in female rabbits. FBC Ltd., UK. Unpublished report No.: R-12614

Anonymous 51 (1986). Technical clofentezine: 6 week dietary investigation of thyroid function in the rat. FBC Ltd., UK. Unpublished report no.: R-12625

Anonymous 52 (1988). Technical clofentezine: 6 week dietary investigation function in the rat (histopathology review of the thyroid gland). FBC Ltd., UK. Unpublished report no.: R-12625

Anonymous 53 (1986). Technical NC 21314 dietary study in the rat: Electron microscopy of anterior pituitary. Huntingdon Research Centre, UK. Unpublished report no.: R-12627

Anonymous 54 (1989). Technical clofentezine: 6 week dietary investigation of thyroid function in the rat. A morphometric study on the thyroid glands. Huntingdon Research Centre, UK. Unpublished report no.: R-12638

Anonymous 55 (1990) Technical NC 21314: Investigation of effect on the thyroid in the rat. Schering Agrochemicals Ltd., UK. Unpublished report No.: R-12640A

Anonymous 56 (1986) Technical clofentezine: 90 day dietary investigation of thyroid function in the rabbit. Schering Agrochemicals Ltd., UK. Unpublished report No: R-12622A

Anonymous 57 (1987) Influence of clofentezine technical on biofunctions. The Biological Research Center for the protection of the environment (B.R.C.P.E), Japan. Unpublished report No.: R-12632

Anonymous 58 (1988) Technical clofentezine: Investigations on the indirect effect of clofentezine on the thyroid of the male rat. Schering Agrochemicals Ltd., UK. Unpublished report No.: R-12637

Anonymous 59 (1989) Technical clofentezine: Further investigations on the indirect effect of clofentezine in the male rat. Schering Agrochemicals Ltd., UK. Unpublished report No.: R-12639

Anonymous 60 (2016) 4-Week Dietary Mechanistic Study in Male Sprague Dawley Rats on Thyroid Effects with Clofentezine technical. ADAMA Irvita NV, Unpublished report No.: R-35460

OECD n°116 guidance document on the conduct and design of chronic toxicity and carcinogenicity studies supporting test guidelines 451,452 and 453, 2nd edition 2012.

Giknis, M.L.A. and C.B. Clifford (2010), Spontaneous neoplastic lesions in the Crl: CD-1 (ICR) mouse in control groups from 18-month to 2 year studies, Charles River laboratories, Wilmington, MA.

FDA (2001) Guidance for industry: Statistical aspects of the design, analysis and interpretation of chronic rodent carcinogenicity studies of pharmaceuticals.

Lin, K.K., and M.W. Ali (1994), "statistical review and evaluation of animal tumorigenicity studies," in Statistic in the Pharmaceutical Industry, Second Edition, Revised and Expanded, edited by C.R. Bunvhrt snf J.Y. Tsay, Marcel Dekker, Inc., New York.

Mode of action Carcinogenicity

Aiges HW, Daum F, Olson M, et al. (1980). The effects of phenobarbital and diphenylhydantoin on liver function and morphology. J Pediatr, 97, 22–6.

Boobis, A.R. et al., 2006. IPCS framework for analyzing the relevance of a cancer mode of action for humans. Crit. Rev. Toxicol. 36, 781–792.

Boobis AR, Cohen SM, Doerrer NG, et al. (2009). A data-based assessment of alternative strategies for identification of potential human cancer hazards. Toxicol Pathol, 37, 714–32.

Capen, C.C., 1992. Pathophysiology of chemical injury of the thyroid gland. Toxicol. Lett. 64–65, 381–388.

Capen C., 1997. Mechanistic Data and Risk Assessment of Selected Toxic End Points of the Thyroid Gland. Department of Veterinary Biosciences, The Ohio State University, 1925 Coffey Road, Columbus, Ohio 43210

Chen, H.J 1984. Age and sex difference in serum and pituitary thyrotropin concentrations in the rat: Influence by pituitary adenoma. Division of Neurobiology, Barrow Neurological Institute, St. Joseph's Hospital and Medical Center, 350 West Thomas Road, Phoenix, AZ 85013, USA.

Choksi N.Y., Gloria D. Jahnke, Cathy St. Hilaire, and Michael Shelby, 2003. Role of Thyroid Hormones in Human and Laboratory Animal Reproductive Health. National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina. Sciences International, Inc., Research Triangle Park, North Carolina. Sciences International, Inc., Alexandria, Virginia

Christianson, D. et al, 1981. The Sex-Related Difference in Serum Thyrotropin Concentration Is Androgen Mediated. Endocrinology, Volume 108, Issue 2, 1 February 1981, Pages 529–535

Cohen, S. M. (2010). Evaluation of possible carcinogenic risk to humans based on liver tumors in rodent assays: the two-year bioassay is no longer necessary. Toxicol. Pathol. 38, 487-501.

Dellarco, V.L., McGregor, D., Berry, C. Sir, Cohen, S.M., Boobis, A.R., 2006. Thiazopyr and thyroid disruption: Cast study within the context of the 2006 IPCS human relevance framework for analysis of a cancer mode of action. Crit. Rev. Toxicol. 36, 793–801.

Dohler, K.D. et al , 1979. The rat as a model for the study of drug effects on thyroid function: consideration of methodological problems. Pharmacolog. Ther. 5, 305-318.

Elcombe CR, Peffer RC, Wolf DC, Bailey J, Bars R, Bell D, et al. (2014). Mode of action and human relevance analysis for nuclear receptor-mediated liver toxicity: a case study with phenobarbital as a model constitutive androstane receptor (CAR) activator. Crit Rev Toxicol, 44, 64–82.

Frith C.H and Manikk Chandra. (1991). Incidence, distribution and morphology of amyloidosis in Charles River CD-1 mice. Tocicol. Pathol. Volumen 19, number 2, 1991.

Goldsworthy TL, Fransson-Steen R. (2002). Quantitaton of the cancer process in C57BL/6J, B6C3H/HeJ mice. Toxicol Pathol, 30, 97-105.

Hard, 1998. Recent developments in the investigation of thyroid regulation and thyroid carcinogenesis. American Health Foundation, Valhalla, NY 10595 USA.

Hill, R.N., Erdreich, L.S., Paynter, O.E., Roberts, P.A., Rosenthal, S.L., Wilkinson, C.F., 1989. Review: thyroid follicular cell carcinogenesis. Fundam. Appl. Toxicol. 12, 629–697.

Hirose Y., Nagahori N., Yamada T. et al, 2009; Comparison of the effects of the synthetic pyrethroid Metofluthrin and phenobarbital on CYP2B form induction and replicative DNA synthesis in cultured rat and human hepatocytes. Toxicology, 258, 64-9

Holsapple et al (2006) Mode of action in relevance of rodent liver tumors to human cancer risk. Toxicol Sci. 2006, 89(1):51-6.

Hurley P, Hill R.N., and Whiting R.J., 1998. Mode of Carcinogenic Action of Pesticides Inducing Thyroid Follicular Cell Tumors in Rodents. Environmental Health Perspectives, Volume 106, Number 8.

IPCS, 2007. IPCS Framework for Analyzing the Relevance of a Cancer Mode of Action for Humans. IPCS Harmonization Project Document No. 4, Part 1, IPCS Framework for Analysing the Relevance of a Cancer Mode of Action for Humans and Case-studies. Accessible at. pp. 10–29. http://www.who.int/ipcs/methods/harmonization/areas/ cancer_mode.pdf.

Junguee Lee, Shinae Yi, Yea Eun Kang, Hyeon-Woo Kim, Kyong Hye Joung, Hae Joung Sul, Koon Soon Kim,1 and Minho Shong, 2016. Morphological and Functional Changes in the Thyroid Follicles of the Aged Murine and Humans.

Klaunig, J.E., Babich, M.A., et all, 2003. PPARα agonist-induced rodent tumors: modes of action and human relevance. Crit.Rev. Toxicol. 33, 655–780.

Klaunig, J.E., 2012. Chapter 8: chemical carcinogenesis. In: Klaassen (Ed.), Casarett & Doull's Toxicology: the Basic Science of Poisons, eighth ed. McGraw-Hill, New York,pp. 330–390.

Kleinstreuer, et al, 2016. Adverse outcome pathways: From research to regulation scientific workshop report. Regul. Toxicol Pharmacol, 76: 39-50.

Kolaja KL, Stevenson DE, Johnson JT, et al. (1996a). Subchronic effects of dieldrin and phenobarbital on hepatic DNA synthesis in mice and rats. Fundam Appl Toxicol, 29, 219–28.

Kolaja KL, Stevenson DE, Johnson JT, et al. (1996b). Dose dependence of phenobarbital promotion of preneoplastic hepatic lesions in F344 rats and B6C3F1 mice: effects on DNA synthesis and apoptosis. Carcinogenesis, 17, 947–54.

Kolaja KL, Stevenson DE, Walborg Jr EF, Klaunig JE. (1996c). Reversibility of promoter induced hepatic focal lesion growth in mice. Carcinogenesis, 17, 1403–9.

La Vecchia, C. and Negri, E. (2014). A review of epidemiological data on epilepsy, phenobarbital, and risk of liver cancer. Eur. J. Cancer Prev. 23 (1), 1-7.

LeBaron MJ, Geter DR, Rasoulpour RJ, Gollapudi BB, Thomas J, Murray J, et al. (2013). An integrated approach for prospectively investigating a mode-of-action for rodent liver effects. Toxicol Appl Pharmacol, 270, 164–73.

Majeed S.K. (1993). Survey on spontaneous systemic amyloidosis in aging mice. Arzneimittelforschung, 43(2):170-8.

Martin P., Riley R., Thompson P., Williams D., Back D., Owen A., 2010. Effect of prototypical inducers on ligand activated nuclear receptor regulated drug disposition genes in rodent hepatic and intestinal cells. Acta Pharmacologica Sinica (2010) 31: 51–65

McClain, R.M., Levin, A.A., Posch, R., Downing, J.C., 1989. The effect of phenobarbital on the metabolism and excretion in rats. Toxicol. Appl. Pharmacol. 99, 216–228.

McClain, R.M., 1992. Thyroid gland neoplasia: non-genotoxic mechanisms. Toxicol.Lett. 64–65, 397–408.

Meek M.E., Bucher J.R., Cohen S.M., Dellarco V., Hill RN., Lehman-Mckeeman L.D. et al., (2003) A framework for human relevance analysis of information on carcinogenic modes of action. Crit. Rev. Toxicol. 33, 591–653.

Meek M.E., Bucher J.R., Cohen S.M., Dellarco V., Hill RN., Lehman-Mckeeman L.D., et al. 2003. IPCS conceptual framework for evaluating a mode of action for chemical carcinogenesis. Regul Toxicol Pharmacol, 34, 146–52.

Meek, M.E. et al, (2014). New developments in the evolution and application of the WHO/IPCS framework on mode of action/species concordance analysis. J Appl. Toxicol. 2014, 34(1): 1-18.

Monro, A. (1993). The paradoxical lack of interspecies correlation between plasma concentrations and chemical carcinogenicity. Regul. Toxicol. Pharmacol. 18, 115-135.

OECD, 2013. Guidance Document on Developing and Assessing Adverse Outcome Pathways. Series on Testing and Assessment, Vol. No. 184, Paris, France.

OECD, 2016.Users'Handbook Supplement to the Guidance Document for Developing and Assessing AOPs. Series on Testing and Assessment, Vol. No. 233, Paris, France.

Orton TC, Doughty SE, Kalinowski AE, et al. (1996). Expression of growth factors and growth factor receptors in the liver of C57BL/10J mice following administration of phenobarbitone. Carcinogenesis, 17,973–81.

Osimitz TG, Lake BG. (2009). Mode-of-action analysis for induction of rat liver tumors by pyrethrins: relevance to human cancer risk. Crit Rev Toxicol, 39, 501–11.

Papineni S., Marty M. S. b, Rasoulpour R J., LeBaron M J., Pottenger LH., Eisenbrandt D., 2014, Mode of action and human relevance of pronamide-induced rat thyroid tumors. Regulatory Toxicology and Pharmacology 71 (2015) 541–551.

Parzefall, W., Erber,E., Sedivy, R. and Schulte-Hermann,R. (1991). Testing for induction of DNA synthesis in human hepatocyte primary cultures by rat liver tumor promoters. Cancer Res. 51, 1143-1147.

Peffer, R., et al., 2017. Adverse Outcome Pathway Number 107. Constitutive Androstane Receptor Activation Leading to Hepatocellular Adenomas and Carcinomas (In Mouse and Rat). AOPwiki available for comment and citation at. https://aopwiki.org/aops/ 107.

Peffer. R.C, LeBaronb. M.J., Battalorac. M, Bomannd, M.J., et al (2018). Minimum datasets to establish a CAR-mediated mode of action for rodent liver tumors. Regulatory Toxicology and Pharmacology 96, 106-120.

Phillips JC, Price RJ, Cunninghame ME, et al. (1997). Effect of piperonyl butoxide on cell replication and xenobiotic metabolism in the livers of CD-1 mice and F344 rats. Fundam Appl Toxicol, 38, 64–74.

Pirttiaho HI, Sotaniemi EA, Ahokas JT, Pitka¨nen U. (1978). Liver size and indices of drug metabolism in epileptics. Brit J Clin Pharmacol, 6, 273–8.

Pirttiaho HI, Sotaniemi EA, Pelkonen RO, Pitka¨nen U. (1982). Hepatic blood flow and drug metabolism in patients on enzyme-inducing anticonvulsants. Eur J Clin Pharmacol, 22, 441–5.

Shizu, R., et al., 2013. Xenobiotic-induced hepatocyte proliferation associated with constitutive active/androstane receptor (CAR) or peroxisome proliferator-activated receptor alpha (PPARalpha) is enhanced by pregnane X receptor (PXR) activation in mice. PLoS One 8, e61802.

Sonich-Mullin C., Fielder R., Wiltse J., Baetcke K., Dempsey J., Fenner-Crisp P, et al. (2001). IPCS conceptual framework for evaluating a mode of action for chemical carcinogenesis. Regul Toxicol Pharmacol, 34, 146–152.

Stanley LA, Horsburgh BC, Ross J, Scheer N, Wolf CR., 2006. PXR and CAR: nuclear receptors which play a pivotal role in drug disposition and chemical toxicity. Drug Metab Rev. 2006;38(3):515-97.

Thatcher, N.J., Caldwell, J., 1994. Origins of hepatomegaly produced by dexamethasone (DEX), pregnenolone 16 alpha-carbonitrile (PCN) and phenobarbitone (PB) in female Sprague-Dawley rats. Biochem. Soc. Trans. 22, 132S.

Timsit, Y.E., Negishi, M., 2007. CAR and PXR: the xenobiotic-sensing receptors. Steroids 72, 231–246.

Whysner J, Ross PM, Williams GM. (1996). Phenobarbital mechanistic data and risk assessment: enzyme induction, enhanced cell proliferation, and tumor promotion. Pharmacol Ther, 71, 153–91.

Williams GM. (1997a). Chemicals with carcinogenic activity in the rodent liver; mechanistic evaluation of human risk. Cancer Lett, 117, 175–88.

Winterhoff, H and Sourgens, H, 1979. The predictive value of animal experiments in the detection of side effects of drugs on thyroid function. Pharmacol Ther B. 1979;5(1-3):325-32.

Willson, T.M., Kliewer, S.A., 2002. PXR, CAR and drug metabolism. Nat. Rev. Drug Discov. 1, 259–266.

Yamada, T., Okuda, Y., Kushida, M., et al, 2014. Human hepatocytes support the hypertrophic but not the hyperplastic response to the murine nongenotoxic hepatocarcinogen sodium phenobarbital in an in vivo study using a chimeric mouse with humanized liver. Toxicol. Sci. 142 (1), 137–157.

Reproductive toxicity

Anonymous 61 (1984). Technical clofentezine: A dietary multigeneration study in the rat. FBC Ltd., UK. Unpublished report no.: R-12620A

Anonymous 62 (1986). Technical clofentezine: A dietary multigeneration study in the rat. Report addendum 1. FBC Ltd., UK. Unpublished report no.: R-12620A_Addendum.

Seamons, M.C. & Crofts, M. (1984). Determination of NC 21314 dietary concentrations in a multigeneration rat reproduction study. FBC Ltd., UK. Unpublished report No. R-12620B

Anonymous 65 (1982). Technical NC 21314: A teratogenicity study in the rat. Plus addendum. FBC Ltd., UK. Unpublished report no.: R-12610B

Crofts, M. (1982a). Determination of NC 21314 concentrations in suspensions for a teratogenicity study with rats. FBC Ltd., UK. Unpublished report no.: R-12610B

Anonymous 66 (1983). Effect of technical NC 21314 on pregnancy of the rabbit (teratology study). Huntingdon Research Centre, UK. Unpublished report no.: R-12613A

Anonymous 67 (1983). Effect of technical NC 21314 on pregnancy of the rabbit (teratology study). Report addendum 1 and 2. FBC Ltd., UK. Unpublished report no.: R-12613A

Crofts, M. (1982b) Effect of technical NC 21314 on pregnancy of the rabbit - Determination of the active ingredient in suspensions. FBC Ltd., UK. Unpublished report no.: R-12613B

Specific target organ toxicity-repeated exposure

Anonymous 68 (1980) The 17 day cumulative oral toxicity of NC 21314 (NC 21314/4) to male and female rats. Company Report No. R-12565 Fisons Ltd, UK Not-GLP, Unpublished.

Anonymous 69 (1982a) The palatability of technical NC 21314 in the diet to the rat Company Report No. R-12611 FBC Ltd., UK Not-GLP, Unpublished.

Snowdon, P.J. (1981b) Determination of NC 12314 concentrations in rodent diet for a palatability study with rats Company Report No. R-12611 FBC Ltd., UK Not-GLP, Unpublished.

Anonymous 70 (1982 a) The palatability of technical NC 21314 to the male and female mouse Company Report No. R-12607 FBC Ltd, UK GLP, Unpublished.

Anonymous 71 (1981) The 17-day cumulative oral toxicity of NC 21314 to the dog Company Report No. R-12601 FBC Ltd., UK Not-GLP, Unpublished.

Anonymous 72 (1980) The palatability of diets containing NC 21314 (CR 20099/5) to the dog Company Report No. R-12566A FBC Ltd., UK Not-GLP, Unpublished.

Anonymous 73 (1983) NC 21314 preliminary oral toxicity study in Beagle dogs Company Report No. R-12616 Huntingdon Research Centre plc, UK Not- GLP, Unpublished.

Anonymous 63 (1982 b). The 90-day dietary toxicity study of pilot plant technical NC 21314 (CR 20099/5) to the rat. FBC Ltd., UK Unpublished report No. R-12604A

Anonymous 64 (1988). Technical NC 21314: 90 day dietary toxicity study in the rat (histopathology of the thyroid gland). FBC Ltd., UK Unpublished report No.: R-12604A

Crofts, M. (1981) Determination of NC 21314 dietary concentrations in a 90 day feeding study with rats Company Report No. R-12604B FBC Ltd., UK Not GLP, Unpublished.

Anonymous 74. (1981) The 90-day dietary toxicity study of technical NC 21314 (CR 20099/5, pilot plant) to the male and female rat. FBC Ltd. Unpublished report No.: R12605A

Anonymous 75 (1983) Technical NC 21314: 90-day dietary toxicity study in the rat – additional examination of the liver histology. FBC Ltd. Unpublished report No.: R-12605C

Anonymous 76 (2006). Neurotoxicity study by dietary administration to CD rats for 13 weeks Huntingdon Life Sciences Ltd, Eye Research, UK. Unpublished report no.: R- 18916.

Anonymous 77 (1982). The 90-day subchronic toxicity of technical (pilot plant) NC 21314 in the diet to the mouse. FBC Ltd., UK. Unpublished report no.: R-12609A

Anonymous 78 (1981). The 90-day subchronic oral toxicity of technical (CR 20099/8) NC 21314 in the diet to the dog. FBC Ltd., UK. Unpublished report no.: R-1260

Anonymous 79 (1984). NC 21314 oral toxicity study in dogs (final report: repeated dietary administration for 52 weeks). Huntingdon Research Centre plc, UK. Unpublished report no.:R- 12621A

14.3 Environment

1.	Bright, A.A.S. & Stalker, A.M. (1990). Clofentezine: Determination of the partition coefficient between N-octanol and water at 25°C, Schering Agrochemicals Ltd, UK. Unpublished report no.: R-12519
2.	Mühlberger, B. (2001). AEB 084866: Solubility in organic solvents at 20°C, Aventis, Germany. Unpublished report no.: R-13315
3.	B.9.2.2/04-05. In DAR (2005). Bioconcentration of Clofentezine in bluegill sunfish: Analysis of radioactivity in fish tissues. Schering Agrochemicals Ltd., Report No. ENVIR/87/48.
4.	Aufderheide, J. (2009). Clofentezine: Life-cycle toxicity test of the saltwater mysid, *Americamysis bahia*, conducted under flow-through conditions. ABC Laboratories, Inc. Unpublished report No.: R-23968.
5.	Aufderheide, J. (2016). Clofentezine: Life-cycle toxicity test of the saltwater mysid, *Americamysis bahia*, conducted under flow-through conditions (final report addendum). ABC Laboratories, Inc. Unpublished report No.: R-23968B.
6.	Barber, I. & Lattimore, A.E. (1992) Determination of the effects of [^{14}C]-Clofentezine on the life cycle of *Daphnia magna*. In DAR (2005). Schering Agrochemicals Limited, UK. Unpublished report No.: R-12679.
7.	Barrett, K.L, Arnold, D.J. 1988a. In DAR 2005. Determination of the acute toxicity of clofentezine technical to *Daphnia magna.*
8.	Brice, A. (2007) [^{14}C]-Clofentezine: Photodegradation and quantum yield in water. Covance Laboratories Ltd, UK. Unpublished report No.: R-18905.
9.	Bright, A.A.S. & Stalker, A.M. (1990) Clofentezine: Determination of the partition coefficient between N-octanol and water at 25°C. Schering Agrochemicals Ltd, UK. Unpublished report No.: R-12519
10.	Clarke, N. (2001) Clofentezine: assessment of ready biodegradability: CO_2 evolution test. SafePharm Laboratories, UK. DAR 2005.Unpublished report No.: R-12854.
11.	Dabrunz, A. 2015a. Metabolite AE C593600. Toxicity to single cell green alga Pseudokirchneriella subcapitata Hinkák under laboratory conditions.
12.	Dabrunz, A. 2015b. Metabolite AE F092117. Toxicity to single cell green alga Pseudokirchneriella subcapitata Hinkák under laboratory conditions.
13.	Dean, G. (2007) Clofentezine (Apollo 50 SC) – Calculation of the real half-lives of aqueous photolysis using GCSOLAR. Huntingdon Life Sciences, UK. Unpublished report No.: R-18905a.
14.	Dorgerloh M. (2003). Acute toxicity of SIR 8514-2-chlorobenzamide (tech) to water fleas (Daphnia magna).
15.	Eser, S. (2015a). Metabolite AE C593600: Toxicity to the water flea Daphnia magna Straus under laboratory conditions (Acute immobilisatios test-static).
16.	Göcer, M. (2016) [^{14}C]-Clofentezine hydrolysis as a function of pH. Eurofins Agroscience Services EcoChem GmbH, Unpublished report No.: R-34979.

17. Hanstveit, A.O. 1987. The effects of Apollo 50 SC on the growth of the alga Selenastrum capricornotum. In DAR (2005). Unpublished report. R-12665.
18. Heinicke, S. (2016). [14C]-Clofentezine phototransformation of [14C]-Clofentezine – soil photolysis. ADAMA Irvita NV, Unpublished report R-34977,
19. B.9.2.1/02. In DAR (2005). Determination of the acute toxicity of NC21314 to bluegill sunfish (*Lepomis macrochirus*). FBC Ltd, UK. Unpublished report No.: R-12649.
20. B.9.2.1/04-05. Determination of the accumulation and elimination of [14C]-Clofentezine in Bluegill sunfish (Lepomis macrochirus). Schering Agrochemicals Limited, UK. Unpublished report No.: R-12666.
21. Ilieva, D. (2016) Aerobic mineralisation of [tetrazine-^{14}C(U)]-clofentezine in surface water. Eurofins Agroscience Services EcoChem GmbH / Eurofins Agroscience Services Ecotox GmbH, Unpublished report No.: R-34980.
22. Kelly, 1985a. The kinetics of the hydrolysis of NC 21314 under acid, neutral and basic conditions. DAR 2005. R-12520
23. Kelly, 1985b. The photodegradation of [14C]-Clofentezine in water under sunlight conditions.
24. (B.9.2.1/07). Acute Toxicity of 2-chlorobenzoic acid to rainbow trout (Oncorhynchus mykiss) in a 96-hour static limit-test
25. Kuhl, R., Deierling, T. (2010b). Toxicity of 2-chlorobenzoic acid to Daphnia magana in a Static 48-h inmobilisation limit-test.
26. Kuhl, R., Deierling, T. (2010c). Toxicity of 2-chlorobenzoic acid to Pseudokirchneriella subcapitata in an algal growth inhibition test.
27. Lines, D. (1981) Determination of the acute toxicity of technical NC21314 to the water flea, *Daphnia magna*. In DAR (2005). Schering Agrochemicals Limited, UK. Unpublished report No. R-15417.
28. Mead, C., Mullee, D.M. (2001). In DAR (2005). 2-Chlorobenzonitrile: algal inhibition test. Accepte in DAR (2005).
29. Mühlberger, B. (2001) AE B084866: Partition coefficient N-octanol/water (HPLC-method). Aventis, Germany. Unpublished report No.: R-13314.
30. Oldersma, H. Hanstveit, A.O. & Pullens, M.A.H.L. (1983) The effect of the product NC21314 technical on the growth of the green alga *Scenedesmus pannonicus*. In DAR (2005). TNO, The Netherlands Unpublished report No.: R-12642.
31. S. Smith, I.D. Kelly, 1985. Characterization of the hydrolysis products of clofentezine in aqueous solution under acid, neutral and basic conditions. R-12522
32. Spickermann, G. (2016) Kinetic evaluation of the decline of clofentezine and its metabolites observed in a hydrolysis study. ADAMA Deutschland GmbH, Unpublished report No.: R-34979b
33. Spickermann (2016b). Kinetic evaluation of the decline of clofentezie and its metabolite 2-CBN (2-chlorobenzonitrile) observed in a soil photolysis study.

34.	Spickermann, G. (2016c) Kinetic evaluation of the decline of Clofentezine observed in two aquatic sediment systems. ADAMA Deutschland GmbH, Unpublished report No.: R-23966b.
35.	Schupner, J.K. & Christ, M.T. (1993) The toxicity of clofentezine technical to early life stages of the rainbow trout *Oncorhynchus mykiss*, in a flow through system. In DAR (2005). NOR-AM Chemical Company. Unpublished report No.: R-12681.
36.	Schupner, J.K., Christ, M.T. (1993). The toxicity of Chlofentezine technical to early life stages of the rainbow trout *Onchorhyncus mykiss,* in a flow through system.
37.	(B.9.2.1/08). Metabolite AE C593600: Toxicity to the Rainbow Trout Oncorhynchus mykiss under laboratory conditions (Acute toxicity test- Semi Static)
38.	(B.9.2.1/09). Metabolite AE F092117: Toxicity to the rainbow trout Oncorhynchus mykiss under laboratory conditions (acute toxicity test – static)
39.	Traub, M. (2016). [Tetrazine-14C(U)]-clofentezine determination of adsorption/desorption behaviour in four soils. ADAMA Irvita NV, Unpublished report No.: R-34978
40.	Turk, R. (2014) [^{14}C]Clofentezine – Aerobic aquatic sediment metabolism. Springborn Smithers Laboratories, Unpublished amended report No.: R-23966a.
41.	van der Gaauw, A. (2001a) Investigation of the volatilisation of [^{14}C]-clofentezine from soil and plant leaf surfaces. RCC Limited, Unpublished report No.: R-13319.
42.	van der Gaauw, 2001. [14C]-Clofentezine hydrolysis at three different pH values. DAR 2005. R-13318
43.	(B.9.2.1/06). In DAR (2005). 2-Chlorobenzonitrile: Acute toxicity to rainbow trout (Oncorhynchus mykiss)
44.	Wetton, P.M., Mullee, D.M. (2001b). In DAR (2005). 2-Chlorobenzonitrile: Acute toxicity to Daphnia magna.

15 ANNEXES

Robust summaries of the studies correspondig to the Annex I of the CLH Report are included in the Renewal Assessment Report (RAR, 2018) of the active substance clofentezine. Volumes B.2, B.6, B.8 and B.9 have been attached for public consultation.

They are also publicly available in the following link:

https://www.efsa.europa.eu/en/consultations/call/181029