Committee for Risk Assessment

RAC

Annex 1
Background document
to the Opinion proposing harmonised classification and labelling at EU level of

benfluralin (ISO); *N*-butyl-*N*-ethyl-α,α,α-trifluoro-2,6-dinitro-*p*-toluidine

EC Number: 217-465-2
CAS Number: 1861-40-1

CLH-O-0000006963-64-01/F

The background document is a compilation of information considered relevant by the dossier submitter or by RAC for the proposed classification. It includes the proposal of the dossier submitter and the conclusion of RAC. It is based on the official CLH report submitted to public consultation. RAC has not changed the text of this CLH report but inserted text which is specifically marked as 'RAC evaluation'. Only the RAC text reflects the view of RAC.

Adopted

18 March 2021

CLH report

Proposal for Harmonised Classification and Labelling

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

International Chemical Identification:

benfluralin (ISO);

N-butyl-*N*-ethyl-*α,α,α*-trifluoro-2,6-dinitro-*p*-toluidine

EC Number: **217-465-2**

CAS Number: **1861-40-1**

Index Number:

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Version number: 3 **Date: October 2019**

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON BENFLURALIN (ISO); *N*-BUTYL-*N*-ETHYL-α,α,α-TRIFLUORO-2,6-DINITRO-*p*-TOLUIDINE

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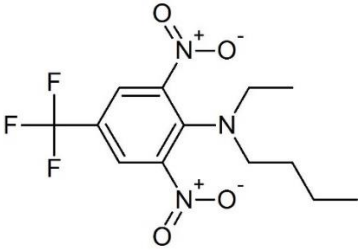
ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON BENFLURALIN (ISO);
N-BUTYL-*N*-ETHYL-α,α,α-TRIFLUORO-2,6-DINITRO-*p*-TOLUIDINE

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

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON BENFLURALIN (ISO);
N-BUTYL-*N*-ETHYL-α,α,α-TRIFLUORO-2,6-DINITRO-*p*-TOLUIDINE

Name(s) in the IUPAC nomenclature or other international chemical name(s)	IUPAC nomenclature: *N*-butyl-*N*-ethyl-*α*,*α*,*α*-trifluoro-2,6-dinitro-*p*-toluidine CA nomenclature: *N*-butyl-*N*-ethyl-2,6-dinitro-4-(trifluoromethyl)-benzenamine
Other names (usual name, trade name, abbreviation)	benefin, balan, binnell, benephin, flubalex, benthrodine, benefex
ISO common name (if available and appropriate)	benfluralin
EC number (if available and appropriate)	217-465-2
EC name (if available and appropriate)	benfluralin
CAS number (if available)	1861-40-1
Other identity code (if available)	CIPAC No: 285
Molecular formula	$C_{13}H_{16}F_3N_3O_4$
Structural formula	
SMILES notation (if available)	O=[N+](O)c1cc(cc([N+](=O)O)c1N(CCCC)CC)C(F)(F)F
Molecular weight or molecular weight range	335.3 g/mol
Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	Benfluralin is not a resolved optical isomer
Description of the manufacturing process and identity of the source (for UVCB substances only)	Benfluralin is not an UVCB substance
Degree of purity (%) (if relevant for the entry in Annex VI)	≥ 96.0%

1.2 Composition of the substance

Table 2: Constituents (non-confidential information)

Constituent (Name and numerical identifier)	**Concentration range (% w/w minimum and maximum in multi-constituent substances)**	**Current CLH in Annex VI Table 3.1 (CLP)**	**Current self- classification and labelling (CLP)**
Benfluralin CAS: 1861-40-1	≥ 96.0%	Not listed	Skin Irrit. 2 (H315) Skin Sens. 1B (H317) Eye Irrit. 2 (H319) Aquatic Acute 1 (H400) M=10 Aquatic Chronic 1 (H410) M=10

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)**	**Current self- classification and labelling (CLP)**	**The impurity contributes to the classification and labelling**
EBNA (ethyl-butyl-nitrosamine) CAS: 4549-44-4	≤ 0.01% *	None	Acute Tox 4 (H302)	No

* The technical specifiactions, either the current or the newly proposed technical specification, are not supported by the toxicological assessment; in addition considering the impurity of known toxicological concern (EBNA) that has been tested up to 0.085 mg/kg in genotoxicity studies. EBNA has structural resemblance to known carcinogens.

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**
No additives	-	-	-	-	-

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	-	-	-								
Dossier submitters proposal		benfluralin (ISO); *N*-butyl-*N*-ethyl-*α*,*α*,*α*-trifluoro-2,6-dinitro-*p*-toluidine	217-465-2	1861-40-1	Carc. 2 Repr. 2 Lact. STOT SE 2 Skin Irrit. 2 Eye Irrit. 2 Skin Sens. 1 Aquatic Acute 1 Aquatic Chronic 1	H351 H361d H362 H371 H315 H319 H317 H400 H410	GHS08 GHS07 GHS09 	H351 H361d H362 H371 H315 H319 H317 H410 		 M=10 M=10	
Resulting Annex VI entry if agreed by RAC and COM		benfluralin (ISO); *N*-butyl-*N*-ethyl-*α*,*α*,*α*-trifluoro-2,6-dinitro-*p*-toluidine	217-465-2	1861-40-1	Carc. 2 Repr. 2 Lact. STOT SE 2 Skin Irrit. 2 Eye Irrit. 2 Skin Sens. 1	H351 H361d H362 H371 H315 H319 H317	GHS08 GHS07 	H351 H361d H362 H371 H315 H319 H317		 M=10 M=10	

					Aquatic Acute 1 Aquatic Chronic 1	H400 H410	GHS09	H410			

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

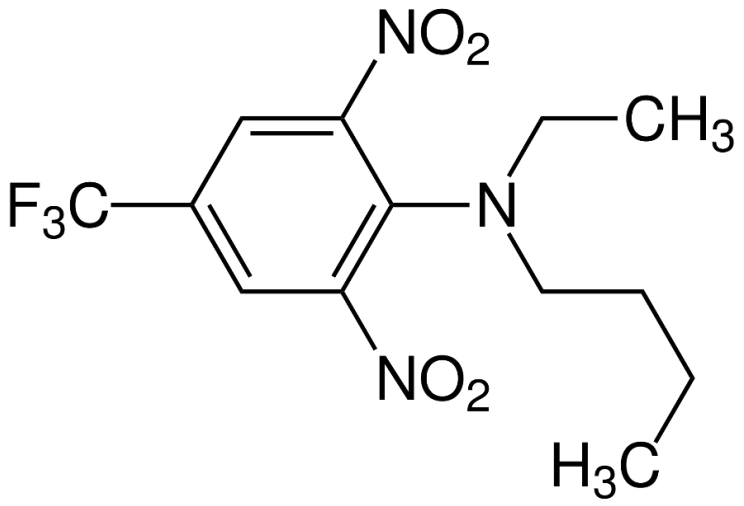
Hazard class	**Reason for no classification**	**Within the scope of public consultation**
Explosives	Data lacking	Yes
Flammable gases (including chemically unstable gases)	Hazard class not applicable	No
Oxidising gases	Hazard class not applicable	No
Gases under pressure	Hazard class not applicable	No
Flammable liquids	Hazard class not applicable	No
Flammable solids	Data conclusive but not sufficient for classification	Yes
Self-reactive substances	Data lacking	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 lacking	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	**Harmonised classification proposed**	Yes
Serious eye damage/eye irritation	**Harmonised classification proposed**	Yes
Respiratory sensitisation	Data lacking	No
Skin sensitisation	**Harmonised classification proposed**	Yes
Germ cell mutagenicity	Data conclusive but not sufficient for classification	Yes
Carcinogenicity	**Harmonised classification proposed**	Yes
Reproductive toxicity	**Harmonised classification proposed**	Yes
Specific target organ toxicity-single exposure	**Harmonised classification proposed**	Yes
Specific target organ toxicity-repeated exposure	Data conclusive but not sufficient for classification	Yes
Aspiration hazard	Hazard class not assessed in this dossier	No
Hazardous to the aquatic environment	**Harmonised classification proposed**	Yes
Hazardous to the ozone layer	Data conclusive but not sufficient for classification	Yes

3 HISTORY OF THE PREVIUOS CLASSIFICATION AND LABELLING

Benfluralin is an active substance in the scope of Regulation (EC) 1107/2009. It is not listed in Annex VI of CLP and has not been considered for harmonised classification and labelling previously.

RAC general comment

Benfluralin is a pre-emergent dinitroaniline herbicide used to control grasses and other weed species. It is registered for uses against weeds in chicory and lettuce crops which have been evaluated in the context of the Plant Protection Products Regulation EC 1107/2009 (EFSA, 2019). Benfluralin affects seed germination and prevents weed growth by inhibition of root and shoot development.



Benfluralin is not listed in Annex VI of CLP and has not been considered for harmonised classification and labelling previously. All hazard endpoints were open for consideration. The proposed classification by the dossier submitter (DS) covers several endpoints; Skin Irrit. 2, Eye Irrit. 2, Skin Sens. 1, STOT SE 2, Carc. 2, Repr. 2 (development), Lact., Aquatic Acute 1 (M=10) and Aquatic Chronic 1 (M=10).

Benfluralin was discussed at the EFSA Pesticides Peer Review Expert's Meeting 182 in September 2018 and at the Pesticide Peer Review Meeting 05 (joint Mammalian toxicology–Ecotoxicology meeting) in May 2019. The final peer review report was published in 2019 (EFSA Journal 2019;17(11):5842).

The oral absorption of benfluralin is limited, around 20%. The active substance is widely distributed, showing some affinity for fat, extensively metabolised and rapidly eliminated, mainly via faeces.

4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

Benfluralin is an active substance in the scope of Regulation 1107/2009.

5 IDENTIFIED USES

Benfluralin is a herbicide. The representative uses evaluated for the formulated product "Bonalan (EF-1553)" were spray applications followed by mechanical incorporation in soil against annual weeds and seedlings of some perennial weeds in chicory and lettuce.

6 DATA SOURCES

Information submitted for the approval of the pesticide active substance.

Renewal Assessmet Report (draft version) August 2018.

7 PHYSICOCHEMICAL PROPERTIES

Table 7: Summary of physicochemical properties

Property	**Value**	**Reference**	**Comment (e.g. measured or estimated)**
Physical state at 20°C and 101,3 kPa	Crystalline solid	Huntley, K., Edgar, L. (1999c)	Visual assessment Purity of test substance: 99.9%
Melting/freezing point	t = 66.4 ºC	Huntley, K., Edgar, L. (1999a)	OECD 102 Purity of test substance: 99.9%
Boiling point	t > 205 ºC (decomposes)	Smith, A.J. (2000a)	OECD 103 Purity of test substance:99.9%
Relative density	$D_4^{21} = 1.42$	Huntley, K. & Edgar, L. (1999b)	OECD 109 Purity of test substance: 99.9%
Vapour pressure	$\rho = 1.8 \times 10^{-3}$ Pa at 20 °C $\rho = 4.3 \times 10^{-3}$ Pa at 25 °C	Dunning, J. (2016a)	OECD 104 Purity of test substance: 99.9%
Surface tension	Not applicable, as substances with a water solubility < 1 mg/L not need to be tested	Dunning, J. (2016e)	-
Water solubility	0.064 mg/L at 20 °C.	Dunning, J. (2016b)	OECD 105 Purity of test substance: 99.9%
Partition coefficient n-octanol/water	Log P_{ow} = 5.27 at 20 °C	Dunning, J. (2016d)	OECD 107 Purity of test substance: 99.9%
Flash point	Not required, as melting point is > 40 °C.	-	-
Flammability	Non-flammable	Garofani, S. (2002)	EC Method A10 Purity of test substance: 97.5%
Explosive properties	Not **explosive**	Garofani, S. (2001)	EC Method A14 Purity of test substance: 96.2%

Property	Value	Reference	Comment (e.g. measured or estimated)
Selfignition temperature	No selfignition temperature < 304 °C	Garofani, S. (2003a)	EC Method A15* Purity of test substance: 97.5% * Since benfluralin presents a low melting point (66.4 °C), EC Method A15 for liquids was preferred instead of A16 used for solids.
Oxidising properties	Non-oxididising	Garofani, S. (2003b)	EC Method A17 Purity of test substance: 97.5%
Granulometry	No data	-	-
Stability in organic solvents and identity of relevant degradation products	No data Solubility in organic solvents at 20°C: n-octanol 23 g/L n-heptane 40 g/L Methanol 41 g/L xylene > 250 g/L 1,2-dichloroethane >250 g/L Acetone >250 g/L Ethyl acetate >250 g/L	Dunning, J. (2016c)	EC Method A6 Purity of test substance: 99.6%
Dissociation constant	pKa = -0.59	Heim, D. (2005)	Estimation method using ACD/pKa DB software (ver. 6.0)
Viscosity	Not relevant, the substance is as solid.		

8 EVALUATION OF PHYSICAL HAZARDS

8.1 Explosives

Table 8: Summary table of studies on explosive properties

Method	Results	Remarks	Reference
EC Method A14	Effect of flame: no thermal sensitivity. Fall Hammer test: no mechanical sensitivity (shock). Friction test: no mechanical sensitivity (friction).	Benfluralin is not explosive within the criteria of this study.	Garofani, S. (2001) a.s. as manufactured, 96.2%

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

There was no evidence of thermal or mechanical (shock or friction) sensitivity when benfluralin was tested in the standard explosivity study A14. Hence, benfluralin was not explosive within the criteria of this study.

Benfluoralin contains groups associated with explosive properties (nitro compounds), it's oxygen balance is -143.2, which is above the limit of -200.

8.1.2 Comparison with the CLP criteria

Benfluoralin did not fulfil the criteria of the screening procedure, hence the acceptance procedure should have been performed. A substance is considered for classification as explosive where a positive result is obtained in a test series as outlined in figure 2.1.2 of Annex I of the CLP regulation, i.e. sensitivity towards heat, shock or friction. The substance was tested only according with EU method A.14, hence despite the negative results, it cannot be conclusively concluded that benfluralin is not explosive,

8.1.3 Conclusion on classification and labelling for explosive properties

Not classified – data lacking

8.2 Flammable gases (including chemically unstable gases)

Hazard class not applicable, substance is a solid.

8.3 Oxidising gases

Hazard class not applicable, substance is a solid.

8.4 Gases under pressure

Hazard class not applicable, substance is a solid.

8.5 Flammable liquids

Hazard class not applicable, substance is a solid.

8.6 Flammable solids

Table 9: Summary table of studies on flammable solids

Method	Results	Remarks	Reference
EC Method A10	Test substance did not ignite but melted, combustion did not propagate along the test pile.	Benfluralin is not highly flammable.	Garofani, S. (2002) a.s. as manufactured, 97.5%

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

The test substance did not ignite but melted, and combustion did not propagate along the test pile. Benfluralin is not highly flammable.

8.6.2 Comparison with the CLP criteria

A substance (non-metal) is classified as a flammable solid when the burning time is < 45 seconds or the burning rate is > 2.2 mm/s. Benfluralin melted, but did not ignite. Therefore, the criteria for classification as a flammable solid are not met.

8.6.3 Conclusion on classification and labelling for flammable solids

Not classified – conclusive but not sufficient for classification

8.7 Self-reactive substances

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

Benfluralin conatins groups associated with explosive properties (nitro compounds), none of the test series E (preliminary procedure) or H was performed.

In the study, conducted in accordance with OECD 103, an endothermic reaction corresponding to a boiling point was not observed with temperatures ≤ 400 ºC (highest test temperature). Decomposition or volatilisation began at 205 ºC.

8.7.2 Comparison with the CLP criteria

The available data are not sufficient to conclude if the substance is self-reactive.

8.7.3 Conclusion on classification and labelling for self-reactive substances

Not classified – data lacking

8.8 Pyrophoric liquids

Hazard class not applicable, substance is a solid.

8.9 Pyrophoric solids

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

No studies are available. Benfluralin has, however, been handled in air in other studies conducted and refered to in this dossier, where no incidences of self-ignition when exposed to air have been reported.

8.9.2 Comparison with the CLP criteria

According to Section 2.10.4.1 of Annex 1 of CLP, classification procedure for pyrophoric solids need not be applied when experience in e.g. handling shows that the substance or mixture does not ignite spontaneously on coming into contact with air at normal temperatures (i.e. the substance is known to be stable at room temperature for prolonged periods of time (days)).

8.9.3 Conclusion on classification and labelling for pyrophoric solids

Not classified – conclusive but not sufficient for classification

8.10 Self-heating substances

Table 10: Summary table of studies on self heating substances

Method	Results	Remarks	Reference
EC Method A15	Selfignition temperature: 304 °C (250 mg sample, 38 s ignition delay time, 997 mbar pressure)	Since benfluralin presents a low melting point (66.4 °C), method A15 for liquids was preferred instead of method A16 used for solids.	Garofani, S. (2003a) a.s. as manufactured, 97.5%

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

A study conducted in accordance with EC method A15 is available. In this study, benfluralin did not selfignite up to a temperature of 304 °C. Since benfluralin presents a low melting point (66.4 °C), method A15 for liquids was preferred instead of method A16 used for solids.

8.10.2 Comparison with the CLP criteria

Studies conducted according to EC Method A15 are generally inappropriate for a sound assessment, and the findings do not lead to a classification. However, substances or mixtures with a low melting point, i.e. < 160 °C, should not be considered for classification in this class since the melting process is endothermic and the substance-air surface is drastically reduced.

8.10.3 Conclusion on classification and labelling for self-heating substances

Not classified – conclusive but not sufficient for classification

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 derived in accordance with the recommended test method in CLP have been provided. Benfluralin has, however, been handled in water in other studies conducted and refered to in this dossier, where no incidences of violent reaction and emission of flammable gases have been reported.

8.11.2 Comparison with the CLP criteria

According to Section 2.12.4.1 of Annex 1 of CLP, the classification procedure for this hazard class need not be applied if the chemical structure does not contain metals or metalloids, or if experience in production or handling shows that the substance does not react with water or if the substance is known to be soluble in water to form stable solution.

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

Not classified – conclusive but not sufficient for classification

8.12 Oxidising liquids

Hazard class not applicable, substance is a solid.

8.13 Oxidising solids

Table 11: Summary table of studies on oxidising solids

Method	Results	Remarks	Reference
EC Method A17	Benfluralin is non-oxidising. The highest burning rate of test mixtures of the test substance with cellulose (3.08 mm/s) were higher than that of the reference mixture of barium nitrate/cellulose 40/60% w/w (0.72 mm/s). The test substance appears to have oxidising properties, however, a false positive result was revealed when the test was repeated using inert kieselguhr (4.76 mm/s) instead of cellulose.	A "Wick effect" was observed, i.e. sample melted when ignition source was applied and became flammable in the presence of a solid support.	Garofani, S. (2003b) a.s. as manufactured, 97.5%

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

In a study conducted according to EC method A17, the maximum burning rate of the test substance/cellulose mixture was determined to be 3.08 mm/s (test substance/cellulose 60/40% w/w). Maximum burning rate of reference mixture was 0.72 mm/s (barium nitrate/cellulose 40/60% w/w). However, when conducted with inert kieselguhr burning rates were even faster than those observed with combustible cellulose; max = 4.76 mm/s (test substance/kieselguhr 70/30% w/w and 60/40% w/w). It was concluded that presence of cellulose was thus not important for burning of mixtures. Instead, "wick effect" was observed, i.e. sample melted when ignition source was applied and became flammable in the presence of a solid support. It is therefore concluded that benfluralin is not an oxidising substance.

8.13.2 Comparison with the CLP criteria

Benfluralin contains oxygen chemically bounded to nitrogen, and should therefore be regarded as potentially oxidising. Test O.1 in Part III, subsection 34.4.1 of the UN Recommendations on the Transport of Dangerous Goods, Manual of Tests and Criteria shall be used for classification. Studies on oxidising properties of benfluralin, however, follow EC Method A.17. Becasue the results generated from the EC Method A.17 are not directly comparable with the CLP criteria, the results can be regarded as inconclusive.

8.13.3 Conclusion on classification and labelling for oxidising solids

Not classified – data lacking

8.14 Organic peroxides

Hazard class not applicable, the substance is not an organic peroxide.

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 studies are available, and the corrosiveness of benfluralin on metals have not been considered.

8.15.2 Comparison with the CLP criteria

The classification criteria (UN Test C.1; test temperature of 55 °C) exclude solids, also having a melting temperature > 55 °C. Benfluralin is a crystalline solid, and has a melting point of 66.4 °C. Further, the water solubility of benfluralin is low, i.e. 0.064 mg/L at 20 °C. Therefore, benfluralin is not expected to materially damage metals.

8.15.3 Conclusion on classification and labelling for corrosive to metals

Not classified – conclusive but not sufficient for classification

RAC evaluation of physical hazards

Summary of the Dossier Submitter's proposal

Explosive

The screening procedure (EC method A.14) was used to derive no thermal sensitivity (effect of flame), no mechanical sensitivity (shock, Fall Hammer test) and no mechanical sensitivity (friction, Friction test). Despite the negative results, the DS stated that the acceptance procedure should have been performed, as benfluralin contains groups associated with explosive properties (nitro compounds). Therefore, it cannot be conclusively concluded that benfluralin is not explosive. The overall DS conclusion was that no classification for explosive is warranted due to lack of data.

Flammable solids

Based on the outcome of EC method A.10, the test substance did not ignite but melted, and combustion did not propagate along the test pile. The DS stated that a substance (non-metal) is classified as a flammable solid when the burning time is < 45 seconds or the burning rate is > 2.2 mm/s. Benfluralin melted but did not ignite. Therefore, the criteria for classification as a flammable solid are not met. The overall DS conclusion was that no classification for flammable solid is warranted based on conclusive but not sufficient for classification data.

Self-reactive substances

Benfluralin contains groups associated with explosive properties (nitro compounds) and none of the test series E (preliminary procedure) or H was performed. In a study, conducted in accordance with OECD TG 103, an endothermic reaction corresponding to a boiling point was not observed with temperatures ≤ 400ºC (highest test temperature). Decomposition or volatilisation began at 205ºC. The overall DS conclusion was that no classification for self-reactive substance is warranted due to lack of data.

Pyrophoric solids

No studies are available. No incidences of self-ignition when exposed to air have been reported. The substance is known to be stable at room temperature for prolonged periods of time (days). The overall DS conclusion was that, based on the CLP criteria, no classification for pyrophoric solids is warranted based on conclusive but not sufficient for classification data.

Self-heating substances

An available study, conducted according to EC method A.15, resulted in a self-ignition temperature of 304°C (250 mg sample, 38 s ignition delay time, 997 mbar pressure). The DS also stated that since benfluralin presents a low melting point (66.4°C), method A.15 for liquids was preferred instead of method A.16 used for solids. The DS acknowledged that studies conducted according to EC method A.15 are generally inappropriate for a sound assessment and the findings do not lead to a classification. However, substances or mixtures with a low melting point, i.e. < 160°C, should not be considered for classification in this class since the melting process is endothermic and the substance-air surface is drastically reduced.

The overall DS conclusion was that, based on the CLP criteria, no classification for self-heating substances is warranted based on conclusive but not sufficient for classification data.

Substances which in contact with water emit flammable gases

No data derived in accordance with the recommended test method in CLP have been provided. Benfluralin has, however, been handled in water in other studies conducted and referred to in the CLH dossier, where no incidences of violent reaction and emission of flammable gases have been reported. The DS stated that according to Section 2.12.4.1 of Annex I of CLP, the classification procedure for this hazard class need not be applied if the chemical structure does not contain metals or metalloids, or if experience in production or handling shows that the substance does not react with water or if the substance is known to be soluble in water to form stable solution. Thus, the overall DS conclusion was that, based on the CLP criteria, no classification for this hazard class is warranted based on conclusive but not sufficient for classification data.

Oxidising solids

In a study conducted according to EC method A.17, the maximum burning rate of the test substance/cellulose mixture was determined to be 3.08 mm/s (test substance/cellulose 60/40% w/w). Maximum burning rate of reference mixture was 0.72 mm/s (barium nitrate/cellulose 40/60% w/w). However, when conducted with inert kieselguhr, burning rates were even faster than those observed with combustible cellulose; max = 4.76 mm/s (test substance/kieselguhr 70/30% w/w and 60/40% w/w). It was concluded that presence of cellulose was thus not important for burning of mixtures. Instead, "wick effect" was observed, i.e. sample melted when ignition source was applied and became flammable in the presence of a solid support.

Benfluralin contains oxygen chemically bounded to nitrogen and should therefore be regarded as potentially oxidising. Test O.1 in Part III, subsection 34.4.1 of the UN Recommendations on the Transport of Dangerous Goods, Manual of Tests and Criteria should have been used for classification. Studies on oxidising properties of benfluralin, however, followed EC method

A.17. Because the results generated from the EC method A.17 are not directly comparable with the CLP criteria, the results can be regarded as inconclusive.

Thus, the overall DS conclusion was that no classification for oxidising solids is warranted due to lack of data.

Corrosive to metals

No studies are available, and the corrosiveness of benfluralin to metals has not been considered. However, according to the DS, the classification criteria (UN Test C.1; test temperature of 55°C) exclude solids having a melting temperature > 55°C. Benfluralin is a crystalline solid, and has a melting point of 66.4°C. Further, the water solubility of benfluralin is low, i.e. 0.064 mg/L at 20°C. Therefore, benfluralin is not expected to materially damage metals.

Thus, the overall DS conclusion was that, no classification for corrosive to metals is warranted based on conclusive but not sufficient for classification data.

Comments received during consultation

A company/manufacturer proposed that the available data should take precedence over the DS's observations that benfluralin contains groups associated with explosive properties. The DS responded that negative results of the EU method A.14 is not sufficient to conclusively exclude explosive properties, and the full screening procedure (Annex I, 2.1.4.2) should be used instead.

Assessment and comparison with the classification criteria

Explosive

RAC agrees with the DS conclusion for **no classification as an explosive due to lack of data**.

Flammable solids

RAC agrees with the DS conclusion for **no classification as flammable solid due to conclusive data.**

Self-reactive substances

RAC agrees with the DS conclusion for **no classification as self-reactive substance due to lack of data.**

Pyrophoric solids

RAC agrees with the DS conclusion for **no classification as pyrophoric solid due to conclusive data**.

Self-heating substances

RAC agrees with the DS conclusion for **no classification as self-heating substance due to conclusive data.**

Substances which in contact with water emit flammable gases

RAC agrees with the DS conclusion for **no classification as flammable gas due to conclusive data.**

Oxidising solids

RAC agrees with the DS conclusion for **no classification as oxidising solid due to lack of data**.

Corrosive to metals

RAC agrees with the DS conclusion for **no classification as corrosive to metals due to conclusive data.**

9 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

9.1 Short summary and overall relevance of the provided toxicokinetic information on the proposed classifications

Absorption
The absorption of benfluralin was relatively rapid with peak plasmatic concentrations achieved at 5-10 hours at the low dose, and at 24 hours at the high dose. The AUC-values indicated that plasmatic concentration increased proportionally to the dose in both males and females. Based on the excretion in urine and residues in tissues and carcass at the low dose, the systemic absorption is 15% for males and 23% for females (approximately 20% as a mean for males and females).

Excretion
The plasmatic half-life at both dose levels was approximately 55 hours for males and approximately 62 hours for females. Most of the radioactivity was eliminated at 48 h. On day 7, males had excreted 14.9% and 78.9% in urine and faeces respectively at the low dose, and 11.8% and 77.7% in urine and faeces respectively at the high dose. Females had excreted 22.6% and 71% in in urine and faeces respectively at the low dose, and 19.9% and 64.6% in urine and faeces respectively at the high dose. The fraction of radioactivity remaining in the animals, seven days after dosing, was very low, accounting for between 0.5 and 1.5% of administered dose. The majority of this was found in the carcass and in the liver.

Biliary excretion in males was 7-8% at both dose levels. In females, this was 13% at low dose and 6% at the high dose. The loss of radioactivity by expired air was negligible.

Distribution
Tissue distribution study showed that benfluralin was widely distributed. Initially carcass, liver, fat and kidneys had the highest percentage of total radioactivity in males and females at both dose levels. Tissue concentration percentages dropped rapidly over the 48 hour study period. The highest residue levels were recovered in the liver (0.1% of the dose) and carcass (0.5-1.5% of the dose), while other tissues contained <0.1%. The RBC concentrations were 2.4-4 times higher than the plasmatic concentrations. In both dose groups, tissue residues were mostly higher in females than in the males. This was most marked in the adipose tissue, indicating an affinity to body fat. Pre-treatment with unlabeled benfluralin had no apparent effect on the absorption, distribution or excretion of the radiolabeled test substance.

Metabolism
Metabolism study showed that benfluralin was the most prominent compound recovered in the faeces, representing 35% of the total dose. Three non-polar metabolites were identified, two in feces and one in urine, indicating that the benfluralin was dealkylated and reduced. In addition, the presence of approximately 100 metabolite fractions were demonstrated, corresponding individually to about 0.05-0.9% of the dose. Further identification of these metabolites was not successful.

A comparative metabolism study showed that all metabolites formed in human liver microsomes >5% of the initial substrate concentration were also formed in mouse, rat, dog, and rabbit liver microsomes. Two metabolites were formed only in human liver microsomes. However, the abundance of these metabolites was <5% of initial substrate concentration. Qualitative and quantitative differences in metabolite formation were observed between mouse, rat, dog, and rabbit. It is to be noted that the abundance of the metabolites in this study was less than seen in *in vivo*, probably due the absence of reductive metabolism in the *in vitro* study.

10 EVALUATION OF HEALTH HAZARDS

Acute toxicity

The acute toxicity, skin and eye irritation and skin sensitisation of benfluralin have been investigated in well conducted, guideline- and GLP-compliant studies. Acute toxicity has been investigated *in vivo* in rats via the oral and inhalation routes, and in rabbits via the dermal route. Skin and eye irritation were conducted *in vivo* in rabbits, while the skin sensitisation potential has been investigated in Guinea pigs in a modified Buehler method and the Maximisation test of Magnusson and Kligman. The studies were performed with different batches of benfluralin, containing 95.64-98.2% of benfluralin.

10.1 Acute toxicity - oral route

Table 12: 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**
OECD TG 401	Rat Fischer 344 (strain) M, F 5/ dose level	Benfluralin (95.8%) Oral Batch No.: TSN100037 Vehicle: 25% suspension in 0.5% w:v hydroxy-propyl methyl-cellulose	M/F: 5000 mg/kg bw	LD_{50} fasted M/F: >5000 mg/kg bw	Author (1996) Report No. DR-0097-3397-006A/ CA 5.2.1/01

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

The acute toxicity of benfluralin (purity 95.8%) was investigated according to OECD 401 (1987), with the minor exeption that dose suspensions were not analyzed for homogeneity, stability and concentration verification. Five male and five female fasted Fischer 344 received a single oral gavage administration of 5000 mg/kg bw (dose volume 20 mL/kg) given as a 25% suspension in 0.5% w:v hydroxypropyl methylcellulose (limit test). No control animals were included in this study. Rats were observed for clinical signs immediately post-dosing, at frequent intervals during Day 1, and at least once daily thereafter for up to 14 days post dosing (study termination Day 15).

Two females died, one during Day 1 and one overnight between Day 1 and 2. The cause of death for both animals was deemed to be a gavage error, resulting in laboured breathing and salivation on Day 1. Necropsy findings in these animals included pulmonary atelectasis, hydrothorax and fibrinous inflammation of the pleural cavity. Clinical signs in the surviving rats included hypoactivity (5/5 males, 3/4 females, day 1), perineal soiling with urine (1/5 males, 4/4 females, day 1-3) and lachrimation (1/5 males, 3/4 females, day 1-2). All animals appeared symptom-free at the end of the 14-day observation period. No significant effect on bodyweight was recorded for either sex, and there were no treatment-related gross pathologic observations in any of the surviving rats. Hence, the acute toxicity of benfluralin after oral administration was relatively low.

10.1.2 Comparison with the CLP criteria

According to the CLP criteria, classification for acute oral toxicity is warranted if the LD50 of a substance is ≤ 2000 mg/kg bw. The acute oral LD50 value to male and female rats was >5000 mg/kg bw. Overall, the available data on benfluralin does not meet the criteria for classification.

10.1.3 Conclusion on classification and labelling for acute oral toxicity

Not classified – conclusive but not sufficient for classification

10.2 Acute toxicity - dermal route

Benfluralin was investigated in two reliable, guideline- and GLP-compliant dermal toxicity studies, conducted in rabbits.

Table 13: 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**
OECD TG 402	Rabbit New Zealand White Lsr: (NZW) M, F 5/dose level	Benfluralin (95.64%) Batch No.: 231EF4 Applied in solid form	M/F: 5000 mg/kg bw	LD50 M/F: >5000 mg/kg bw	Author (1990) Report No. B04990/ CA 5.2.2/01
OECD TG 402	Rabbit New Zealand White (strain) M, F 5/dose level	Benfluralin (95.8%) Batch No.: ACD13683 Vehicle: Moistened with 5.0 ml of 0.5% aqueous methylcellulose	M/F: 5000 mg/kg bw	LD50 M/F: >5000 mg/kg bw	Author (1997) Report No. 971155/ CA 5.2.2/02

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

Benfluralin was either administrated in solid form (purity 95.64%) or moistened with aqueous methylcellulose (BALAN technical: 95.8% purity) in two well conducted studies (OECD 402). In both studies, the acute dermal and systemic toxicity of benfluralin was assessed in five male and five female New Zealand White rabbits. Benfluralin was applied at a dose of 5000 mg/kg bodyweight to the clipped dorsum of each rabbit. The dose was applied to approximately 10% of the body surface and test material was maintained in place for 24 hours. No control animals were included in the studies.

Rabbits were observed for clinical signs one hour after removal of the wrap, and daily for the subsequent remainder 28 days after dermal administration of benfluralin in solid form. None of the treated rabbits died and no overt signs of toxicity were noted during the 28 day post–exposure observation period. The test material stained the treated sites yellow for up to 23 days. Desquamation and signs of skin irritation (moderate to severe erythema and oedema), which cleared in all animals within 28 days, were observed in the animals. No treatment–related pathological changes were evident during necropsy, but the kidneys of one female rabbit contained slight multiple depressed pale foci characteristic of incidental renal encephalitazoonosis. The acute dermal LD_{50} value to male and female rats was >5000 mg/kg bw.

Upon dermal administration of benfluralin moistened with aqueous methylcellulose, rabbits were observed for clinical signs and/or dermal reactions on the day of dosing and then daily (on work days) for the remainder of the 15 day observation period. All animals survived and there were no effect on body weight. Some degree of erythema and oedema (males and females), burns and fissures at the site (males) and scale formation and scabs (males and females) was observed. The scab formation was not reversed during the study as only one of the ten rabbits had normal skin by Day 15. Macroscopic abnormalities were limited to observation of a moderate crust present at the dermal test site of several rabbits. The acute dermal LD_{50} value to male and female rats was >5000 mg/kg bw.

10.2.2 Comparison with the CLP criteria

According to the CLP criteria, a substance is classified for acute dermal toxicity if the LD_{50} value is ≤ 2000 mg/kg bw. In the available studies, benfluralin was found to have an LD_{50} value of >5000 mg/kg bw. Hence, benfluralin does not meet the criteria for classification.

10.2.3 Conclusion on classification and labelling for acute dermal toxicity

Not classified – conclusive but not sufficient for classification

10.3 Acute toxicity - inhalation route

One reliable, acute inhalation toxicity study is available, which was conducted in rats in accordance with OECD TG 403 (1981). The study is presented in section 10.11 in the CLH report (classification for STOT-SE category 2 is proposed).

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

Please refer to section 10.11.

10.3.2 Comparison with the CLP criteria

Acute inhalation toxicity means those adverse effects occurring following an inhalation exposure of 4 hours and classification is generally assigned on the basis of evident lethality (LC_{50} value), or, where the potential to cause lethality can be concluded from evident toxicity (e.g. from the fixed dose procedure). In the available acute inhalation study, the LC_{50} (4 hr, aerosol) for male and female rats was >2.16 mg/L air, which was the highest technically attainable concentration (two males and one female died during the exposure). The Mass Median Aerodynamic diameter (MMAD) was 25.88±4.06 µm and 23.72±3.59 µm. According to the CLP guidance, results from studies in which substances with particle size with a MMAD > 4 µm have been tested can generally not be used for classification. Hence, benfluralin does not meet the criteria for classification.

10.3.3 Conclusion on classification and labelling for acute inhalation toxicity

Not classified – conclusive but not sufficient for classification

RAC evaluation of acute toxicity

Summary of the Dossier Submitter's proposal

Acute Oral Toxicity

The DS did not propose classification. There was one guideline compliant (OECD TG 401, 1987) acute oral study available as shown in table below:

Table: *Summary of the Acute oral toxicity studies*

Study, guideline, animal strain	**Test substance,**	**Dose levels, duration of exposure**	**Value LD_{50}**	**Reference**
OECD TG 401 (1987) GLP: Yes Fischer 344	Benfluralin (95.8%) Vehicle: 0.5% w/v hydroxy-propyl methyl cellulose	5/sex/dose (5000 mg/kg bw)	LD_{50} > 5000 mg/kg bw	B.6.2.1/ RAR, 2019 Anonymous, 1996

All animals at the highest dose of 5000 mg/kg bw survived except for 2 females where the cause of death was determined to be as the result of a gavage error.

Animals showed a number of clinical signs including hypoactivity (5/5 males, 3/4 females, day 1), perineal soiling with urine (1/5 males, 4/4 females, day 1-3) and lachrymation (1/5 males, 3/4 females, day 1-2). All animals appeared symptom-free at the end of the 14-day observation period.

No significant effect on bodyweight was recorded for either sex, and there were no treatment-related gross pathological observations in any of the surviving rats.

Acute Dermal Toxicity

The DS proposed no classification. Benfluralin was investigated in two reliable, guideline- (OECD TG 402, 1987) and GLP-compliant dermal toxicity studies, conducted in rabbits.

Benfluralin was either administrated in solid form (purity 95.64%) or moistened with aqueous methylcellulose (95.8% purity) and was tested in 5 animals/sex at 5000 mg/kg bw.

Study 1: Anonymous, 1990. Benfluralin applied in solid form (not moistened with water)

The dose, under a damp semi-occlusive wrap, covered 10% of the body surface and treatment was maintained for 24 hours. Rabbits were observed for clinical signs one hour after removal of the wrap, and daily for the subsequent 28 days. None of the treated rabbits died and no overt signs of toxicity were noted during the 28-day post-exposure observation period. The test material stained the treated sites yellow for up to 23 days. Desquamation and signs of skin irritation (moderate to severe erythema and oedema), were observed in these animals and cleared in all animals within 28 days. No treatment-related pathological changes were evident during necropsy.

Study 2: Anonymous, 1997. Benfluralin applied moistened with aqueous methylcellulose

The test substance was moistened with 5.0 mL of 0.5% aqueous methylcellulose and placed on the dorsum under a semi-occlusive gauze/cotton patch. Rabbits were observed for clinical signs and/or dermal reactions on the day of dosing and then daily (on workdays) for the remainder of the 15-day observation period. All rabbits survived the 5000 mg/kg bw dose level. Some degree of erythema and oedema (males and females), burns and fissures at the site (males) and scale formation and scabs (males and females) was observed. The scab formation was not reversed during the study as only one of the ten rabbits had normal skin by day 15. Macroscopic abnormalities were limited to observation of a moderate crust present at the dermal test site of several rabbits.

According to the DS, there was no evidence for treatment related lethality and the acute lethal dermal dose LD_{50} of benfluralin was greater than 5000 mg/kg bw. No classification was proposed.

Acute Inhalation Toxicity

The DS proposed no classification for acute toxicity via inhalation. One reliable, acute inhalation toxicity study was available, which was conducted in Fisher 344 rats in accordance with OECD TG 403 (1981), with 10 animals/sex/dose (vs. 5 animals/sex/dose recommended according to the most current guideline). The 10 males and 10 females were exposed in a 4-hour, nose only exposure study (Anonymous, 1986), to solid particulate aerosols of technical benfluralin containing up to 2.16 mg/L air benfluralin. This was the highest technically achievable concentration for benfluralin (97.3%). The Mass Median Aerodynamic Diameter (MMAD) was 25.88 ± 4.06 µm and 23.72 ± 3.59 µm at target concentrations of 1.12 or 2.16 mg/L air. A significant respirable fraction (with particles that can reach all regions of the respiratory tract) was not achievable. The DS concluded that benfluralin did not meet the criteria for acute inhalation toxicity classification. However, two males and one female exposed to 2.16 mg/L died on day 1 and on the basis of necropsy findings, the DS proposed STOT SE classification (see further down).

The acute inhalation LC_{50} (4h, aerosol) for male and female rats in the study was determined by the DS to be > 2.16 mg benfluralin/L air.

Comments received during consultation

There was one comment from a Member State Competent Authority (MSCA). They agreed that the study results do not support classification of benfluralin for acute toxicity. However, it was noted that the two dermal studies in rabbits support classification of the test substance as a skin irritant. They also highlighted concerns with respect to the acute inhalation study, suggesting that the data did not support classification because a respirable fraction of the tested substance was not generated.

Assessment and comparison with the classification criteria

Acute Oral Toxicity

According to the CLP criteria, classification for acute oral toxicity is warranted if the LD_{50} of a substance is ≤ 2000 mg/kg bw. The acute oral LD_{50} value for male and female rats was > 5000 mg/kg bw. Overall, the available data on benfluralin does not meet the criteria for classification. The ATE is considered > 5000 mg/kg bw. RAC agrees with the DS proposal of **no classification for acute oral toxicity**.

Acute Dermal Toxicity

The LD_{50} of benfluralin was greater than 5000 mg/kg bw. RAC agrees with the DS that **no classification for acute dermal toxicity is warranted.**

Acute Inhalation Toxicity

According to ECHA guidance on the application of the CLP criteria, v.5.0 (CLP guidance), results from studies in which substances show particle sizes with a MMAD > 4 µm can generally not be used for classification while at the same time recognising that expert judgement is required where there are indications of high toxicity.

In the available acute inhalation study, industry brought to attention the technical difficulty with generating an inhalable dust fraction from a wet cake of benfluralin. The highest technically attainable concentration of benfluralin was 2.16 mg/L air but the MMAD was 25.88 ± 4.06 µm. A respirable fraction was not achieved at any tested concentration of benfluralin. The number of animals used was satisfactory according to the guidelines in force at the time of the study (OECD TG 403, 1981 stipulated at least 5 animals of each sex but did not indicate an upper limit to the number of animals that may be used). There were three animal deaths in total at the highest dose tested; two males (2/10) and one female (1/10); necropsy revealed hepatic and pulmonary congestion, and this is considered further under classification for STOT SE. The three deaths occurred during the exposure period on day 1. There were no indications of high toxicity. The effects following inhalation exposure were not severe enough to result in mortality in one half or more of the animals. The study data may be considered inconclusive for classification purposes due to the difficulties in generating a significant respirable particle size fraction (e.g. MMAD ≤ 2.6 µm accounted for 5.7% of the total particles) for the solid aerosol.

The LC_{50} for male and female rats in the study could not be determined, but it is reasoned to be > 2.16 mg/L. A respirable fraction was not achieved at any tested concentration. RAC agrees with the DS that **no classification is warranted due to inconclusive data.**

10.4 Skin corrosion/irritation

Two guideline- and GLP-compliant skin irritation studies are available, which was conducted in rabbits using two different batches of benfluralin.

Table 14: 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**
OECD TG 404	Rabbit New Zealand White M, F 3/ dose level	Benfluralin (purity 95.64%) Batch No.: 231EF4	M/F: 0.5 g (not moistened)	Individual mean scores for dermal irritation after 24, 48 and 72 h were as follows: Erythema: M: 1.0/2.0/1.7 F: 2.0/2.0/2.0 Oedema: M: 1.0/1.0/1.0 F: 1.0/1.0/1.3 Erythema persists in 3 out of 6 rabbits at the end of the study (day 15).	Author (1990) Report No. B09690/ CA 5.2.4/01
OECD TG 404	Rabbit New Zealand White, (Lsr:(NZW) M, F 3/ dose level	Benfluralin (purity 95.8%) Batch No.: ACD13683	M/F: 0.5 g (moistened with 0.5 mL of 0.5% aqueous methylcellulose)	The mean scores for dermal irritation after 24, 48 and 72 h were as follows: Erythema: M: 0/0/0.3 F: 0.7/0.7/0.3 Oedema: M: 0.7/0.3/1.0 F: 2.0/0.3/1.3 Scaliness observed in 5 out of 6 rabbits at study termination (day 9).	Author (1997) Report No. 971153/ CA 5.2.4/02

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

Two skin irritation studies were performed in New Zealand White rabbits according to guideline OECD TG 404. The complete evaluation of the studies are given in the Annex 1 to the CLH report (RAR, section B.6.2.4). In the first study, benfluralin (not moistened) was applied and maintained for four hours as 0.5 g dose under a damp semi-occlusive wrap, approximately 6 cm^2 in area, to the clipped dorsum of each rabbit. The animals were observed after one hour after removal and daily for the subsequent 14 days. The degree of dermal irritation erythema and oedema was assessed according to the method of Draize.

In the second study, benfluralin (moistened with 0.5 mL of 0.5% aqueous methylcellulose) was applied as 0.5 g dose on the clipped dorsum of each rabbit and covered with a gauze patch with cotton backing. The dose was maintained in situ for four hours by an elastic jacket. The application sites were graded for erythema and oedema within thirty minutes, and 24, 48 and 72 hours after removal of the patches and on test days 7, 8 and 9. The study was terminated at day 9.

In both studies, benfluralin caused moderate skin irritation, and the mean scores of 24-72 hours were below the trigger for classification according to CLP Regulation (EC) No. 1272/2008. However, erythema persisted in 3 out of 6 rabbits at the end of the observation period (when applied as not moistened), and scaliness was

observed in 5/6 animals at the end of the observation period (when applied as moistened). According to OECD TG 404, reversibility of dermal lesions should be considered in evaluating irritant responses.

10.4.2 Comparison with the CLP criteria

According to the CLP criteria for classification of skin irritation, a substance is irritant to the skin when it produces reversible damage to the skin following its application up to 4 hours. A substance should be classified for skin irritation category 2 if any of the following criteria are met:

(1) mean value of ≥ 2.3 - ≤ 4.0 for erythema/eschar or for oedema in at least 2 of 3 tested animals from gradings at 24, 48 and 72 hours after patch removal or, if reactions are delayed, from grades on 3 consecutive days after the onset of skin reactions; or

(2) inflammation that persists to the end of the observation period normally 14 days in at least 2 animals, particularly taking into account alopecia (limited area), hyperkeratosis, hyperplasia, and scaling; or

(3) in some cases where there is pronounced variability of response among animals, with very definite positive effects related to chemical exposure in a single animal but less than the criteria above.

Benfluralin meets criteria 2, but not criteria 1 and 3.

Therefore, based on the persistent erythema in 3/6 animals until the end of the study and on the scaliness observed in 5/6 animals at termination, the test substance should be classified as 'Skin Irrit. Cat. 2' (H315: Causes skin irritation). This classification proposal is in accordance with EFSA's conclusion on the previous evaluation (EFSA Scientific Report (2008) 127, 1-82, Conclusion on the peer review of benfluralin), where benfluralin was proposed to be classified as a R38 skin irritant. However, the previous proposal was based on the 21-day studies in rabbits, described in Annex 1 to the CLH report (RAR, section B.6.3). At the time of the previous evaluation, the findings from the available acute skin irritation studies did not trigger classification as a skin irritant.

10.4.3 Conclusion on classification and labelling for skin corrosion/irritation

Classification as 'Skin Irrit. Cat. 2' (H315) is considered appropiate

RAC evaluation of skin corrosion/irritation

Summary of the Dossier Submitter's proposal

Two skin irritation studies were performed in New Zealand White (NZW) rabbits according to GLP and OECD TG 404 (1981). Both were acceptable to the DS.

Anonymous, 1990

The skin irritation potential of benfluralin (purity: 95.64%, batch # 231EF4) was assessed in 6 animals in total (3 male and 3 female NZW rabbits). Benfluralin (not moistened with water) was applied topically as a 0.5 g dose under a damp semi-occlusive wrap, approximately 6 cm^2 in area, to the clipped dorsum of each rabbit. The dose was maintained *in situ* for 4 hours by an elastic sleeve. After removing the semi-occlusive wrap, the treatment sites were rinsed

with warm water. Animals were observed for signs of toxicity 1 hour after removal of the semi-occlusive wrap and daily for the subsequent 14 days.

Anonymous, 1997

The skin irritation potential of benfluralin (purity: 95.8%, batch # ACD13683) was assessed in 6 animals in total (3 male and 3 female NZW rabbits). Benfluralin (moistened with 0.5 mL of 0.5% aqueous methylcellulose) was applied topically as a 0.5 g dose to the clipped dorsum of each rabbit and covered with a gauze patch with cotton backing. The dose was maintained *in situ* for 4 hours by an elastic jacket. After removing the patches, the treatment sites were rinsed with warm water. Animals were observed for signs of toxicity within 30 minutes, and 24, 48 and 72 hours after removal of the patches and on test days 7, 8 and 9. Animals were sacrificed on day 9.

Results

In both studies, mean gradings for severity of damage, i.e. erythema/eschar, or for oedema in at least 4 of 6 tested animals at the 24, 48- and 72-hour time points (after patch removal) were not sufficient to support a classification for skin irritation.

The DS outlined that the criteria for classification according to CLP Regulation (Annex I: 3.2.2.8.2) were in fact met when consideration was given to reversibility of skin lesions, in particular the persistence of inflammation to the end of each study period. In Anonymous (1990), erythema persisted in 3 out of 6 rabbits at the end of the observation period (15 days). In Anonymous (1997), the erythema and oedema were unremarkable but scaliness was observed in 5/6 animals at the end of the observation period (9 days).

The DS also noted that EFSA (2008) supported skin irritation classification previously based on results from two 21-day dermal subchronic toxicity studies in rabbits (Anonymous, 1986; Anonymous, 1993). Both studies were GLP and OECD TG 410 (1981) compliant with treatment groups comprised of 5 rabbits/sex/dose. The dermal application of benfluralin to the rabbit over a period of 21 days caused dermatitis at all doses, and an associated inflammatory increase of leukocytes and thrombocytes at 500 mg/kg bw/d and higher. Dermal irritation indices were calculated for the different treatment groups, and it appeared that a dose- and time-related increase of very significant dermal irritation was observed. Corrosivity was not supported by the data because animals did not generally show fissuring, scabs (crusts) or necrosis.

The DS also described significant and severe dermal irritation in two acute dermal toxicity tests. Desquamation and signs of skin irritation (moderate to severe erythema and oedema), were observed and cleared in all animals within 28 days in the Anonymous (1990) study. In the Anonymous (1997) study, erythema and oedema (males and females), burns and fissures at the site (males) and scale formation and scabs (males and females) were observed. The scab formation was not reversed during the study as only one of the ten rabbits had normal skin by day 15. The DS proposed Skin Irrit. 2; H315 (Causes skin irritation).

Comments received during consultation

There was a single comment from an MSCA supporting classification for skin irritation (Skin Irrit. 2; H315).

Assessment and comparison with the classification criteria

According to the CLP criteria, a substance is irritant to the skin under a number of conditions including inflammation that persists to the end of the study observation period, in at least 2 animals exhibiting alopecia (limited area), hyperkeratosis, hyperplasia, and scaling of the skin.

Both studies presented for the assessment of skin irritation appear to support skin irritation classification based on this aspect of the criteria rather than on the standard reliance on severity of damage as indicated by the degree of erythema and/or oedema. The persistence of clear signs of skin irritation to the end of each study observation period is clear. What is not so clear from the CLP guidance are:

1. Does the observation period adhere strictly to 14 days? Study observation periods for skin irritation studies are normally referred to as being 14 days in length but individual studies can differ in this respect. There is no further explanation in the guidance text if the end of the study observation period applies to all skin irritation studies regardless of the length of their observation period following substance removal from the skin, i.e. is a 9-day observation period as valid as a 14-day one?
2. The guidance specifies effects seen to persist in at least two animals. The text, following on from a brief description of erythema and oedema scores in a 3-animal study would therefore presume persistent effects in two animals out of three. However, there is no explicit confirmation that this is indeed the case. Does the guidance actually mean 2 animals out of 3 in a study or 2 animals regardless of the number of animals used in the study? The two dermal irritancy tests were conducted with 6 animals.

Regardless of these uncertainties, there is ample evidence for supporting a classification for skin irritancy; see table below.

***Table**: Summary of the evidence for skin irritation*

Test guideline / GLP	Animals	Purity	Dose	Results	Reference
Skin irritation studies					
OECD TG 404 / GLP: Yes	Rabbit NZW 3M/3F	95.64%	[1]0.5 g active	Erythema persisted in **3/6** rabbits at the end of the study (day 15).	Anonymous, 1990 Report No. B09690
OECD TG 404 / GLP: Yes	Rabbit NZW 3M/3F	95.8%	[2]0.5 g active	Scaliness observed in **5/6** rabbits at the end of the study (day 9).	Anonymous, 1997 Report No. 971153
Acute dermal toxicity studies					
OECD TG 402 / GLP: Yes	Rabbit NZW 5M/5F	95.64%	[3]5000 mg/kg bw	28-day post-exposure observation period. By day 5 or 6, a moderate to severe erythematous and oedematous response was evident. Desquamation was present from day 6, and 2 females developed coriaceous or cracked dermal treatment sites from day 7 or 10.	Anonymous, 1990 Report No. B04990

				Irritation cleared in all animals by 28 days.	
OECD TG 402 / GLP: Yes	Rabbit NZW 5M/5F	95.8%	[4]5000 mg/kg bw	15-day post-exposure observation period. Evidence of erythema and oedema present from day 2 to day 15. Females showed some scale formation and scabs developed from day 7. The males had burns and fissures at the site from day 2 to 4 with more fissures developing on day 10/11; scaling was seen from day 7 and scab formation from day 10. Nine of the 10 rabbits showed persistent skin irritation up to day 15.	Anonymous, 1997 Report No. 971155
Subchronic (21-day) dermal toxicity studies					
OECD TG 410 / GLP: Yes	Rabbit NZW 5M/5F	97.3%	[5]0, 100, 325 or 1000 mg/kg bw	Exposure was for 6h/d for 21 days. A dose- and time-related persistent increase of dermal irritation was observed. At 325 mg/kg bw/d: moderate irritation progressed to severe erythema and slight oedema to severe oedema. At 1000 mg/kg bw/d: slight irritation progressed to severe erythema and moderate oedema progressed to severe oedema. In all treatment groups desquamation occurred within 5-20 days of treatment, followed by epithelisation (without scar tissue or other indication of corrosive effects). The skin exhibited a coriaceous, cracking and bleeding appearance.	Anonymous, 1986 Report No. 02185
OECD TG 410 / GLP: Yes	Rabbit NZW 5M/5F	95.8%	[6]0, 100, 500 or 1000 mg/kg bw	Exposure was for 6 h/d for 5 d/week for 21 days. A dose-dependent increase of erythema, eschar, oedema and scaling was observed from the lowest dose in both the males and the females. At the highest doses, necrosis, ulcers and suppurative lesions occurred. Underlying tissues were also affected, as inflammation and oedema of the dermis, and sebaceous gland hyperplasia was observed at all doses.	Anonymous, 1993 Report No. DR-0097-3397-002

[1] not moistened, but covered with moistened dressing.
[2] moistened with 0.5 mL of 0.5% aqueous methylcellulose.
[3] not moistened, but applied in solid form under a damp semi-occlusive wrap.
[4] moistened with 5.0 mL of 0.5% aqueous methylcellulose.
[5] not moistened, but covered with a moistened gauze pad.
[6] moistened with 1 mL of water per gram of test material.

The data from several studies indicates that the inflammation persists to the end of the study period. This fulfils one of the main CLP criteria for classification of skin irritation, that of a substance which is irritant to the skin and produces reversible damage to the skin following its application for a period up to 4 hours. Based on a weight of evidence assessment, RAC considers that there is sufficient and clear evidence and that **classification as Skin Irrit. 2; H315 is warranted**.

10.5 Serious eye damage/eye irritation

The eye irritating potential of benfluralin was investigated in a study in rabbits.

Table 15: Summary table of animal studies on serious eye damage/eye 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**
OECD TG 405	Rabbit New Zealand White M, F 3/ dose level	Benfluralin (purity 95.8%)	0.1 g aliquot. The eyes of all rabbits remained unwashed post treatment	All eyes were examined 1, 24, 48 and 72 hours after dosing and again on Days 7 and 14. Mean scores/animal: Corneal opacity: 1.06 Iris lesion: 0.61 Conjunctiva redness: 2.33 Conjunctiva chemosis: 2.44 Reactions had largely resolved by Day 7 and all eyes were overtly normal by Day 14.	Author, (1997) Report No. 971154/ CA 5.2.5/01

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

The eye irritation potential of benfluralin was assessed in three male and three female New Zealand White rabbits. Benfluralin was instilled as a finely ground 0.1 g aliquot into the conjunctival sac of the right eye of the rabbit and scored at 1, 24, 48 and 72 hours after dosing and again on Days 7 and 14. The results are summarized in table 16 and 17. The complete evaluation of the study is given in the RAR (section B.6.2.5).

Table 16: Eye irritation scores of Benfluralin according to OECD TG 405 – male rabbits

Time	**Cornea**			**Iris**			**Conjuctiva**					
							Redness			Chemosis		
Animal number	97A22-868	97A22-869	97A22-870	97A22-868	97A22-869	97A22-870	97A22-868	97A22-869	97A22-870	97A22-868	97A22-869	97A22-870
After 1 hour	0	0	0	0	0	0	2	2	2	3	3	4
After 24 hours	1	1	1	0	1	0	2	2	2	3	2	4
After 48 hours	1	1	1	0	1	1	3	2	2	2	1	3

Time	**Cornea**			**Iris**			**Conjuctiva**					
After 72 hours	1	1	1	0	1	1	2	2	2	1	1	2
Mean scores 24-72 hours	1	1	1	0	1	0.7	2.3	2	2	2	1.3	3
After 1 week	0	0	1	0	0	0	0	0	1	0	0	1
After 14 days	0	0	0	0	0	0	0	0	0	0	0	0

Table 17: Eye irritation scores of Benfluralin according to OECD TG 405 – female rabbits

Time	**Cornea**			**Iris**			**Conjuctiva**					
							Redness			Chemosis		
Animal number	97A22-871	97A22-872	97A22-873	97A22-871	97A22-872	97A22-873	97A22-871	97A22-872	97A22-873	97A22-871	97A22-872	97A22-873
After 1 hour	0	0	0	0	0	0	2	2	2	3	4	4
After 24 hours	1	1	1	0	0	1	2	2	2	3	3	4
After 48 hours	1	1	1	1	1	1	3	3	3	2	2	4
After 72 hours	1	1	2	1	0	1	2	3	3	1	2	4
Mean scores 24-72 hours	1	1	1.3	0.7	0.3	1	2.3	2.7	2.7	1.7	2.3	4
After 1 week	1	2	2	0	0	0	1	1	1	1	1	1
After 14 days	0	0	0	0	0	0	0	0	0	0	0	0

Ocular instillation of benfluralin had no effect on bodyweight gain. Some corneal opacity was evident for all rabbits from Day 2, persisting at Day 7 in four eyes. Slight reddening of the iris was seen in several cases on Days 2 to 4. All treated eyes produced some discharge sufficient to moisten the hairs adjacent to the eyelids within an hour of dosing. Over the next 72 hours the incidence and extent of the discharge reduced. Conjunctival reactions in the first 72 hours following instillation included a diffuse crimson or beefy red coloration and moderate or marked swelling (Day 1 and 2) which reduced to slight or moderate reactions by Day 3 or 4. Reactions had largely resolved by Day 7 and all eyes were overtly normal by Day 14.

The study follows the OECD TG 405 (adopted 2nd October, 2012), with the minor deviation that no information on the ocular anaesthetic used was provided.

10.5.2 Comparison with the CLP criteria

According to the CLP criteria, substances that have the potential to induce reversible eye irritation shall be classified in Category 2 (eye irritation). A substance is classified as an eye irritant if the following criteria are met in at least 2 of 3 animals:

Corneal opacity ≥ 1 and/or

Iritis ≥ 1 and/or

Conjunctival redness ≥ 2 and or

Conjunctival oedema (chemosis) ≥ 2

In the available study, scores were equal or above 1 in 6/6 animals for corneal opacity, in 2/6 animals for iris lesion, and equal or above 2 in 6/6 animals for conjunctival redness, in 4/6 animals for chemosis. Overall,

following grading at 24, 48 and 72 hours after installation of the test material, the 3 out of 4 scores are above the trigger for classification as a Category 2 eye irritant. Therefore, benfluralin meets the criteria for classification.

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

Classification as 'Eye Irrit. Cat. 2' (H319) is considered appropriate

RAC evaluation of serious eye damage/irritation

Summary of the Dossier Submitter's proposal

The DS proposed to classify benfluralin as Eye Irrit. 2; H319 based on a single GLP and guideline compliant study (OECD TG 405, 1987), in three male and three female NZW rabbits (Anonymous, 1997, RAR B.6.2.5, 2018). Benfluralin (95.8%) was instilled as a finely ground 0.1 g aliquot into the conjunctival sac of the right eye of each rabbit. The left eye remained untreated and served as a control. An ocular anaesthetic was used for all rabbits (both eyes) after discomfort was observed in the first dosed rabbit. All eyes were examined 1, 24, 48 and 72 hours after dosing and again on days 7 and 14. The eyes of all rabbits remained unwashed post treatment. Results are presented in the table below.

Table: *Summary of rabbit ocular data*

Mean values for ocular lesions 24, 48 and 72 hours after instillation

Animals	Corneal opacity	Iridial lesions	Conjunctival	
			Redness	Chemosis
1. male 97A22-868	**1**	0	**2.3**	**2**
2. female 97A22-871	**1**	0.7	**2.3**	1.7
3. male 97A22-869	**1**	**1**	**2**	1.3
4. female 97A22-872	**1**	0.3	**2.7**	**2.3**
5. male 97A22-870	**1**	0.7	**2**	**3**
6. female 97A22-873	**1.3**	**1**	**2.7**	**4**
CLP Criteria: Eye Irrit. (Cat. 2)	≥ 1	≥ 1	≥ 2	≥ 2
CLP Criteria: Eye Dam. (Cat. 1)	≥ 3	> 1.5	na	na

According to the criteria defined in the CLP Regulation, the mean scores for corneal opacity, conjunctival redness and conjunctival chemosis (oedema), following grading at 24, 48 and 72 hours after installation of the test material, are above the trigger for classification as a category 2 eye irritant. All six animals are positive for at least two indicators supporting classification. The DS therefore proposed classification of benfluralin as Eye Irrit. 2; H319.

Comments received during consultation

There was a single comment from an MSCA supporting classification for eye irritation (Eye Irrit. 2; H319).

Assessment and comparison with the classification criteria

According to the CLP criteria, substances that have the potential to induce reversible eye irritation shall be classified in Category 2. In the single available study (Anonymous, 1997), scores were equal or above 1 in 6/6 animals for corneal opacity, in 2/6 animals for iris lesion, and equal or above 2 in 6/6 animals for conjunctival redness, and in 4/6 animals for chemosis. The criteria specifying at least 4 animals out of 6 with scores in indicators of reversible irritation above the trigger values was fulfilled. RAC agrees with the DS that **benfluralin should be classified as Eye Irrit. 2; H319**.

10.6 Respiratory sensitisation

No studies available.

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

No studies available.

10.6.2 Comparison with the CLP criteria

No studies available.

10.6.3 Conclusion on classification and labelling for respiratory sensitisation

No conclusion can be reached on respiratory sensitisation owing to the lack of available data.

RAC evaluation of respiratory sensitisation

Summary of the Dossier Submitter's proposal

Not assessed, as no study data was available. No classification is thus warranted, due to lack of data.

10.7 Skin sensitisation

The skin sensitisation potential of benfluralin has been investigated in two GLP-compliant studies according to OECD guideline 406 (one modified Buehler method and one Maximisation test of Magnusson and Kligman), using Guinea pigs.

Table 18: Summary table of animal studies on skin sensitisation

Method, guideline, deviations if any	**Species, strain, sex, no/group**	**Test substance,**	**Dose levels duration of exposure**	**Results**	**Reference**
OECD TG 406, modified Buehler method (supplementary)	Guinea pig Hartley F 12 (test) 6 (control)	Benfluralin (purity 98.2%) Batch No.: X 35746	Dermal induction 6h: 0.2 mL doses of 5% benfluralin (three treatments per week for two weeks). Challenge 6h: 0.2 mL doses of 5% benfluralin	Sensitisation rate 75%	Author (1984) Report No. G01183/ CA 5.2.6/01
OECD TG 406, Maximisation test of Magnusson and Kligman	Guinea pig Crl:(HA)BR Sex not stated 20 (test) 20 (control)	Benfluralin (purity 95.8%) Batch No.: ACD13	Intradermal injection: 5% Dermal induction: 25% Challenge: 10%	Sensitisation rate 95%	Author (1998) Report No. 8202485/ CA 5.2.6/02

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

The potential of benfluralin to cause skin sensitisation was evaluated in female albino Hartley guinea pigs using a modified Buehler method (considered to be supplementary) and in twenty test and twenty control Crl:(HA)BR guinea pigs according to the Maximisation test of Magnusson and Kligman.

In the modified Buehler method both the dermal induction and the challenge doses were 5% of benfluralin, whilst in the maximisation test, the intradermal injection dose was 5%, the dermal induction 25% and the challenge 10% benfluralin. The results are summarized in table 19 (modified Buehler method) and 20 (maximisation test). Further details including method, guideline (and deviations if any), doses, study duration, exposure route and a description of the results are given in the text below or is included in Annex 1 of the CLH report (RAR, section B.6.2.6).

Table 19: Challenge results, modified Buehler method with 5% test article in 95% ethanol

Group	**No. of animals**	**Incidence of dermal responses (score ≥ 1)**						**Total responders**
		24 hours		**48 hours**		**72 hours**		
		Erythema	Oedema	Erythema	Oedema	Erythema	Oedema	
Control	6	0	0	1	0	0	0	1 (17%)
Test	12	7	5	9	8	9	7	9 (75%)

Table 20: Responses to challenge applications with 10% benfluralin in petrolatum - maximisation test

Group	**No. of animals**	**Incidence of dermal responses**		**Total responders**
		24 hours	**48 hours**	
Control	20	2	1	2
Test	20	7	20	20

The modified Buehler method follows the OECD TG 406 (updated July 17th 1992), with the exception of the use of 12 test and 6 control animals, instead of at least 20 test and ten control animals, which for this test is

strongly recommended in order to conclude that the test substance is a sensitiser. Further, there are no results for preliminary testing/dose ranging investigations to justify dose selection. The positive control, dinitrochlorobenzene (DNCB), responded as expected (100% sensitisation rate). In view of the confirmatory positive response obtained in the maximisation test, however, it was not necessary to repeat the study.

In the maximisation test, all animals exposed to 10% w:v benfluralin were positive. At 24h, 20/20 animals exhibited erythema score 2 (moderate and diffuse redness) or 3 (intense redness and swelling), and at 48h, 1/20 animals exhibited score 1, while 19/20 exhibited score 2 or 3. The study follows the OECD TG 406 (updated July 17th 1992), with the following exception; the use of sodium lauryl sulphate prior to topical induction is normally only required when the maximum practical test substance concentration to be applied is non-irritating. In this case, the pre-treatment was applied even though the selected dose concentration was moderately irritating. The deviation is not considered to have affected the positive result obtained in the study.

10.7.2 Comparison with the CLP criteria

In accordance with the CLP criterica, a substance is classified if there are positive results from an appropriate animal test. In the available studies, a strong sensitisation reaction was elicited, both in the topical and in the maximisation assay, and a classification is therefore proposed for benfluralin. Since a high concentration for intradermal induction (> 1 (% w/v)) was used and the incidence of sensitised guinea pigs was high in the maximisation assay, the data are not sufficient for sub-categorisation in Cat 1A or Cat 1B. It is not possible to exclude that benfluralin is a strong sensitiser (Cat 1A) since that would require to test benfluralin in a low concentration range of >0.1-≤1.0 (%w/v). Therefore and according to the CLP criteria, the data obtained from the skin sensitiation assays suggests that benfluralin should be classified in Category 1.

10.7.3 Conclusion on classification and labelling for skin sensitisation

Classification as 'Skin Sens. 1' (H317) is considered appropriate.

RAC evaluation of skin sensitisation

Summary of the Dossier Submitter's proposal

The skin sensitisation potential of benfluralin (95.8%) was investigated in two GLP-compliant studies according to OECD TG 406 (one modified Buehler method, Anonymous, 1984; and one Magnusson and Kligman Maximisation test, Anonymous, 1998).

The Buehler study was considered acceptable but only as supporting data by the DS due to two main shortcomings: (1) the use of 12 test and 6 control animals, instead of at least 20 test and ten control animals, and (2) there were no results for preliminary testing/dose ranging investigations to justify the dose selection. Both the dermal induction and the challenge doses were 5% benfluralin. As consistent positive results were observed throughout the observation period, and the sensitisation rate was 75%, the DS considered this test highly indicative of sensitising potential. The positive control, dinitrochlorobenzene, responded as expected (100% sensitisation rate).

The maximisation test was the key study and performed according to guidelines with one small caveat; pre-treatment with sodium lauryl sulphate prior to topical induction was not required, the selected dose concentration was already moderately irritating.

A pretest identified a 5% w/v benfluralin formulation in mineral oil as suitable for intradermal injection; a 25% w/v paste in petrolatum as suitable for the topical phase of the induction period and 10% w/v benfluralin in petrolatum as the maximum non-irritating concentration for use in the challenge phase. There was a typo in the CLH report, all 20 animals exposed to 5% w/v benfluralin intradermal induction were positive at both the 24-hour and 48-hour time points following challenge. At 24h, 20/20 animals exhibited erythema score 2 (moderate and diffuse redness) or 3 (intense redness and swelling), and at 48h, 1/20 animals exhibited score 1, while 19/20 exhibited score 2 or 3. Only 2 control group animals responded to application of benfluralin and the reactions did not exceed transient moderate erythema.

According to the criteria defined in CLP Regulation, the DS considered that benfluralin is a skin sensitiser and proposed classification as Skin Sens. 1; H317 with no sub-categorisation.

Comments received during consultation

There was a single comment from an MSCA supporting classification for skin sensitisation (Skin Sens. 1; H317) with no sub-categorisation.

Assessment and comparison with the classification criteria

In the two available studies, strong sensitisation reactions were elicited. According to the CLP criteria, a substance may be classified as a skin sensitiser on the basis of positive test results in either the mouse LLNA, Guinea pig Buehler assay or Guinea pig maximisation test. The data for benfluralin supporting classification for skin sensitisation was available from both a Buehler and Maximisation study. The Maximisation study was the key study. Using an intradermal induction with 5% w/v benfluralin resulted in a 100% sensitisation incidence (20/20 for test animals, 2/20 in controls) at both the 24-hour and 48-hour time points following challenge.

Based on the Maximisation study results benfluralin can be categorised as a moderate sensitiser. The Buehler or occluded patch test used less than the recommended number of test animals, which could lead to a potential reduction in accuracy. However, the results from the Buehler test are sufficiently robust to support the results from the Maximisation test. Potency on the basis of the Guinea pig maximisation test was determined by comparison with the criteria for a moderate sensitiser:

- A sensitisation incidence ≥ 30%
- An intradermal induction using > 1.0% (w/v) test substance

The results of the Maximisation test (5% intradermal induction concentration with a 100% nt sensitisation incidence) support that benfluralin is a moderate sensitiser with a predicted sub-category of 1B.

The concentration of benfluralin used for intradermal induction was high, 5%. This makes it impossible to exclude the possibility of the substance being a Category 1A sensitiser since that would require to test benfluralin in the low concentration range of > 0.1 - ≤ 1.0%. On this basis RAC cannot propose sub-categorisation for benfluralin.

Specific concentration limits for sensitisation generally apply for the most potent skin sensitisers classified in Category 1A. There was no adequate or reliable scientific information available showing that skin sensitisation warranted deviations from the GCL for classification.

RAC supports the classification proposal by the DS, that benfluralin should be **classified as Skin Sens. 1; H317 without sub categorisation**.

10.8 Germ cell mutagenicity

The genotoxicity of benfluralin has been investigated in several guideline- and GLP-compliant *in vitro* tests and three *in vivo* bone marrow micronucleus tests using different batches of benfluralin.

In vitro

The gene mutation potential of benfluralin has been investigated *in vitro* in bacterial gene mutation studies (Ames tests) and in mammalian cells (mouse lymphoma cells), whilst the clastogenic and aneugenic potential of benfluralin was investigated *in vitro* in human lymphocytes (micronucleus assay). A non-acceptable chromosomal aberration assay in the CHO cells was also conducted (not included in the summary table), as well as an unscheduled DNA synthesis test which was considered supplementary.

Table 21: Summary table of mutagenicity/genotoxicity tests in vitro

Method, guideline, deviations if any	**Test substance,**	**Relevant information about the study including rationale for dose selection (as applicable)**	**Observations**	**Result**	**Reference**
Guideline: not stated Supplementary GLP Deviation: TA102 or E. coli WP2uvrA were not included	Benfluralin (97.3%) Batch No.: 231EF4	S. typhimurium (TA98, TA100, TA1535, TA1537, TA1538), plate incorporation assay, 0 to 0.75 mg/plate, ± S9, DMSO (three replicates)	Precipitation at 100 µg/plate (-S9) or 50 µg/plate (+S9). Cytotoxicity at 5000 µg/plate Positive controls induced the appropriate increases in mutant frequencies	Negative ±S9	Author (1985b) Report No. 850624AMS25898 and 850708AMS2598/ CA 5.4.1/02
OECD 471 GLP	Benfluralin (96.7%) Batch No: 650/0	S. typhimurium (TA98, TA100, TA1535, TA1537, TA1538) and E. coli (WP2uvrA), plate incorporation and pre-incubation assay, 0 to 5 mg/plate, ± S9, DMSO. (two replicates)	No cytotoxicity up to 5000 µg/plate Positive controls induced the appropriate increases in mutant frequencies.	Negative ±S9	Author (2002) Report No. 842142 and 730900/ CA 5.4.1/03
OECD 471 GLP	Benfluralin (96.2%) Batch No: 2228	Benfluralin Technical, Batch 2228: Bacterial Reverse Assay. S. typhimurium (TA98, TA100, TA1535, TA1537 and TA102), plate incorporation and pre-incubation assay, 0 to 5 mg/plate, ± S9, DMSO (three replicates)	Precipitation at 1600 - 5000 µg/plate No cytotoxicity up to 5000 µg/plate *A single vehicle control revertant counts in the absence and presence of metabolic activation in strain TA102 (plate incorporation assay) fell above the historical control range, while the mean plate counts fell within the historical range. Appropriate positive & solvent controls gave the expected results	Negative* ±S9	Author (2017a) Report No. 8367644/ CA 5.4.1/07
OECD 471 GLP Deviation: plates were incubated for 5	Benfluralin (96.6%) Batch No: 2614	Benfluralin Technical, Batch 2614: Bacterial Reverse Assay. S. typhimurium (TA98, TA100,	Precipitation at 1000 – 5000 µg/plate No cytotoxicity up to 5000 µg/plate *There were two exceptions	Negative* ±S9	Author (2017b) Report No. 8367647/ CA 5.4.1/08

Method, guideline, deviations if any	**Test substance,**	**Relevant information about the study including rationale for dose selection (as applicable)**	**Observations**	**Result**	**Reference**
days in the plate incorporation assay.		TA1535, TA1537 and TA102), plate incorporation and pre-incubation assay, 0 to 5 mg/plate, ± S9, DMSO (three replicates)	where single vehicle control revertant plate counts fell slightly outside the historical range, while the mean plate counts fell within the historical range. Appropriate positive & solvent controls gave the expected results		
Guideline: not stated. GLP	Benfluralin (97.3) Batch No: 231EF4	Lymphoma cells L5178Y/TK+/-, 0 0.02 mg/mL (± S9), DMSO (two replicates)	Cytotoxicity at 250 µg/mL or higher with S9 *An increase in the mutation frequency was noted in the activated test. The increase did not reach the laboratory's acceptance criteria for a positive response. Positive controls induced the appropriate increases in mutant frequencies.	Negative* ±S9	Author (1985) Report No. 850612MLA2598 and 850724MLA2598/ CA 5.4.1/05
OECD 476 GLP	Benfluralin (96.2%) Batch No: 2228	Lymphoma cells L5178Y/TK+/-, 0 0.02 mg/mL (± S9), DMSO Benfluralin Technical, Batch 2228: In vitro L5178Y Gene Mutation assay at the hprt locus. Lymphoma cells L5178Y/tk+/-, 0 0.15 mg/mL (-S9), 0-0.2 mg/mL (+ S9), DMSO (two replicates)	Precipiation at ≥100 µg/mL Cytotoxicity: highest tested concentration at 150 µg/mL, -S9 (14% RS). *Non-significant increases in MF compared to negative controls, with the exception of 200 µg/mL in the presence of S9, which fell above the laboratory's historical MF range (in the presence of precipitation, 44% RS). Appropriate positive & solvent controls gave the expected results	Negative* ±S9	Author (2017c) Report No. 8367645/ CA 5.4.1/09
OECD 476 GLP	Benfluralin (96.6%) Batch No: 2614	Benfluralin Technical, Batch 2614: In vitro L5178Y Gene Mutation assay at the hprt locus. Lymphoma cells L5178Y/tk+/-, 0-0.1 mg/mL (- S9), 0-0.175 mg/mL (+ S9), DMSO	Precipitation from 90 µg/mL (-S9) and 100 µg/mL (+S9) Cytotoxicity: highest observed with –S9 at 50 µg/mL (≥10% RS) in the range-finder study and at 80 µg/mL (15% RS) in the main study *Non-reproducible changes in osmolality were reported at 150 µg/mL in the Mutation	Negative* ±S9	Author (2017d) Report No. 8367648/ CA 5.4.1/10

Method, guideline, deviations if any	**Test substance,**	**Relevant information about the study including rationale for dose selection (as applicable)**	**Observations**	**Result**	**Reference**
		(two replicates)	Experiment Appropriate positive & solvent controls gave the expected results		
OECD 487 GLP	Benfluralin (96.2%) Batch No: 2228	Benfluralin Technical, Batch 2228: In vitro Human Lymphocyte Micronucleus Assay. ♂Human lymphocytes, 3h; 0-0.08 mg/mL (-S9), 0-0.14 mg/mL (+S9), 24h; 0-0.08 mg/mL, DMSO (pooled duplicates)	Precipitation in the Micronucleus Experiment at ≥50 µg/mL (-S9) and at ≥60 µg/mL (+S9) Cytotoxicity: The highest concentrations analysed achieved 50-60% cytotoxicity, based on RI. *One single treatment (-S9) in the results exceeded the historical control data while the mean micronucleated binucleate cell frequency fell within the historical control range. Positive and negative controls gave the expected results	Negative* ±S9	Author (2017e) Report No. 8367646/ CA 5.4.1/11
OECD 487 GLP	Benfluralin (96.6%) Batch No: 2614	Benfluralin Technical, Batch 2614: In vitro Human Lymphocyte Micronucleus Assay. ♂Human lymphocytes, 3h; 0-0.08 mg/mL (-S9), 0-0.14 mg/mL (+S9), 24h; 0-0.08 mg/mL, DMSO (pooled duplicates)	Precipitation at ≥40 µg/mL in the Micronucleus Experiment (±S9) Cytotoxicity: the highest concentrations analysed achieved 50-60% cytotoxicity, based on RI. Positive and negative controls gave the expected results	Negative ±S9	Author (2017f) Report No. 8367649/ CA 5.4.1/12
Guideline: not stated GLP Supplementary	Benfluralin (97.3%) Batch No: 231EF4	Rat, hepatocytes (ex vivo), UDS, 0-1 mg/mL, DMSO	Precipitation ≥ 500 µg/mL Cytotoxicity: ≥ 50 µg/mL Positive control gave the expected results	Negative	Author (1985) Report No. 850716UDS2598 and 850723UDS2598/ CA 5.4.1/06

Benfluralin was negative in studies investigating gene mutations in vitro in bacteria and in mammalian cells either with or without metabolic activation. In one in vitro gene mutation assay at the hprt locus, the mean

mutation frequency at the highest concentration tested (200 µg/ml) in the presence of S9, was slighlty above the distribution of the historical negative control data and the result was not significantly negative. The positive effect was observed within acceptable level of cytotoxicity (44% relative survival). However, since positive effects observed in the presence of precipitation could be artificial, the study was considered negative. Benfluralin showed no evidence of clastogenic potential in vitro in human peripheral blood lymphocytes both in the presence and absence of S9 mix, even when tested up to cytotoxic concentrations.

In vivo

The potential of benfluralin to induce chromosomal damage in rodents has been investigated in vivo in three bone marrow micronucleus tests (rats and mice) conducted with three different batches. A supplementary in vivo bone-marrow Sister Chromatid exchange test, not included in the recommended test battery according to regulation (EC) 283/2013, is also available in Annex 1 (RAR, section B.6.4.2.1).

Table 22: Summary table of mutagenicity/genotoxicity tests in mammalian somatic or germ cells in vivo

Method, guideline, deviations if any	**Test substance,**	**Relevant information about the study (as applicable)**	**Observations**	**Results**	**Reference**
OECD 474 GLP Deviation: 2000 immature erythrocytes per animal were scored for the incidence of micronucleated immature erythrocytes	Benfluralin (95.8%) Batch No.: ACD13683	Mouse [CD-1 (1-CR)BR)], bone marrow micronucleus, 2000 mg/kg bw (Six mice/dose/sex)	No reduction of the PCE:NCE ratio. The mean value of the treated group (2.6 ‰ MNPC) was outside the range of the mean values of the historical data (0.3-2.2 ‰ MNPC) The positive control induced a marked increase	Equivocal	Author (2004) Report No. 031084/ CA 5.4.2/02
OECD 474 GLP Deviations: The bone morrow exposure was not demonstrated in this study. Lack of information regarding the HCD data	Benfluralin (96.2%) Batch No.: 2228	Benfluralin Technical, Batch 2228: Rat Bone Marrow Micronucleus Assay Six male rats/dose [young adult out-bred Sprague Dawley (Crl:CD(SD] 0, 500, 1000 or 2000 mg/kg bw	No clinical signs of toxicity noted No notable effect of treatment on body weights was observed No evidence of any test article-induced toxicity to the bone marrow, but available information from ADME studies supports the exposure of the bone marrow. Statistically significant increases MN PCE frequencies (P≤0.05) at 500 and 2000 mg/kg bw/day. Positive and negative controls gave the expected results	Negative	Author (2017a) Report No. 8368604/ CA 5.4.2/03
OECD 474. GLP. Deviations: The bone morrow	Benfluralin (96.6%) Batch No.: 2614	Benfluralin Technical, Batch 2614: Rat Bone Marrow Micronucleus Assay Six male rats/dose	No clinical signs of toxicity noted No notable effect of treatment on body weights was observed No evidence of any test article-	Negative	Author (2017b) Report No. 8368605/ CA 5.4.2/04

Method, guideline, deviations if any	**Test substance,**	**Relevant information about the study (as applicable)**	**Observations**	**Results**	**Reference**
exposure was not demonstrated in this study Lack of information regarding the HCD data		[young adult out-bred Sprague Dawley (Crl:CD(SD] 0, 500, 1000 or 2000 mg/kg bw	induced toxicity to the bonemarrow, but available information from ADME studies supports the exposure of the bone marrow Positive and negative controls gave the expected results		

Benfluralin was investigated for its ability to induce micronucleated immature erythrocytes in the bone marrow of [CD-1 (1-CR)BR)] mice and Sprague Dawley (Crl:CD(SD] rats.

In the *in vivo* mouse bone marrow micronucleus study, six mice/dose/sex were exposed to benfluralin dissolved in 0.5% w:v methylcellulose, at the dose level of 2000 mg/kg bw by gavage (dosing volume 10 mL/kg). Animals were sacrificed 24h and 48h after dosing. Both analytical verification of concentration and homogeneity in administered samples was performed, and were satisfactory (93% of target concentration). Positive control was obtained by treating with cyclophosphamide dissolved in a.d. (CP, 120 mg/kg, sacrifice time 24h; 1♀ mouse was excluded from mean calculations because of a possible dosing error). Negative control was obtained by treating with the vehicle. In males, the mean value of micronucleated PCE was slightly high (due to 5/6 animals exhibiting 2.5-3.5‰ MNPC) at 24 h sampling, but the effect did not attain statistical significance. These values were within the range of historical control data. However, the mean value of the treated group (2.6 ‰ MNPC) was outside the range of the mean values of the historical data (0.3-2.2 ‰ MNPC), suggesting a possible biological significant effect. There was no reduction of the PCE:NCE ratio. It is argued that the bone marrow should have been exposed as the substance is shown to be widely distributed in the ADME studies in the rats. It is however questionable if this can be used as evidence for bone marrow exposure in the mouse bone marrow micronucleus test since no ADME studies in mice are available. It should be noted that the study do not fully comply with the latest OECD guideline (TG 474, 2016) in that at least 4000 immature erythrocytes per animal should be scored for the incidence of micronucleated immature erythrocytes.

In the two rat bone marrow micronucleus tests, doses of 500, 1000 and 2000 mg/kg bw were administrated twice (0 and 24 hours) by gavage to male rats (six rats/dose), before bone marrow was sampled at 48 hours. Cyclophosphamide (20 mg/kg bw) provided the positive control, whilst methylcellulose served as the vehicle (negative) control. In the first study (Batch 2228) the mean MN PCE frequencies of the groups treated with benfluralin were increased compared to vehicle control. The increases were statistically significant ($P \leq 0.05$) at 500 and 2000 mg/kg bw. The study authors consider that the statistical significance at 500 and 2000 mg/kg bw is of no biological relevance as all individual animal and group mean micronucleus frequencies fell within the laboratory's historical vehicle control 95% reference range, and because there was no evidence of a dose-related effect. In the second study (Batch 2614) there was no statistical increase in the mean MN PCE frequencies. However the mean MN PCE frequency was higher at high dose when compared with the concurrent control.

In both rat studies, more information should have been provided regarding the historical control data (HCD) (e.g. date of the study, housing and environmental conditions, diet, vehicle and information about how the 95% CI of the HCD was calculated). Further, no clinical signs of toxicity were noted and no effect of treatment on body weights was observed. There was no evidence of any test article-induced toxicity to the bone marrow in either of the tests (no decrease in %PCE values compared to the vehicle control group). Results from ADME studies in rats (data show that benfluralin is present in blood and bone marrow) do however support that the bone marrow was exposed. The studies were conducted in males, since there is no difference in toxicity between females and males. Still, it can be argued that the studies should have been conducted in females since

the pharmacokinetics studies show that benfluralin is more systemically available with a relatively longer half-live and higher residues in females than males.

Overall, under the conditions of the studies, they were considered acceptable and benfluralin was not genotoxic *in vivo* in rats.

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

Benfluralin has been tested for potential genotoxic properties in a standard battery of *in vitro* and *in vivo* assays.

There was no evidence that the different batches of benfluralin was mutagenic or clastogenic in the well conducted, reliable *in vitro* tests.

The genotoxicity of benfluralin was tested *in vivo* in two acceptable bone marrow micronucleus tests conducted in rats.

There is no evidence from the available data set that benfluralin is a somatic cell mutagen. There is therefore no reason to believe that benfluralin would have the potential to induce mutations in germ cells.

It should be noted, however, that the batches used in the gentotoxicty studies had lower content of EBNA than the current approved specification (0.1 mg /kg). Furthermore it has not been conducted any genotoxicity studies with batches of benfluralin containing a higher level than 0.085 mg/kg EBNA (batch 2228). Considering that the impurity of EBNA is genotoxic, it can be concluded that it is not clearly demonstrated that benfluralin (with the current specification of 0.1 mg EBNA/kg) is devoid of genotoxic potential.

10.8.2 Comparison with the CLP criteria

In accordance with the CLP criteria, benfluralin did not demonstrate any genotoxic potential in eight *in vitro* and two *in vivo*, guideline- and GLP-compliant studies and therefore the criteria for classification are not met.

10.8.3 Conclusion on classification and labelling for germ cell mutagenicity

Not classified - data conclusive but not sufficient for classification

RAC evaluation of germ cell mutagenicity

Summary of the Dossier Submitter's proposal

The genotoxicity of benfluralin was investigated in several guideline- and GLP-compliant *in vitro* tests and *in vivo* bone marrow micronucleus tests (see table 21 and 22 of the CLH report). No germ cell mutagenicity tests were conducted. The DS reported on the following:

In vitro assays:

- 4 × Ames tests (reverse mutation assay with *Salmonella typhimurium* and *Escherichia coli*) (Rexroat, 1985; Deparade, 2002; Lloyd, 2017a, 2017b),
- 3 × mammalian cell gene mutation tests (mouse lymphoma L5178Y cells) (Bewsey, 1985; Lloyd, 2017c, 2017d),
- 2 × chromosome aberration tests (mammalian cell micronucleus tests, human lymphocytes) (Lloyd, 2017e, 2017f),
- 1 × UDS test (Hill, 1985).

In vivo assays:

- 2 × micronucleus assays in rat bone marrow (Anonymous, 2017a, 2017b),
- 1 × micronucleus assay in mouse bone marrow (Anonymous, 2004).

In vitro results

Benfluralin was found to be negative in studies investigating gene mutations *in vitro* in bacteria and in mammalian cells either with or without metabolic activation. The newer *in vitro* genotoxicity studies were clearly negative except for the *in vitro* gene mutation assay at the *hprt* locus (Lloyd, 2017c), tested with benfluralin containing the highest specification of the genotoxic impurity ethyl-butyl-nitrosamine (EBNA, Batch 2228, 0.085 mg/kg). The mean mutation frequency (10.04 M/F), at the highest concentration tested (200 µg/mL) in the presence of S9, was not within the distribution of the historical control data (HCD, 1.29-9.08 M/F). The result was difficult to interpret due to the observed precipitation at this concentration and the DS deferred to the conclusions of the EFSA peer review and final assessment report. Here experts noted that within the OECD TG 476 it states that positive effects observed in the presence of precipitation *could* be artefactual. On this basis the DS agreed with the final EFSA conclusion that the overall result for the Lloyd (2017c) study should be considered negative. Benfluralin showed no evidence of clastogenic potential, the *in vitro* human lymphocyte micronucleus studies were negative both in the presence and absence of S9 mix. An unscheduled DNA synthesis test *in vitro* in rat hepatocytes was negative.

In vivo results

The clastogenic effect of benfluralin was further investigated *in vivo* in three bone marrow micronucleus tests (2 in Sprague Dawley rats and 1 in CD-1 mice) conducted with three different batches. An extra study available in the RAR (2018) was considered supplementary but not assessed by the DS (Female Chinese hamster bone-marrow Sister Chromatid exchange test, Anonymous, 1985).

In the *in vivo* mouse bone marrow micronucleus study (Anonymous, 2004), six mice/dose/sex were exposed by gavage (dosing volume 10 mL/kg bw), to benfluralin dissolved in 0.5% w/v methylcellulose, at a dose level of 2000 mg/kg bw. Animals were sacrificed 24h and 48h after dosing. There was no evidence of bone marrow toxicity, the PCE/NCE ratio was not reduced. In males, the mean value of micronucleated PCE (MN PCE) showed a non-statistically significant increase in the treatment group, however, this was within the historical control range. The positive control induced a marked statistically significant increase confirming the sensitivity of the assay. The DS noted that this study was conducted with a benfluralin batch containing a lower content of EBNA (0.04 mg/kg) than the current specification for the active substance (0.1 mg/kg). The DS also noted some concern over actual bone marrow exposure by benfluralin.

In the two rat bone marrow micronucleus tests, doses of 500, 1000 and 2000 mg/kg bw were administrated twice (0 and 24 hours) by gavage to male rats (six rats/dose), before bone marrow was sampled at 48 hours. There was no evidence of any test article-induced toxicity to the bone marrow in either of the tests (no decrease in %PCE values compared to the vehicle control group). Results from ADME studies in rats provided evidence that the bone marrow was exposed.

In the first rat bone marrow micronucleus study (Anonymous, 2017a, substance batch 2228, EBNA content 0.085 mg/kg), the mean micronucleated PCE frequencies of treatment groups were increased compared to the vehicle control. The increases were statistically significant ($p \leq 0.05$) at 500 and 2000 mg/kg bw (but not at 1000 mg/kg bw) and did not follow a dose response. The DS also noted that all individual animal and group mean micronucleus frequencies fell within the laboratory's historical vehicle control 95% reference range and that the small and variable increases in MN PCE were not treatment related.

In the second study (Anonymous, 2017b, substance batch 2614, EBNA content 0.058 mg/kg) all doses exhibited MN PCE frequencies that were similar to the concurrent vehicle control group and that fell within the laboratory's historical vehicle control 95% reference range.

Conclusion

The DS noted that the batches of benfluralin used in the genotoxicity studies had a lower content of the genotoxic impurity EBNA than the current approved specification (0.1 mg /kg). The highest level of EBNA tested was 0.085 mg/kg. According to the DS, there was no evidence from the available data set that benfluralin is a somatic cell mutagen. The DS did not propose to classify benfluralin as mutagenic.

Comments received during consultation

There was a single comment from an MSCA that supported the DS conclusion that benfluralin was unlikely to be genotoxic. Despite some deficiencies, they accepted the study database for benfluralin.

Assessment and comparison with the classification criteria

Summary

The genotoxicity of benfluralin was investigated in several guideline- and GLP-compliant *in vitro* tests and three *in vivo* bone marrow micronucleus tests using different batches of benfluralin (table below). Benfluralin generally gave negative responses in studies investigating gene mutations *in vitro* in bacteria and in mammalian cells. However, in one case a negative conclusion is not as certain as described by the DS. The studies presented by the RMS in the RAR (2019), section B.6.4 Genotoxicity, table B.6.4-01 "Summary of studies on genotoxicity" detail the complete genotoxicity database. There are extra studies incorporated in this table not described by the DS and this is supported by RAC since the original RMS concluded in these cases that the studies for various reasons were not acceptable from a regulatory point of view. However, one of the newer *in vitro* mammalian gene mutation assays at the hprt locus (Lloyd, 2017c), and tested with benfluralin containing the highest specification of the genotoxic impurity EBNA (Batch 2228, 0.085 mg/kg), should be best described as equivocal rather than negative for genotoxicity as it showed a weak positive result in the presence of precipitation.

Along with three *in vivo* bone marrow micronucleus studies there was also a Chinese Hamster, bone marrow Sister Chromatid Exchange test described by the RMS in the RAR (2019) under section B.6.4.2.1 (Anonymous, 1985). This was briefly noted by the DS as a supplementary study but not assessed. RAC agrees with the RMS that deficiencies in the study (use of only 3 animals/dose, all female; GLP but not an OECD guideline study and is not included in the

recommended test battery according to regulation (EC) 283/2013), relegate it as a supplementary study, but one with a clear but limited result; benfluralin did not increase the frequency of chromosome aberrations (sister chromatid exchange).

The DS described in detail the *in vivo* mouse bone marrow micronucleus study (Anonymous, 2004) and concluded the results were equivocal. The DS included a statement speculating on whether the data from the toxicokinetic studies in rats could be used as evidence for bone marrow exposure in the mouse micronucleus test. The concern was raised because no ADME studies on the mouse were available to prove bioavailability. OECD TG 474 (1997) states that the test is unsuitable only if there is evidence of the substance not reaching the target tissue. The mouse 90-day study, using a different strain but dosed at near comparable levels, showed clear systemic effects illustrating that the limit dose approach was correct for the *in vivo* mouse MN test. In principle, data from ADME and toxicokinetic studies in an animal species such as the rat are applied to human risk assessment scenarios. This indicates it is a reasonable assumption that ADME data from the rat can also be used to justify bioavailability in the mouse as long as there are no substantial differences in the delivery or route of administration of the tested substance.

The CD-1 mouse study was GLP- and guideline- (OECD TG 474, 1997) compliant. A single limit dose (2000 mg/kg bw) was tested and no effects on body weight or other indications of toxicity were noted during the in-life portion of the micronucleus test. The vehicle used was 0.5% methyl cellulose, the same as used in the two rat *in vivo* MN tests. The original rat ADME studies used a 10% acacia solution (gum Arabic) as vehicle which is comparable to methyl cellulose and compatible with oral toxicity studies as a suitable test vehicle.

In contrast to the position of the DS, RAC considers that bone marrow exposure in the mouse was highly probable, taking into account all the available data including that from the rat metabolism studies. According to the available studies, benfluralin, while poorly absorbed (approximately 20% of an oral dose), does exhibit extensive distribution and is found in the blood which perfuses the bone marrow.

According to the DS, the results of the mouse micronucleus assay were described as equivocal based purely on the mean value of the treated group (2.6‰ MN PCE) being outside the range of the mean values of the HCD (0.3-2.2‰ MN PCE). However, the mean of the treatment group (2000 mg/kg bw) was not statistically significantly different from the control group and in no case did any of the individual animal results (range 2.5-3.5‰ MN PCE) exceed the range of the laboratory HCD (range 0-6‰ MN PCE). A significant biological effect is not supported. There was no reduction of the PCE:NCE ratio. The positive control induced a marked increase confirming the sensitivity of the assay. RAC considers this study to be valid and negative for the induction of micronuclei.

In vitro tests

Several well conducted studies investigating gene mutations *in vitro* in bacteria and in mammalian cells were available for assessment of mutagenicity (table below). RAC concludes that the available data does not support a mutagenic potential for benfluralin.

Table: *Summary of genotoxicity tests with benfulralin (and content of EBNA) adapted from table 21 and 22 in the CLH report, and table B.6.4-01 in the RAR (2019).*

Study	Result	Test System	Reference
***In vitro* studies**			
Bacterial mutagenicity *[EBNA] = 0.31 mg/kg*	**negative**	GLP, non-guideline *Salmonella* Strains: TA98, TA100, TA1535, TA1537, TA1538	Rexroat, 1985 Supplementary
Bacterial mutagenicity *[EBNA] = 0.04 mg/kg*	**negative**	GLP, OECD TG 471 (1997) *Salmonella* Strains: TA98, TA100, TA1535, TA1537 *E. coli* strain WP2uvrA$^-$	Deparade, 2002 Acceptable
Bacterial mutagenicity *[EBNA] = 0.085 mg/kg*	**negative**	GLP, OECD TG 471 (1997) *Salmonella* Strains: TA98, TA100, TA102, TA1535, TA1537	Lloyd, 2017a Acceptable
Bacterial mutagenicity *[EBNA] = 0.058 mg/kg*	**negative**	GLP, OECD TG 471 (1997) *Salmonella* Strains: TA98, TA100, TA102, TA1535, TA1537	Lloyd, 2017b Acceptable
Mammalian cell mutagenicity *[EBNA] = 0.31 mg/kg*	**negative**	GLP, Guideline not stated. Mouse Lymphoma L5178Y Cells (Thymidine Kinase locus)	Bewsey, 1985 Acceptable
Mammalian cell mutagenicity *[EBNA] = 0.085 mg/kg*	**equivocal**	GLP, OECD TG 476 (2016) Mouse Lymphoma L5178Y Cells (*hprt* locus)	Lloyd, 2017c Acceptable
Mammalian cell mutagenicity *[EBNA] = 0.058 mg/kg*	**negative**	GLP, OECD TG 476 (2016) Mouse Lymphoma L5178Y Cells (*hprt* locus)	Lloyd, 2017d Acceptable
Clastogenicity *[EBNA] = 0.085 mg/kg*	**negative**	GLP, OECD TG 487 (2016) Human Lymphocyte Micronucleus Assay	Lloyd, 2017e Acceptable
Clastogenicity *[EBNA] = 0.058 mg/kg*	**negative**	GLP, OECD TG 487 (2016) Human Lymphocyte Micronucleus Assay	Lloyd, 2017f Acceptable
Primary DNA damage *[EBNA] = 0.31 mg/kg*	**negative**	GLP, Guideline not stated. rat hepatocyte (*ex vivo*) UDS	Hill, 1985 Supplementary
***In vivo* studies**			
Biomarker exposure/repair *[EBNA] = 0.31 mg/kg*	**negative**	GLP, non-guideline bone-marrow Sister Chromatid exchange test	Anonymous, 1985 Supplementary
Clastogenicity *[EBNA] = 0.04 mg/kg*	**negative**	GLP, OECD TG 474 (1997) Mouse [CD-1], bone marrow micronucleus	Anonymous, 2004 Acceptable
Clastogenicity *[EBNA] = 0.085 mg/kg*	**negative**	GLP, OECD TG 474 (2016) Rat [SD], bone marrow micronucleus	Anonymous, 2017a Acceptable
Clastogenicity *[EBNA] = 0.058 mg/kg*	**negative**	GLP, OECD TG 474 (2016) Rat [SD], bone marrow micronucleus	Anonymous, 2017b Acceptable

In vivo tests

The genotoxicity of benfluralin was tested *in vivo* in three acceptable bone marrow micronucleus tests conducted in rodents. In addition, a non-standard but acceptable Sister Chromatid Exchange (SCE) test was also available from the RAR (2019). There was no evidence from the available data set that benfluralin is a somatic cell mutagen.

Classification Assessment

No human data are available for benfluralin, therefore a classification with Muta. 1A is not supported. Benfluralin is negative in acceptable *in vitro* tests and negative in *in vivo* somatic cell mutagenicity guideline tests in mammals. Data are not available illustrating the induction of mutagenic effects in germ cells (a criterion for Category 1B). RAC does not support classification with Muta. 1A or B.

The overall weight of evidence for benfluralin supports no potential for genotoxicity in bacterial or somatic cells from a battery of *in vivo* and *in vitro* GLP and guideline compliant studies. Therefore, no classification in Category 2 is warranted. RAC notes that the genotoxic impurity EBNA, was tested up to a level of 0.31 mg/kg in older studies, all negative, but mainly supplementary by regulatory standards. In newer studies, EBNA was tested up to a level of 0.085 mg/kg.

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

10.9 Carcinogenicity

The carcinogenic potential and chronic toxicity of benfluralin have been investigated in a standard set of studies in rats and mice. An additional study has been conducted to investigate the mode of action (MoA) and human relevance (see Annex 1, section B.6.8.2).

Table 23: Summary table of animal studies on carcinogenicity

Study	Dose levels	Results
OECD TG 453 2 years, rat Oral (dietary) CDF®(F-344)Crl BR 60 M/F Author (1996) Report No. CHV 174-133/ CA 5.5/02	Benfluralin (95.8%) Batch No.: ACD13683 0, 10, 100, 2500, 5000 ppm Equivalent to: Males: 0, 0.5, 5.4, 136.3 and 274.8 mg/kg/bw/day Females: 0, 0.7, 6.8, 167.9 and 331.3 mg/kg bw/day	**NOAELs** Toxicity: 10 ppm (0.5 mg/kg bw/day). Carcinogenicity: 100 ppm (5.4 mg/kg bw/day). **Chronic phase (12 months)** *Mortality* There were no treatment-related effects on survival rates; 100% survival rate was reported for all doses except at 2500 ppm M (98%) & control F (98%). *BW and BWG* ↓BW in F at 2500 ppm and in M & F at 5000 ppm. Food efficiency was marginally lowered at the doses where BW effects were affected (data were only present for the first 3 months of treatment). *Haemotology* 100 ppm: ↑Platelet count (Plt) in M (5%) 2500 ppm: ↓Red blood cell count (RBC) in M (-7%) & F (-8%). ↓Hemoglobin (Hb) in M (-9%) & F (-11%). ↓Hematocrit (Hct) in M (-11%) & F (-13%). ↑Plt in M (16%) & F (7%). 5000 ppm: ↓RBC in M (-9%) & F (-10%). ↓Hb in M (-13%) & F (-15%). ↓Hct in M (-14%) & F (-17%).

Study	Dose levels	Results
		↑Plt in M (23%) & F (11%). *Clinical chemistry* 100 ppm: ↓Alanine aminotransferase (ALT) in M & F. ↓Aspartate aminotransferase (AST) in M. 2500 ppm and 5000 ppm: ↑blood urea nitrogen in M & F. ↑Creatinine in M. ↑Total cholesterol in M & F. ↑Total protein in M & F. ↑Albumin in M & F. ↑Globulin in M & F. ↑Alkaline phosphatase (ALP) in M, only at 5000 ppm in F. ↓ALT in M & F. ↓AST in M & F. *Organ weight* ↑Liver weight in M & F at 2500 ppm and above. ↑Thyroid weight in M & F at 2500 ppm and above. ↑Adrenal weight in F at 2500 ppm and above. **Carcinogenicity phase (24 months)** *Mortality* Decrease in survival rates in M at 100 ppm to 62%, 66% at 2500 ppm and to 64% at 5000 ppm. Decrease in survival rates in F: 92% at 2500 ppm. *FC, BW and BWG*↓FC & BW in F at 2500 ppm and in M & F at 5000 ppm. ↓BWG in whole treatment period in M (-15%) and F (-32%) at 2500 ppm and in M (-30%) and F (-50%) at 5000 ppm). *Clinical signs* Signs of poor antemortem condition including hunched posture, thin appearance, prostration or entire body paleness were noted frequently at 2500 ppm and above in M. Effects on skin/pelage in F (alopecia, discolorlation) and urine stains in M & F at 2500 ppm and above. Slight increase of swollen appearances of the scrotum at 5000 ppm in M. *Ophthalmology* Increased incidence of yellow/orange hue in the internal eye structures at 2500 ppm and above in M & F (bilirubin levels were unaffected). More incidences of cataract in F or cloudy cornea at 5000 ppm (associated with neovascularisation). *Haemotology* 2500 ppm: ↓RBC in F (-5%). ↓Hb in F (-11%). ↓Hct in F (-9%). ↑Plt in M (17%) & F (18%). 5000 ppm: ↓RBC in F (-7%). ↓Hb in M (-11%) & F (-13%). ↓Hct in M (-10%) & F (-12%). ↑Plt in M (22%) & F (31%).

Study	Dose levels	Results
		Clinical chemistry 100 ppm: ↓AST in M. 2500 ppm and 5000 ppm: ↑blood urea nitrogen in M & F. ↑Creatinine in M & F. ↑Total cholesterol in F. ↑Total protein in F. ↑Globulin in F. ↑ALP only at 5000 ppm in M. ↓ALT in F. ↓AST only at 5000 ppm in F. *Urinalysis* Slight decrease (1%) in urine specific gravity from wk 52 in M at 2500 ppm and above. *Organ weight* ↑Liver weight in M & F at 2500 ppm and above. ↑Thyroid weight in F at 2500 ppm and above. ↑Adrenal weight in M & F at 2500 ppm and above. *Gross pathology* Gross necropsy lesions in the kidney and the liver in M & F at 2500 ppm and above. Effects were observed in lung (pale area), stomach (dark area), uterus (cysts) and ovary (cysts) and testes (enlarged) at 2500 ppm and above. Dark discolored adipose tissue at 2500 ppm and above. ***Non-neoplastic findings*** Thyroid: ↑follicular cysts in M & F at 2500 ppm and above. ↑Hypertrophy in F at 5000 ppm. ↑follicular cell hyperplasia in M & F at 2500 and above. Liver: ↑Centrilobular/diffuse hypertrophy in M & F at 100 ppm and above. ↑Hepatocellular pigmentation in F at 100 ppm and above in M & F. ↑Sinusoidal cell pigmentation in M at 5000 ppm. ↑Hepatocellular necrosis in M at 2500 ppm and above. Kidney: ↑Chronic progressive nephropathy (CPN similar among all groups, severity increased in F at 2500 ppm and above). ↑Hyaline droplets in F at 10 ppm and in M & F at 100 ppm and above. ↑Tubule cell karyomegaly in M & F at 100 ppm and above. ↑Transitional cell hyperplasia in M & F at 100 ppm and above. ↑Large pelvis calculus in M & F at 100 ppm and above. ↑Free renal pelvic calculus in M & F at 100 ppm and above. Other findings: ↑Sciatic nerve degeneration in M & F at 2500 ppm, severe at 5000 ppm. ↑Skeletal muscle degeneration at 2500 ppm and above. ↑Lung – chronic inflammation in F at 2500 ppm and above in M & F. ↑Stomach – erosion/ulcer in M at 2500 ppm and above. ↑Endometrial cysts in 14/50 F at 5000 ppm (control incidence 6/50). ↑Follicular cysts in 4/50 F also showing uterus cysts at 5000 ppm (control incidence 1/49).

Study	Dose levels	Results
		Neoplastic findings Thyroid:↑Trend for follicular cell adenoma in M at 2500 ppm and above. ↑Follicular cell adenoma/carcinoma in M & F at 2500 ppm and above. Liver: ↑Hepatocellular adenoma in M at 5000 ppm. ↑Adenoma/carcinoma in M at 5000 ppm. Kidney: Tubule cell adenoma in M (2/50) at 5000 ppm. Testes: High incidence of intertestial cell tumour in in all exposure groups, including control animals.
OECD TG 451 2 years, mouse Oral (dietary) B6C3F1/Crl 60 M/F	Benfluralin (95,25%) Batch No.: 231EF4 0, 50, 300, 1500 ppm Equivalent to: Males: 6, 36.4 and 184.7 mg/kg bw/day Females: 6.9, 41.8 and 223.5 mg/kg bw/day	**NOAELs** Toxicity: 50 ppm (6 mg/kg bw/day). Carcinogenicity: 50 ppm (6 mg/kg bw/day). *Mortality* Survival rates in control animals were 81.7% in M and 86.7% in F. In M, survival rates were 78.3% at 1500 pmm, while a decrease were noted to 75% at 300 ppm and to 76.7% at 1500 ppm in F. *Clinical signs* Chromaturia was evident from wk 5 or 7 at 1500 ppm in M & F and persisted throughout the study. *FC, BW & BWG* ↓BW at 1500 ppm F at termination. ↓BWG at 1500 ppm in F at termination. FC was not affected by treatment (only a limited number of animals were monitored). *Haematology – 1500 ppm* ↑RBC in M (12 mo) ↑Hb in M (12 and 18 mo) ↑Hct in M (12 mo) ↑Mean corpuscular volume (MCV) in M (12 mo) ↑Mean corpuscular hemoglobin (MCH) in M (12 mo) ↑White blood cells (WBC) in M (12 mo) ↑Monocytes % in M & F (12 and 18 mo) *Clinical chemistry (24 mo)* 300 ppm: ↑BUN in F 1500 ppm: ↑AP in F ↑ALT in F ↑BUN in F *Organ weight* ↑Liver weight in F at 300 ppm and above in M & F. *Gross pathology* ↑Mouse Urologic Syndrome in M at 1500 ppm. ↑Gross liver nodules in F at 1500 ppm. ***Non-neoplastic findings***

Study	Dose levels	Results
		Histopathology ↑Focal hepatocellular hyperplasia in F at 1500 ppm. ↑Multifocal hepatocellular hyperplasia in M at 300 ppm and above in M & F. ***Neoplastic findings*** *Liver* ↑Statistical significant trend of increase of combined hepatocellular adenoma/carcinoma in F at 1500 ppm. Increased incidence of cellular carcinomas at 50 ppm and onwards in F.

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

Two acceptable long term toxicity and carcinogenicity studies were performed in rats and mice. The results are summarized in table 23 and include all effects observed with statistical significance (indicated by arrows). Further details (if not presented here) including study design, a description of the results (including information on incidences and severities of findings and extent of changes relative to controls, etc.) are given in the RAR (section B.6.5).

Rat

In a two-year combined chronic and carcinogenicity study in rats, benfluralin (purity 95.8%) was administred with dose levels in the diet of 0, 10, 100, 2500 or 5000 ppm (achieved test article intake: 0, 0.5, 125, or 250 mg/kg bw/day). On week 52, 10 rats/sex/dose were sacrified. The doses given equated to mean intake 0, 0.5, 5.4, 136.3 and 274.8 mg/kg/bw/day in males and 0, 0.7, 6.8, 167.9 and 331.3 mg/kg bw/day in females. The liver, the thyroid and the kidneys were the target organs after long-term administration of benfluralin to the F344 rat.

Significantly reduced survival rates were apparent in the males at 100 ppm and higher, and signs of poor antemortem condition including hunched posture, thin appearance, prostration or entire body paleness were noted more frequently (at the two highest doses, the onset of death was more rapid than at the lowest doses). Survival in the females was not dose-dependently affected. The majority of deaths was associated with haematopoietic or pituitary neoplasms, but in decedents, there was no apparent relationship of tumour prevalence with the treatment. At 2500 ppm and above, effects on skin/pelage in the females (alopecia, discoloration), and urine stains in both sexes were observed. At the highest doses, a slight increase of swollen appearances of the scrotum was also noted. Food consumption and body weight was consistently lowered at 2500 ppm (females) and above (males, females) throughout the study period, and was more marked in the females. Body weight changes were more markedly decreased during the second half of the study in these dose groups. For the whole treatment period, significant decreases in body weigth gain compared to controls amounted to 15% (males) and 32% (females) at 2500 ppm, and to 30% (males) and 50% (females) at the top-dose. Food efficiency was marginally lowered at the doses where body weight effects were affected, but data were only present for the first 3 months of treatment. There was an increased incidence of rats showing a yellow/orange hue in the internal eye structures (raw data not provided) at 2500 ppm and above (interpreted as the presence of test substance; according to the notifier, the yellow hue was unlikely to represent a clinical sign of icterus, as bilirubin levels were not excessively high). At the top-dose, slightly more rats showed cataract (females) or cloudy cornea (often associated with neovascularisation). The notifier stressed that the effects were secondary to the presence of corneal dystrophy at pre-dose stage (the lesion being a common in F344 rats).

There were treatment-related effects on some haematological parameters: red blood cell parameters were decreased in both the males and the females at 2500 ppm and above. Hct and Hb decreased in a slightly greater magnitude than the drop in RBC number, suggesting that the rats suffered a slight microcytic anemia. The etiology of it was unclear, as the finding could be related to the general underfeeding status of animals. The thrombocytosis at the highest dose was considered treatment-related.

With regard to clinical chemistry changes, (see table 24), the increased levels of urea nitrogen and creatinin at 2500 ppm and above were indicative of the nephropathy observed at these doses. The elevated cholesterol levels illustrated either the effect on the kidney and/or on the liver. Further indication of hepatotoxicity was found in the increased total bilirubin values. On the contrary, the lowered alkaline phosphatase and transaminase activities were interpreted as a changed liver metabolism pattern, as liver toxicity would be expected to be accompanied by a rise rather than a decrease of these enzymes. The increased levels of total proteins, more particularly albumin and globulin, were partly explained by the slight dehydrated status of the animals at the high doses (based upon the observed urine hypervolemia and decreased urine density).

Table 24: Summary of selected clinical chemistry findings in rats following a two-year dietary administration of benfluralin

Clinical chemistry parameter	Males					Females				
	Week	26	52	78	104	Week	26	52	78	104
	Dose (ppm)					Dose (ppm)				
Blood urea nitrogen	0	14	15	15	18	0	14	16	16	15
	10	14	15	15	17	10	15	15	16	16
	100	14	15	15	17	100	14	15	16	18
	2500	17*	17*	19	33*	2500	17*	19*	19*	21*
	5000	17*	19*	23*	37*	5000	20*	20*	22*	27*
Creatinine	0	0.6	0.4	0.6	0.7	0	0.7	0.5	0.7	0.6
	10	0.6	0.4	0.6	0.7	10	0.7	0.4	0.7	0.6
	100	0.7*	0.3	0.6	0.7	100	0.6	0.4	0.6	0.6
	2500	0.7*	0.5*	0.8*	0.8*	2500	0.7	0.5	0.7*	0.7*
	5000	0.7*	0.5*	0.8*	0.8	5000	0.7*	0.5	0.8*	0.7*
Total cholesterol	0	62	83	112	162	0	101	124	128	141
	10	66	91	118	152	10	98	125	135	137
	100	61	88	124	154	100	110	123	131	145
	2500	95*	116*	134	119	2500	158*	194*	187*	182*
	5000	112*	131*	132	171	5000	185*	207*	234*	284*
Total protein	0	6.7	7.0	6.6	6.6	0	7.4	7.5	7.6	7.2
	10	7.2	7.0	6.8	6.8	10	7.2	7.5	7.7	7.3
	100	7.1	7.0	6.9	6.7	100	7.4	7.5	7.5	7.4
	2500	7.6*	7.5*	7.4*	6.9	2500	7.8*	8.1*	8.3*	7.9*
	5000	7.8*	7.7*	7.2*	6.9	5000	8.2*	8.3*	8.6*	7.8*
Albumin	0	4.4	4.8	4.3	4.0	0	5.0	5.4	5.5	5.1
	10	4.7	4.7	4.4	4.1	10	4.9	5.4	5.5	5.1
	100	4.7	4.7	4.4	4.0	100	5.1	5.4	5.3	5.1
	2500	4.9*	5.1*	4.6	4.0	2500	5.2	5.7*	5.8	5.2
	5000	5.1*	5.2*	4.4	4.0	5000	5.3*	5.7*	5.9*	4.8
Globulin	0	2.3	2.2	2.3	2.6	0	2.4	2.1	2.1	2.2
	10	2.5	2.3	2.4	2.7	10	2.3	2.1	2.2	2.3
	100	2.5	2.3	2.4	2.7	100	2.3	2.0	2.2	2.2
	2500	2.7*	2.4*	2.8*	2.9	2500	2.6*	2.4*	2.5*	2.7*
	5000	2.7*	2.5*	2.8*	3.0	5000	2.8*	2.6*	2.7*	3.0*
Alkaline phosphatase	0	72	89	74	94	0	56	53	53	72
	10	71	85	72	66	10	51	5	51	78
	100	70	84	74	61	100	48	50	59	99
	2500	63	67*	68	71	2500	48	45	42	46
	5000	56*	58*	86	61*	5000	49	42*	35*	55
Alanine aminotransferase	0	75	83	66	71	0	50	52	57	58
	10	64	74	59	52	10	45	58	58	58
	100	61	62*	52	49	100	47	44*	60	95
	2500	40*	47*	41	59	2500	37*	42*	47	45*
	5000	40*	47*	38*	60	5000	33*	36*	39*	45*
Aspartate aminotransferase	0	110	109	111	183	0	86	81	78	85
	10	92	95	90	87	10	81	86	84	113
	100	88	89*	78	73*	100	85	74	87	186
	2500	70*	72*	70	128	2500	73*	65*	86	70
	5000	69*	66*	72	101	5000	70*	65*	62*	58*

* Significantly different from control value, $p < 0.05$

Males, treated at 2500 ppm and above, showed a slightly decreased urine specific gravity (-1%) from wk 52 on (although absolute values were also slightly higher on wk 52, 78 and 104 than on wk 26). The report further stated (data not provided) that urine volume was high at 2500 ppm and above at most time-points. Further, there was an increased incidence of hyaline casts (indicating protein loss through the glomeruli) or fine granular casts (cell casts that have arisen in the renal tubules) in the high-dose animals on wk 52. The effects showed no clear time-dependency, as no consistent findings were observed on either wk 78 or wk 104. The darker appearance of the urine at the high doses was merely related to excreted test article and/or metabolites (also observed in other studies), as urine concentration would be incompatible with the observed hypervolemia.

The urinalysis findings were considered treatment-related, in the view of the effects on the kidney (chronic progressive nephropathy, CPN).

Organs from the animals at the 52 and 104 week scheduled kills were weighed and subjected to a gross necropsy (see table 25). Liver, thyroid and adrenal weights were increased at 2500 ppm and 5000 ppm. An enlarged appeareance of the testes observed during necropsy indicated that also the weights of testes were increased at 2500 ppm or 5000 ppm.

Table 25: Selected organ weights of rats at interim and terminal sacrifice points

Sex and sacrifice point	Dose (PPM)	Final body weight	Liver			Thyroid			Adrenal		
			Organ weight (g)	Organ: body weight ratio	Organ: brain weight ratio	Organ weight (g)	Organ: body weight ratio	Organ: brain weight ratio	Organ weight (g)	Organ: body weight ratio	Organ: brain weight ratio
Males											
Interim	0	348.3	8.63	2.481	4.455	0.025	0.0071	0.0127	0.049	0.0142	0.0255
	10	360.7	8.97	2.485	4.612	0.025	0.0069	0.0128	0.051	0.0142	0.0262
	100	366.3	9.07	2.477	4.676	0.026	0.0070	0.0133	0.055	0.0150	0.0281
	2500	345.9	10.93*	3.166*	5.659*	0.029*	0.0084*	0.0150*	0.056	0.0162	0.0291
	5000	344.0	13.33*	3.869*	7.053*	0.032*	0.0092*	0.0167*	0.055	0.0160	0.0292
Ter-mination	0	324.8	10.08	3.136	4.970	0.034	0.0106	0.0170	0.069	0.0213	0.0339
	10	334.1	9.98	3.014	4.953	0.033	0.0101	0.0164	0.070	0.0212	0.0348
	100	341.4*	10.44	3.073	5.186	0.035	0.0105	0.0177	0.086	0.0251	0.0431
	2500	296.9*	12.04*	4.050*	6.157*	0.053	0.0176	0.0275	0.081*	0.0275*	0.0414*
	5000	264.5*	12.81*	4.840*	6.776*	0.046	0.0173	0.0244	0.093*	0.0352*	0.0502*
Females											
Interim	0	193.5	5.13	2.654	2.850	0.019	0.0101	0.0108	0.050	0.0263	0.0280
	10	186.9	5.06	2.707	2.878	0.020	0.0105	0.0112	0.058	0.0309	0.0328
	100	187.9	5.20	2.768	2.876	0.021	0.0111	0.0115	0.050	0.0269	0.0278
	2500	172.9	6.84*	3.953*	3.830*	0.022	0.0127*	0.0123	0.065*	0.0377*	0.0364*
	5000	165.6*	7.81*	4.721*	4.549*	0.025*	0.0151*	0.0146*	0.066*	0.0398*	0.0383*
Ter-mination	0	222.4	6.40	2.885	3.436	0.041	0.0182	0.0219	0.065	0.0295	0.0352
	10	224.7	6.34	2.829	3.597	0028	0.0128	0.0164	0.067	0.0302	0.0381
	100	224.7	6.47	2.891	3.618	0.024	0.0105	0.0132	0.101	0.0469	0.0586
	2500	179.5*	7.79*	4.343*	4.491*	0.045	0.0257*	0.0270	0.072	0.0405*	0.0419*
	5000	156.2*	9.18*	5.888*	5.303*	0.038	0.0243*	00216	0.074	0.0477*	0.0438*

* $p < 0.05$, values statistically significantly different from control. (two tailed Dunnett t on raw data).

Gross necropsy lesions were observed at 2500 ppm and higher, and were essentially confined to the liver and the kidney. The effects in lung (pale area), stomach (dark area), testis (enlarged), uterus (cysts) and ovary (cysts) were corroborated by histopathological findings. Adipose tissue of animals at the highest dose-levels (2500 and 5000 ppm) was dark (yellow discolored) in appearance, but no histological data were available to confirm the finding, and it was probably due to substance accumulation.

Non-neoplastic findings

Histopathological changes considered attributable to treatment were recorded in the thyroids, the liver, the kidney (see table 26) and the testes.

The effects in the liver included hepatocellular pigmentation and centrilobular hypertrophy at 100 ppm and above, along with hepatocellular necrosis and sinusoidal cell pigmentation at the highest doses. At 100 ppm and above, kidney calculi were observed, which were believed to be the primary cause, both the exacerbation of nephropathy (CPN) and of transitional cell hyperplasia, possibly leading to tubular cell adenoma in two males at the top-dose. In the thyroid, follicular hyperplasia was increased at 2500 ppm and above and this was considered a first stage of the neoplastic changes. Another major finding was a dose-dependent increase in incidence and of severity of sciatic nerve degeneration and concomitant skeletal nerve degeneration at 2500 ppm and higher. Muscle degeneration was characterised by slightly atrophic fibers with prominent sarcolemmal nuclei. Sciatic nerve fibers showed also prominent nuclei. In addition, infiltration of foamy lipid laden macrophages, along with occasional cholesterol clefts in the intramyelin spaces was observed. The relevance of the sciatic nerve degeneration was discussed at the PRAPeR Expert Meeting 34 (22 – 26 October

2007), which considered the effects of sciatic nerve degeneration not relevant. No clear mode of action for the sciatic nerve degeneration was provided and it was pointed out that the effects were only observed at the high dose levels at the end of treatment, and associated with high systemic toxicity. Furthermore, the finding was linked to aged rats only, and was not seen e.g. in the dog study. It was also pointed out that in general the active substance is not considered a neurotoxin.

Table 26: Selected non-neoplastic findings in rats following a two-year dietary administration of benfluralin

Dose (ppm)		**0**		**10**		**100**		**2500**		**5000**	
	week	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
N° of animals examined	*52*	*10*	*10*	*10*	*10*	*10*	*10*	*10*	*10*	*10*	*10*
	104	*50*	*50*	*50*	*50*	*50*	*50*	*50*	*50*	*50*	*50*
THYROIDS											
follicular cyst	52	1	0	2	0	0	0	1	0	**2**	**2**
	104	8	4	4	2	5	4	**11**	**12**	**10**	**7**
follicular cell hypertrophy	52	0	0	0	0	0	0	1	0	**7**	**4**
	104	0	0	0	0	0	0	0	0	0	**3**
follicular cell hyperplasia	104	1	0	1	0	0	1	**3**	**2**	**3**	**4**
LIVER											
centrilobular/diffuse hypertrophy	52	0	0	0	0	0	0	**10**	0	**10**	**10**
	104	0	1	2	1	**3**	**6**	**27**	**34**	**31**	**42**
mean severity grade		*0*	*0*	*0*	*0*	*0.1*	*0.1*	*0.9*	*0.6*	*1.1*	*1.3*
hepatocellular pigmentation	52	0	0	0	0	0	0	**3**	**10**	**9**	**10**
	104	0	5	0	6	0	**24**	**18**	**44**	**30**	**41**
mean severity grade		*0*	*0.1*	*0*	*0.1*	*0*	*0.4*	*0.1*	*1.2*	*0.4*	*1.2*
sinusoidal cell pigmentation	104	2	7	2	5	5	6	2	2	**12**	3
mean severity grade	*104*	*0.2*	*0.3*	*0.1*	*0.4*	*0.2*	*0.5*	*0.1*	*0.2*	*0.4*	*0.1*
hepatocellular necrosis	104	2	5	6	7	5	11	**15**	5	**27**	9
mean severity grade	*104*	*0.1*	*0.2*	*0.5*	*0.2*	*0.2*	*0.6*	*0.6*	*1.2*	*0.7*	*0.6*
KIDNEY											
chronic progressive nephropathy (CPN)	52	10	2	9	2	10	2	10	3	10	**7**
	104	48	44	50	47	39	44	49	**49**	50	**49**
mean severity grade		*2.2*	*1.0*	*2.2*	*1.1*	*2.3*	*1.1*	***2.6***	***1.6***	***2.6***	***2.5***
hyaline droplets	52	0	0	0	0	**10**	**10**	**10**	**10**	**10**	**10**
	104	10	5	9	**12**	**33**	**47**	**43**	**49**	**48**	**47**
mean severity grade		*0.3*	*0.1*	*0.2*	*0.2*	*1.0*	*1.3*	*1.4*	*2.2*	*1.7*	*2.0*
tubule cell karyomegaly	52	0	0	0	0	0	0	10	10	10	10
	104	1	0	2	0	**25**	**1**	**50**	**49**	**49**	**49**
mean severity grade		*0*	*0*	*0*	*0*	*0.4*	*0*	*1.6*	*1.5*	*1.8*	*1.7*
transitional cell hyperplasia	52	0	0	0	0	0	0	0	0	2	7
	104	0	0	0	0	**4**	**1**	**49**	**41**	**50**	**47**
mean severity grade		*0*	*0*	*0*	*0*	*0.1*	*0*	*1.7*	*1.1*	*2.3*	*1.8*
large pelvis calculus	52	0	0	0	0	0	3	2	3	2	7
	104	3	6	1	7	**4**	**8**	**37**	**20**	**47**	**26**
free renal pelvic calculus	104	19	45	24	38	**26**	46	**42**	48	**48**	41
SCIATIC NERVE: degeneration	104	0	0	2	1	0	0	**26**	**26**	**30**	**41**
mean severity grade	*104*	*0*	*0*	*0.4*	*0*	*0*	*0*	***0.2***	***0***	***0.7***	***0.8***
SKELETAL MUSCLE: degeneration	104	1	0	2	0	0	0	**33**	**32**	**35**	**44**
mean severity grade	*104*	*0*	*0.1*	*0*	*0*	*0*	*0*	***0.7***	***0.6***	***0.9***	***1.1***
LUNG: chronic inflammation	52	3	0	1	0	0	1	2	2	4	**3**
	104	13	7	7	11	8	11	10	**33**	**20**	**37**
ABDOMINAL CAVITY congestion	52	0	0	0	0	0	0	**6**	**3**	**7**	**2**
STOMACH *n° examined*	*104*	*48*		*8*		*20*		*28*		*50*	
erosion/ulcer	104	4		3		4		**11**		**10**	
UTERUS *n° examined*	*104*		*50*		*20*		*28*		*19*		*49*
endometrial cyst	104		6		3		5		2		**14**

Incidence on 10 (wk 52) or on 50 (wk 104, including deaths on test or unscheduled sacrifices), except mentioned otherwise; the severity grade of findings are mean values of observations in all animals (n=60), except stated otherwise; Statistically significant modification, Fisher-Irwin exact test *p<0.05.

Neoplastic findings

An overview of selected neoplastic findings is presented in table 27.

At 2500 ppm and above, the combined incidence of hepatocellular adenoma and carcinoma was increased in the males. Both benign and malignant tumors occurred at 2500 ppm and the high dose. In the kidney, the tubule cell adenoma at 5000 ppm was considered secondary to the observed CPN and associated tubule cell hyperplasia. Thyroid follicular cell adenoma and carcinoma were increased at 2500 ppm and the high dose. A significant trend was demonstrated for the males (adenoma) and for the males and females (combined neoplastic incidence).

Table 27: Selected neoplastic findings of rats following a two-year dietary administration of benfluralin

Dose (ppm)	**week**	**0**		**10**		**100**		**2500**		**5000**	
		♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
N° of animals examined	*52*	*10*	*10*	*10*	*10*	*10*	*10*	*10*	*10*	*10*	*10*
	104	*48*	*50*	*50*	*50*	*48*	*50*	*50*	*50*	*50*	*50*
THYROIDS											
follicular cell adenoma[b]	52	0	0	0	0	0	0	0	1	1	0
[α]	104	1	0	1	0	1	0	**3**	**3**	**5**	**2**
follicular cell carcinoma[m]	52	0	0	0	0	0	0	1	0	0	0
	104	0	0	0	0	0	1	**4**	**2**	**3**	**2**
combined adenoma+carcinoma [β]		*1*	*0*	*1*	*0*	*1*	*1*	***8****	***6***	***9****	***4***
LIVER											
hepatocellular adenoma[b]	52	0	0	0	0	0	0	1	0	0	0
	104	1	2	2	0	1	1	**3**	1	**9***	2
hepatocellular carcinoma[m]	104	1	0	0	0	0	0	**2**	0	**2**	0
combined adenoma+carcinoma		*2*	*2*	*2*	*0*	*1*	*1*	***6***	*1*	***11****	*2*
KIDNEY											
tubule cell adenoma	104	0	0	0	0	0	0	0	0	**2**	0
TESTES ***n° examined***	*52*	*10*		*10*		*10*		*10*		*10*	
	104	*48*		*49*		*45*		*50*		*50*	
interstitial cell tumour[b]	52	1	-	0	-	0	-	0	-	**4**	-
	104	44	-	49	-	45	-	47	-	48	-

Incidence on 10 (wk 52) or on 50 (wk 104, including deaths on test and unscheduled sacrifices); m: malignant, b: benign; Statistically significant modification, Fisher-Irwin exact test *p<0.05; significant (p<0.05) trend for ♂ (α) and for ♂+♀(β)

At the top-dose, interstitial cell tumour incidence was increased at interim kill. However, at termination the incidence was not markedly higher than the concurrent incidence in the controls. Overall, the incidence in all animals was 52/60 (87%), which was slightly above the control value of 45/60 (75%).

There was a slight increase of malignant granulosa/theca cell tumours in the ovary (3/14) and uterus carcinoma (4/12) at 2500 ppm, but the finding was not elevated at the top-dose (1/50 for the ovary tumours, and 2/49 for the uterine tumours).

In conclusion, liver and thyroid tumors were observed in the rat (along with a slight increase of kidney adenoma and Leydig cell tumors). It should be noted that the increased incidence of Leydig cell tumours was detected at the top dose, albeit only in the 1-year sacrifice group.

The carcinogenicity NOAEL should be 100 ppm = 5.4 mg/kg bw/day, based on the increase in combined incidence of adenoma and carcinoma in the liver and thyroids at higher doses. The study follows OECD TG 453 with two deviations (variation of weight at start slightly in males (> 20%) and there were no summary data for ophthalmology and urinanalysis). The deviations were not considered to have affected the setting of carcinogenic endpoints. It should be noted that benfluralin, with a technical specification limited to a level of 0.085 mg/kg for the impurity EBNA is unlikely to be genotoxic (section 10.8.2). Genotoxicity as a mechanism for carcinogenicity in the rat is therefore unlikely since the rat study was conducted with a batch of benfluralin containing a lower level than 0.085 mg/kg EBNA.

Mouse

Benfluralin (purity 95.25%-96.15%) was administred to males and females for 104 weeks at doses of 0, 50, 300 or 1500 ppm, which equated to 6, 36.4 and 184.7 mg/kg bw/day in males and 6.9, 41.8 and 223.5 mg/kg bw/day. The study was conducted as two replicates (designated MO2785 and MO2885), with initiation dates separated by 15 day interval. The results were combined for the report (designated MC2729). The study was acceptable and follows OECD TG 451 (September 7th 2009) with the above mentioned exception that the study was conducted as two independent experiments (15d apart) and that food consumption was determined only on 9 animals/sex/dose on months 1, 3, 6, 12, 18 and 24. The target organs in the mouse were the kidney and the liver.

The survival was slightly impaired in the female animals, treated at 300 ppm and above. The causes of death were similar among study groups, except for a slightly increased incidence of mouse urologic syndrome (MUS) in the males of the two highest doses. Decreases in body weight were noted from 50 ppm and throughout the study, but at termination results indicated only slight changes at 300 ppm (females) and above (males, females). It was of note that the differences were more marked in study MO2885 (see table 28). The body weight gain was markedly depressed at 300 ppm and above, but occasional decreases were already observed at 50 ppm. There were no clinical signs attributable to treatment, except the observed chromaturia in the high dose animals which is a common finding in animals given high doses of dinitroanaline compounds.

Table 28: Summary of growth in mice following a two-year dietary administration of benfluralin

Dose (ppm)		**0**		**50**		**300**		**1500**	
		♂	♀	♂	♀	♂	♀	♂	♀
Survival (%)[§]		82(2)	87	87(2)	80	77(4)	75	78(7)	77
Body weight	**24 mo**					-	↓6%	↓4%	↓7%*
	24 mo[2]					-	↓15%*	↓11%	↓14%*
Mean weight (g)	**0 mo**	20.1	17.0	19.5	17.0	20.2	16.7	20.0	16.7
Mean weight (g)	**24mo**	41.0	37.7	40.3	36.6	41.2	35.5	39.2	35.1*
Body weight gain	**6 mo**			↓9%**	↓6%	↓12%***	↓8%	↓11%**	↓9%*
	9 mo			↓10%**	-	↓11%**	↓8%	↓14%**	↓14%**
	12 mo			↓7%*	-	↓10%**	↓7%	↓13%**	↓13%**
	15 mo			↓7%*	-	↓11%**	↓8%	↓12%**	↓14%**
	18 mo			-	-	↓7%*	↓4%	↓8%*	↓5%
	24 mo			-	-	-	↓10%	↓9%	↓11%*
Mean weight gain (g)	**24mo**	21.1	20.7	20.8	19.7	20.9	18.7	19.2	18.4*

Combined values, except when annotated [2]:study MO2885; Statistically significant modification: Dunnett's t-test *p<0.05, **p<0.01;
[§]: indices refer to the n° of ♂ animals dying or humanely killed, showing mouse urologic syndrome

In the males, most red blood cell parameters were significantly increased at 12 months. At 18 months, the increases were restricted to marginally increased Hb and Hct levels. The findings were unremarkable at termination. No effects were observed in the females at any sampling times. As the RBC compartment was affected in other species, a relationship with treatment was suspected.
The observed increased levels of monocytes at 12 month were not confirmed at 18 month or 24 month, and were thus considered fortuitous. The decreased leukocyte counts in the males at intermediate sampling times was small (15-28%) and was not corroborated by shifts in the differential count, or was unremarkable at termination, and was therefore considered irrelevant. Likewise, the increased eosinophil count at 24 months in

the females was also considered incidental as it was present in only one replicate and at one time-point. An increase of alkaline phosphatase (top-dose) and of alanine aminotransferase (300 ppm and above) was obvious in the females, indicating hepatic dysfunction. It was of note that extreme values were recorded in the top-dose males bearing liver neoplasms (see histopathology). At 300 ppm and above, blood urea nitrogen levels were significantly increased. However, dose-dependency was not evident, and as no renal effects were observed, the relationship with treatment is unclear. The other parameters (glucose, creatinine and total bilirubin) were unaffected.

Table 29: Summary of haemotology and clinical chemistry parameters in mice following a two-year dietary administration of benfluralin

Dose (ppm)		**0**		**50**		**300**		**1500**	
		♂	♀	♂	♀	♂	♀	♂	♀
Haematology									
RBC	**12 mo**							↑6%*	-
Hb	**12 mo**							↑8%*	-
	18 mo							↑4%*	-
Hct	**12 mo**							↑7%*	-
	18 mo							↑2%	-
MCV	**12 mo**							↑1%*	-
MCH	**12 mo**							↑2%*	-
WBC (10^3/mm^3)	**12 mo**	8.69±2.60						6.30±2.00*	
	18 mo	11.36±2.38						9.60±2.71	
monocytes (%)	**12 mo**	0.20±0.42	0.20±0.42					2.40±2.63*	1.40±1.35*
	18 mo	3.70±3.56	4.70±4.74					0.50±0.85*	0.70±1.25*
eosinophils (%)	**24 mo**	-	0.23±0.65					-	0.52±0.75
Clinical chemistry									
AP (I.U./L)	***24 mo***		*856±3230*				*461±258*		*535±260**
AP[1] (I.U./L)	**24 mo**	-	413±253			-	430±199	-	491±277 ↑19%
AP[2] (I.U./L)	**24 mo**	-	398±200▪			-	488±302 ↑23%	-	566±248 ↑42%
ALT (I.U./L)	***24 mo***	-	*25±15*			-	*49±74*	-	*94±182** ↑276%
ALT[1] (I.U./L)	**24 mo**	-	27±19	-	▪▪	-	46±52 ↑70%	-	62±119 ↑130%
ALT[2] (I.U./L)	**24 mo**	-	24±9	-	-	-	52±91 ↑117%	-	116±215* ↑383%
BUN (mg/dL)	**24 mo**	-	18.7±10.9	-	-	-	26.4±22.3* ↑41%	-	21.5±6.1* ↑15%

Combined values, except when annotated as [1]:study MO2785 or [2]:study MO2885; Shaded areas: no data;
Statistically significant modification: Dunnett's t-test *p<0.05;
▪: outlier (control animal #72) excluded (23420 I.U./L considered analytical error);
▪▪: value 29±31 I.U./L, comparable with controls, excluding one outlier (50 ppm animal #1080: 3360 I.U./L considered analytical error)

At necropsy, the top-dose males had developed the Mouse Urologic Syndrome at a higher frequency than those of the control group. At the lower doses, the prevalence was also moderately high. Gross liver nodules were detected at a higher incidence in the top-dose females, but not in the males.

The treatment-related non-neoplastic lesions were essentially confined to the liver. At 300 ppm and higher, hepatotoxicity occurred supported by alkaline phosphatase (AP) and alanine transamine (ALT)-activities, and increased liver weights. Increased incidences of adenoma (1500 ppm) and of carcinoma (50 ppm, 300 ppm and 1500 ppm) were observed in females. The combined incidence was also increased at 300 ppm onwards in the females, and a statistically significant trend was detected when the Peto's survival adjusted trend test was applied (p=0.018). At termination, the incidences in the survivors were 1/52 (1.9%), 2/49 (4.1%), 3/45 (6.7%) and 5/47 (10.6%) at 0, 50, 300 and 1500 ppm respectively. These incidences were then compared to an in-house contemporary historical control incidence of liver neoplasias in the females, based on studies conducted

from 1980 onwards. It appeared that both the incidence of adenomas and carcinomas separately (5.1%), and the combined incidence (10%), was outside the range (stated being 3.3% and 6.9%, respectively). In the males, a high number of combined adenomas and carcinomas was observed (1500 ppm), but no statistically increase in the combined incidence was detected due to a high number of hepatocellular carcinomas in the control group. An overwiew of the histopathological findings is presented in table 30.

Table 30: Liver weight, necropsy and histopathology of mice following a two-year dietary administration of benfluralin

Dose (ppm)		**0**		**50**		**300**		**1500**	
		♂	♀	♂	♀	♂	♀	♂	♀
Liver weight	**a**					-	↑19%	↑5%*	↑21%*
	r					-	↑26%	↑10%*	↑31%*
Number examined	**1**	30	30	30	30	30	30	30	29
	2	30	30	30	30	30	30	30	30
	T	*60*	*60*	*60*	*60*	*60*	*60*	*60*	*59*
Mouse urologic syndrome	**T**	5	-	12 *(20%)*	-	11 *(18%)*	-	**18** *(30%)*	-
Liver gross pathology :nodules	**1**	11	2	9	5	9	**9**	13	**10**
	2	13	5	7	1	6	3	12	**15**
	T	*24*	*7*	*16*	*6*	*15*	***11***	*25*	***25***
Liver histopathology									
Focal hepatocellular hyperplasia	**T**	20	6	10	5	11	4	14	**15**
Multifocal hepatocellular hyperplasia	**T**	1	1	2	0	**5**	1	**8**	**8**
Hepatocellular adenoma	**T**	2	1	1	1	3	1	4 (6.7%)	3 (5.1%)
Hepatocellular carcinoma	**T**	7	0	7	2	5	3 (5%)	8 (13%)	3 (5.1%)
combined incidence adenoma/carcinoma		*9*	*1*	*8*	*3*	*6* *(10%)*	*4* *(6.7%)*	*12* *(20%)*	*6#* *(10%)*
Cholangiocarcinoma	**T**							0	1 (1.7%)
Haemangiosarcoma	**T**					1	0	0	1 (1.7%)
Haemangioma	**T**							1 (1.7%)	0

Combined values (T), except when annotated as 1:study MO2785 or 2:study MO2885; a: absolute, r; relative weight;
Statistically significant modification: Dunnett's t-test *p<0.05;
#: value showed a statistical significant trend (p<0.05; see text) when expressed as the incidence in the survivors on week 104.

Due to the results in the females, benfluralin should be considered carcinogenic in the mouse liver based on the hepatocellular carcinomas. Further, the increased incidence of carcinomas at the lowest dose (6 mg/kg bw/day) in females should not be disregarded. It should be noted that the mouse study was conducted with a batch of benfluralin containing a higher level of EBNA (0.31 mg/kg) than the highest technical specification (0.085 mg/kg) tested in the standard genotoxicity battery of *in vitro* and *in vivo* assays.

In summary, and based on the results from the presented studies, benfluralin should be considered carcinogenic in the rat liver and thyroid and in the mouse liver.

Summary of supporting data for the key events of proposed Mode of Action

There are possible mechanistic explanations for the carcinogenic effect in rats and mice. The notifier submitted a study (RAR, section B.6.8.2) to examine the possible MoA for benfluralin-induced liver tumors in F344/DuCrl rats. In this study, male F344/DuCrl rats (6/dose/time period) were exposed to 0 and 5000 ppm benfluralin in the diet for 7 or 14 days. Based on the results from this study, the notifier pointed out that based on new mechanistic data, the observed benfluralin-induced liver and thyroid tumours are caused by the constitutive androstane receptor (CAR) - and UDPglucuronsyltransferase (UGT)-mediated mode of actions, respectively, both of which are not relevant to humans. The tumors seen in the mice are proposed to be caused by the same rodent MoA. The results from the study are summarized in table 31.

Table 31: Benfluralin-induced molecular, cellular, and biochemical changes in male F344/DuCrl rats

<table>
<tr><th>Type of study/data, aim of study</th><th>Test substance, test system</th><th>Conc/dose, replicates, duration of exposure</th><th>Results</th></tr>
<tr>
<td>Repeated dose mode-of-action study in rats
Non GLP compliance

Author (2010)
Report no: 090246/CA 5.8.2/01</td>
<td>Benfluralin (97.5%)
Lot/Batch No: ACD13683 (TSN100037)
Male rats, F344/DuCrl (6/dose/time period)</td>
<td>0 & 5000 ppm
Equivalent to: 0 and
7-day group: 449 mg/kg/day
14-day group: 436 and 435 mg/kg/day (at 7 and 14 days)
7 & 14 days’ exposure
<u>Endpoints:</u>
Serum: clinical chemistry and thyroid hormone analysis; Liver: histopathology, targeted gene expression, cytochrome P450 enzymatic activity, and hepatocellular proliferation; Thyroid: histopathology and cell proliferation.
Indicators of nuclear-receptor activation: Cyp2b1, Cyp2b2, Cyp3a23/3a1, Cyp4a1, Ugt1a6, Ugt2b17</td>
<td>General toxicity: There were no treatment-related deaths or clinical signs of toxicity.

Effects on body weight, body weight gain and food consumption
<table>
<tr><th></th><th colspan="2">7 Day exposure</th><th colspan="2">14 day exposure</th></tr>
<tr><td>Dose (ppm)</td><td>0</td><td>5000</td><td>0</td><td>5000</td></tr>
<tr><td>Number of rats</td><td>6</td><td>6</td><td>6</td><td>6</td></tr>
<tr><td>Body weight (kg)</td><td>151.9</td><td>145.8 (↓4%)</td><td>181.5</td><td>174.0 (↓4.1%)</td></tr>
<tr><td>Body weight gain (kg)</td><td>31.7</td><td>24.5 (↓22.7%)</td><td>61.0</td><td>53.6 (↓12.1%)</td></tr>
<tr><td>Food consumption</td><td>13.1</td><td>12.0 * (↓8.4%)</td><td>15.1</td><td>14.2 (↓6.1%)</td></tr>
</table>
Clinical chemistry parameters
<table>
<tr><th></th><th colspan="2">7 Day exposure</th><th colspan="2">14 day exposure</th></tr>
<tr><td>Dose (ppm)</td><td>0</td><td>5000</td><td>0</td><td>5000</td></tr>
<tr><td>Number of rats</td><td>6</td><td>6</td><td>6</td><td>6</td></tr>
<tr><td>Cholesterol (mg/dl)</td><td>84</td><td>104*</td><td>83</td><td>102*</td></tr>
<tr><td>Triglycerides (mg/dl)</td><td>207</td><td>133*</td><td>242</td><td>125*</td></tr>
<tr><td>Alkaline phosphatase (ALP) (u/l)</td><td>245</td><td>186*</td><td>322</td><td>235*</td></tr>
<tr><td>Gamma-glutamyl transpeptidase (GGT) (u/l)</td><td></td><td></td><td>2</td><td>3*</td></tr>
<tr><td>Aspartate aminotransferase (AST) (u/l)</td><td></td><td></td><td>80</td><td>67*</td></tr>
<tr><td>Alanine aminotransferase (ALT) (u/l)</td><td></td><td></td><td>53</td><td>37*</td></tr>
<tr><td>Total protein (g/dl)</td><td></td><td></td><td>6.1</td><td>6.4*</td></tr>
<tr><td>Albumin (g/dl)</td><td></td><td></td><td>3.8</td><td>4.0*</td></tr>
</table>
*Statistically different from controls at alpha = 0.05

Serum concentrations of thyroid hormones
<table>
<tr><th></th><th colspan="2">7 Day exposure</th><th colspan="2">14 day exposure</th></tr>
<tr><td>Dose (ppm)</td><td>0</td><td>5000</td><td>0</td><td>5000</td></tr>
<tr><td>Number of rats</td><td>6</td><td>6</td><td>6</td><td>6</td></tr>
</table>
</td>
</tr>
</table>

Type of study/data, aim of study	Test substance, test system	Conc/dose, replicates, duration of exposure	Results

T3 (ng/dl)	115.28	93.64*	118.07	106.09
T4 (ug/dl)	5.57	2.15*	4.95	2.55*
Thyroid stimulating hormone (TSH)	3.64	5.34	4.80	4.63

*Statistically different from controls at alpha = 0.05

Absolute and relative organ weights

	Liver		**Thyroid gland**	
Dose (ppm)	**7 days of treatment**			
	g	g/100	g	g/100
0	6.87	4.52	0.0083	0.0055
5000	8.575*	5.876*	0.0097	0.0066*
	14 days of treatment			
0	7.70	4.24	0.0086	0.0047
5000	10.024*	5.76*	0.01*	0.006*

*Statistically different from controls at alpha = 0.05

Histopathological observations

		7 Day exposure		**14 day exposure**	
Dose (ppm)		0	5000	0	5000
Liver	Number examined	6	6	6	6
	Hypertrophy with altered tinctorial properties; hepatocyte; centrilobular/midzonal, very slight	0	6	0	6
	Extramedullary Haematopoiesis; multifocal, very slight			1	0
	Necrosis; hepatocyte; focal, very slight			1	0
	Mitotic Alteration; increased; hepatocyte; multifocal, very slight	4	3	3	3
Thyroid gland	number examined	6	6	6	6
	Hypertrophy, follicular cell, diffuse Very slight	1	3	0	4
	Ectopic Tissue; focal	0	1	1	0

Targeted gene expression (expressed as fold-change compared to control

	7 Day exposure		**14 day exposure**	
Dose (ppm)	0	5000	0	5000

Type of study/data, aim of study	Test substance, test system	Conc/dose, replicates, duration of exposure	Results

Cyp 2b1	1	474.79*	1	343.77*
Cyp 2b2	1	11.45*	1	12.09*
Cyp 3a3	1	9.40*	1	7.48*
Cyp 4a1	1	0.89	1	0.97
Ugt 1a6	1	7.43*	1	6.94*
Ugt 2b17	1	7.33*	1	8.30*

* Significantly different at P = 0.05 and ≥ 1.5 fold-change criterions.

Liver Enzyme Activity (picomole/min/mg protein)

	7 Day exposure			**14 day exposure**		
	Mean activity		**Fold change**	**Mean activity**		**Fold change**
Dose (ppm)	0	5000		0	5000	
EROD	12.74	44.81	3.52*	12.30	40.36	3.28*
PROD	1.45	9.51	6.55*	1.81	8.09	4.46*
UGT	1.26	3.09	2.46*	1.11	2.96	2.67*

* Significantly different at P = 0.05 and ≥ 1.5 fold-change criterions.

Liver Proliferation as Measured by BrdU

	7 Day exposure			**14 day exposure**		
	Mean activity		**Fold change**	**Mean activity**		**Fold change**
Dose (ppm)	0	5000		0	5000	
centrilobular	26.4	28.0	1.06	21.8	30.6	1.4*
Midzonal	38.3	45.1	1.18	46.8	57.0	1.22*
Periportal	29.3	48.5	1.66*	40.1	62.4	1.56*
Total	31.3	40.6	1.29	36.2	50.0	1.38*

Score: Mean% positive hepatocytes based on 1000 cells/zone/animal, *=statistically identified, Dunnett's test (α≤0.05)

Thyroid Proliferation as Measured by BrdU

	7 Day exposure			**14 day exposure**		
	Mean activity		**Fold change**	**Mean activity**		**Fold change**
Dose (ppm)	0	5000		0	5000	
Follicular	6.43	9.97	1.55*	5.05	6.05	1.20

Score: Mean% positive thyroid follicular cells based on 1000 cells/animal, *=statistically identified, Dunnett's test (α≤0.05).

The results from the study indicate that CAR may be involved, due to elevated CAR-related transcripts and activities of the hepatic enzymes pentoxyresorufin O-dealkylase (PROD) and UGT. Hepatocellular proliferation was also demonstrated. However, as also elevated levels of EROD were demonstrated, a mechanism involving activation of the Ah-receptor cannot be excluded. In the study report, it is suggested that the elevated EROD activity is likely produced through a rat specific AhR-independent mechanism. The notifier argument is however not supported by studies with benfluralin showing no activation in mice. It is to be noted that in the previous evaluation (DAR, 2006), CYP1-450 induction assayed by determining the p-nitroanisole O-demethylase activity, was detected in the short term toxicity study with mice. This indicates Ah-receptor activation in the mice. The mechanism of liver tumours induced by benfluralin has therefore not yet been clearly demonstrated.

With respect to the possible mechanism for thyroid tumours, UGT activation and as well as decrease in T4 was demonstrated in the MoA study. However, as TSH was not affected, the possible mechanism that the decrease in T4 stimulates the pituitary to release more TSH that drives the thyroid to make more T4, was not demonstrated.

Other plausible mechanisms

The notifier has also pointed out that benfluralin caused liver and thyroid tumours in rats at doses above a MTD and that the thyroid tumours in male rats are not relevant to humans, due to the absence of a high-affinity plasma protein for binding thyroid hormones in the blood of rodents. Furthermore, the notifier refers to studies conducted with the dinitroaniline analogues pendimethalin and trifluralin, in which thyroid tumours were seen. According to the notifier, the effects observed from various studies conducted with pendimethalin provides evidence of an effect on thyroid hormone homeostasis secondary to liver enzyme induction, and that consequently the secondary effects of liver enzyme induction on thyroid function are also responsible for the thyroid tumours in male Fischer 344 rats following chronic exposure to benfluralin.

Notably, the carcinogenic potential of benfluralin (liver and thyroid in rats, liver in mice) and the relevance of trifluralin assays to assess benfluralin toxicological properties, was discussed at the PRAPeR Expert Meeting 34 (22 – 26 October 2007). Overall, it appeared that the long-term and carcinogenic effects of benfluralin and trifluralin in the rat (at approximately the same doses) were not identical for both substances. Trifluralin exerts a more adverse effect on the kidney, while the effect on the liver was less clear. On the contrary, the adverse effects of benfluralin towards the kidney were less pronounced, and no bladder effects were evident. Although some metabolites are common, the RMS of the previous evaluation pointed out that it was impossible to declare both pathways identical based on the available information. Taking into account all uncertainties, the meeting proposed to classify as carcinogen Cat. 3, R40, taking into account the tumours observed in the thyroid and the liver (in two species).

Relevance to humans

The involvement of CAR as a MoA has not yet been fully investigated as apparently no studies have been conducted with human cells to study hepatocellular proliferation, or with CAR knockout mice. Therefore it is considered that the relevance to humans of benfluralin-mediated CAR activation has not been adequately excluded.

10.9.2 Comparison with the CLP criteria

Based on the results from the presented studies, benfluralin is considered carcinogenic in the rat liver and thyroid and in the mouse liver. The findings should therefore be evaluated further with respect to classification for carcinogenicity.

Table 32 presents the CLP criteria for classification as a carcinogen.

Table 32

CLP regulation
A carcinogen means a substance which induces cancer or increases its incidence and a substance is classified according to their potential to cause cancer in humans. Direct evidence on humans can be derived from epidemiological studies, but in most cases the availiable evidence is derived from animal studies and the relevance to humans must be considered. CATEGORY 1 Substances which are known or presumed human carcinogens are classified in Category 1. A substance is classified in Category 1 for carcinogenicity on the basis of epidemiological and/or animal data. A substance may be further distinguished as: *Category 1A*: known to have carcinogenic potential for humans, classification is largely based on human evidence or *Category 1B:* presumed to have carcinogenic potential for humans, classification is largely based on animal evidence. The classification in Category 1A and 1B is based on strength of evidence together with additional considerations (see section 3.6.2.2). Such evidence may be derived from: - human studies that establish a causal relationship between human exposure to a substance and the development of cancer (known human carcinogen); or - animal experiments for which there is sufficient (see section 3.6.2.2.4.) evidence to demonstrate animal carcinogenicity (presumed human carcinogen). In addition, on a case-by-case basis, scientific judgement may warrant a decision of presumed human carcinogenicity derived from studies showing limited evidence of carcinogenicity in humans together with limited evidence of carcinogenicity in experimental animals. CATEGORY 2 Substances which are suspected human carcinogens are classified in Category 2. The placing of a substance in Category 2 is done on the basis of evidence obtained from human and/or animal studies, but which is not sufficiently convincing to place the substance in Category 1A or 1B, based on strength of evidence together with additional considerations (see section 3.6.2.2). Such evidence may be derived either from limited (see section 3.6.2.2.4) evidence of carcinogenicity in human studies or from limited evidence of carcinogenicity in animal studies.

There are no relevant data from epidemiological studies available and consequently no classification with Cat1A according to CLP regulation is proposed.

In rat treated for 2 years with benfluralin, both males and females (treated with 2500 or 5000 ppm), had higher incidences of follicular cell adenoma and carcinoma of the thyroid. A clear dose-related increase of the combined incidence of benign and malignant tumors was recorded on study conclusion. The finding was supported by the increased occurence of focal hyperplasia at these doses. In the liver, both benign and malignant tumors occurred at 2500 ppm and higher in the males. The combined incidence was increased in a dose-related way and a statistical significance was attained at the top dose. Further, several histological liver changes (hepatocellular cell pigmentation, sinusoidal cell pigementation, hyalin droplets, hypertrophy, necrosis) were noted in males and/or females, some occuring from 100 ppm (hypertophy and pigmentation).

Two top-dose males showed tubule cell adenoma of the kidney, which was considered secondary to the observed CPN and associated tubule cell hyperplasia. Other findings in other organs were not considered treatment-related.

The available mechanistic data proposes a rodent specific explanation for the observed tumours as benfluralin induced activites of PROD and UGT, induced hepatocellular proliferation and caused elevated levels of EROD in rats exposed to 5000 ppm for 7 or 14 days (MoA study). These findings suggest that the tumours in rat are caused by CAR and UGT-mediated mode of actions and through rat specific Ahr-independent mechansism. The suggested mechanism for the thyroid tumours through the decrease in T4 levels did not give corresponding

increase in TSH levels in the MoA study, but in the pubertal assays conducted with benfluralin (please refer to Annex I, section B.6.8.3) decreased T4 levels corresponded with increases in TSH levels. In the female pubertal assay, decreased T4 levels were demonstrated in the mid (75 mg/kg bw/day)- and high-dose (300 mg/kg bw/day) groups with corresponding increases in TSH levels in the highest dose group, but there were no corresponding changes in thyroid weight and histopathology. In the males, T4 levels were reduced in all dose groups (25, 100 and 400 mg/kg bw/day) with corresponding increases in TSH levels at the two highest dose groups, significantly increased thyroid weights and altered thyroid histopathology (increased follicular cell height and decreased amount of colloid) at 400 mg/kg bw/day. However, since TSH was not affected in the MoA study and was not measured in the 2 year rat study, the possible mechanism for thyroid tumours has not been clearly demonstrated.

In mice treated for 2 years with benfluralin, increased incidences of hepatocellular carcinomas were observed in females from 50 ppm and onwards. A significant trend of combined incidence of adenoma/carcinoma was detected at the top dose (1500 ppm). Findings in the liver were corroborated by focal hyperplasia in females at 1500 ppm and multifocal hyperplasia at 300 ppm in males and in both sexes at 1500 ppm.

In contrast to the rat, no EROD activation has been demonstrated in mice and benfluralin caused induction of CYP1-450 in a short term toxicity study in mice which indicates that the tumours are caused through Ah-receptor activation in mice (DAR, 2006).

Taken together, a relevant physiological route of exposure (oral exposure) was used in the rat and the mouse studies. Benfluralin was not genotoxic/mutagenic *in vitro* or *in vivo* in the available studies, but the mouse study was conducted with a batch of benfluralin containing a higher level of EBNA than the highest technical specification tested in the standard genotoxicity battery of *in vitro* and *in vivo* assays. Liver tumours were observed in two species, albeit in the male rat and in the female mouse. Thyroid tumors were observed in one species, however in both sexes of the rat. No ADME data in humans are available, and a comparison with the respective animal data is not possible. The animals suffering with liver cancer had also reduced survival rates. Hence survival rate in the male rat were reduced with all doses, except with the lowest dose, and in the female mouse with the next highest dose and above. Body weight in the top dose group in the female mice was low compared to control group, and in the rat it was lowered at next highest dose (females) and the top dose (males and females) throughout the study period.

The available mechanistic data suggest an explanation for the occurence of the observed tumours and the mode of action, but the relevance to humans has not yet been adequately excluded until a MoA potentially relevant for humans (stimulation of hepatocyte cell division and CAR-knock out mice) has been sufficiently investigated.

Taking into account the factors for increasing or decreasing level of concern, and deciding whether there is "limited evidence for carcinogenicity" there is enough evidence to establish a positive association between exposure and cancer and to suspect that benfluralin is carcinogenic. In summary, the studies provide sufficient evidence that benfluralin should be considered carcinogenic in rat (liver and thyroid) and in mice (liver), but with the available data, there is not enough evidence to establish a causal relationship between exposure to benfluralin and human cancer. Hence no classification with Cat1B according to CLP regulation is proposed.

Classification for Cat 2 is therefore proposed.

10.9.3 Conclusion on classification and labelling for carcinogenicity

Classification as 'Carc. 2' (H351) is considered appropriate

RAC evaluation of carcinogenicity

Summary of the Dossier Submitter's proposal

Two guideline- and GLP compliant long-term oral (dietary) toxicity/carcinogenicity studies were reported by the DS: a 2-year combined chronic toxicity/carcinogenicity study in the Fischer 344 rat (Anonymous, 1996) and a 2-year carcinogenicity study in the B6C3F1 mouse (Anonymous, 1988). Study details were summarised in Table 23 in the CLH report. Benfluralin had treatment-related neoplastic findings in rats, the most significant being liver (males only) and thyroid tumours (both sexes, along with a slight increase of kidney adenoma (males) and Leydig cell tumours). An increased incidence of hepatocellular adenoma and carcinoma was observed in female mice. An additional study was conducted to investigate the MoA and human health relevance of the rodent tumours. This was also part of the RAR (2019) and described by the DS. Several additional and new studies were conducted to further investigate the MoA and human health relevance of the rodent tumours. These were made available after the EFSA peer review of the active substance and after completion of the CLH report. They have not been previously assessed by either the RMS or the DS. Please refer to the section on Additional key elements for the RAC assessment.

In vivo animal studies

Rat 2-year dietary toxicity/oncogenicity study

Benfluralin was assessed in a rat GLP- and OECD TG 453 (1981) compliant carcinogenicity dietary study (Anonymous, 1996). Significantly reduced survival rates were apparent in the males at ≥ 5.4 mg/kg bw/d (81%, 84%, 62%*, 66%*, and 64%* in controls, 0.5, 5.4, 136 and 275 mg/kg bw/d dose groups respectively). Signs of poor antemortem condition including hunched posture, thin appearance, prostration or entire body paleness were noted more frequently. Fischer 344 strain rats were divided into treatment groups and scheduled kills were conducted after 12 months treatment for 10 animals/sex/group and at study termination after 24 months treatment for the remaining nominal 50 animals/sex/group.

Table: *Mean dose of benfluralin (purity 95.8%) received (mg/kg bw/d)*

Dietary concentration of benfluralin (M/F ppm)	0	10	100	2500	5000
Males	0	0.5	5.4	136.3	274.8
Females	0	0.7	6.8	167.9	331.3

The liver, the thyroid and the kidneys were the target organs after long-term administration of benfluralin to the F344 rat. The maximum tolerated dose (MTD) was achieved. Significant treatment-related effects were observed. General toxicity was evident as significantly lower body weight gain compared to controls which amounted to 15% (males) and 32% (females) at 136/168 mg/kg bw/d, and to 30% (males) and 50% (females) at the top-dose of 275/331 mg/kg bw/d (in males and females respectively). Several other treatment effects were particularly noticeable at the mid to top dose groups and affected haematological parameters,

clinical chemistry and urinalysis, but not to an extent that could support classification for STOT RE category 1 or 2.

Dose-related increased liver weights (males, females) and adrenal weights (females) at 136/168 mg/kg bw/d and higher were observed both at the interim and terminal sacrifice, while thyroid weight increase from this dose level was obvious at interim sacrifice. At terminal sacrifice there was still a strong response, i.e. substance related, but in males a dose response between the two top dose levels was not so clear and lacked statistical significance. In the thyroid, follicular hyperplasia was increased at 136/168 mg/kg bw/d (2500 ppm) and above and this was considered the first stage for neoplastic change.

The Fischer 344 rat is naturally susceptible to chronic progressive nephropathy (CPN) with high background incidences common in this strain making the interpretation of the condition difficult. While the incidence was similar across all groups, the severity of lesions associated with CPN were increased with the dose of benfluralin, both at the interim sacrifice and at the final sacrifice at levels of ≥ 5/7 mg/kg bw/d (100 ppm). The presence of hyaline and/or fine granular casts in the urine of animals at doses ≥ 136/331 (M/F) mg/kg bw/d correlated with the treatment-related increase in severity of CPN. Several other lesions were noted (such as hyaline droplets in the renal tubule cells; tubule cell karyomegaly; transitional cell hyperplasia of the renal papilla) but may be associated with exacerbation of CPN by benfluralin and/or the significant presence of calculi in the renal pelvis. The DS postulated that the kidney calculi were the primary cause of both the exacerbation of nephropathy and of transitional cell hyperplasia, possibly leading to tubular cell adenoma in two males at the top-dose of 275 mg/kg bw/d.

Neoplastic findings

According to the CLH report there were three main tumour types of concern following benfluralin treatment:

1. Thyroid follicular adenoma and carcinoma in both sexes
2. Hepatocellular adenoma and carcinoma in males
3. Renal tubular cell adenomas in 2 males from the top dose group

The liver, the thyroid and the kidneys were the target organs after long-term administration of benfluralin to the F344 rat.

Thyroid follicular cell adenoma and carcinoma were increased at 136/168 (M/F) mg/kg bw/d (2500 ppm) and the high dose (table below 'RAC Overview of thyroid tumours in the long-term rat study'). A significant trend in neoplastic incidence was demonstrated for the males (adenoma) and for males and females combined.

At 136 mg/kg bw/d (2500 ppm) and above, the individual and combined incidences of hepatocellular adenoma and carcinoma were increased in males only (table below 'RAC Overview of liver tumours in the long-term rat study'). Both benign and malignant tumours occurred at greater than or equal to this level.

In the kidney the DS noted tubule cell adenoma (table below 'RAC overview of kidney tumours in the long-term rat study'), at the top dose of 275 mg/kg bw/d (5000 ppm), also confined to males (2/50, 4%). This was considered secondary to the observed CPN and associated tubule cell hyperplasia arising from irritation by pelvic calculi.

There were no HCD provided by the DS. Leydig cell tumours were also noted by the DS. At the top dose, the incidence of these tumours was slightly elevated, but only in the 1-year sacrifice group. These tumours occur with a naturally high incidence in F344 rats and cannot be considered to arise from a treatment related effect.

There were some minor deviations from OECD TG 453, but the DS considered the study acceptable for regulatory purposes. The DS noted the rat study was conducted with a batch of benfluralin containing a lower level of the impurity EBNA (0.04 mg/kg) than that tested in the mutagenicity studies (0.085 mg/kg) and concluded that benfluralin was unlikely to be genotoxic. However, the DS supported the RMS conclusion that benfluralin was to be considered carcinogenic in the rat liver and thyroid.

***Table**: RAC overview of thyroid tumours in the long-term rat study (Anonymous, 1996)*

Males					
Dose (mg/kg bw/d)	**0**	**0.5**	**5.4**	**136.3**	**274.8**
N. of animals week 104)	48	50	48	50	50
Follicular cell adenoma Follicular cell carcinoma	1 0	1 0	1 0	3 4	5 3
Combined	1	1	1	7	8
Females					
Dose (mg/kg bw/d)	**0**	**0.7**	**6.8**	**167.9**	**331.3**
N. of animals (week 104)	50	50	50	50	50
Follicular cell adenoma Follicular cell carcinoma	0 0	0 0	0 1	3 2	2 2
Combined	0	0	1	5	4

***Table**: RAC overview of liver tumours in the long-term rat study (Anonymous, 1996)*

Males					
Dose (mg/kg bw/d)	**0**	**0.5**	**5.4**	**136.3**	**274.8**
N. of animals (week 104)	48	50	48	50	50
Hepatocellular adenoma Hepatocellular carcinoma	1 1	2 0	1 0	3 2	9 2
Combined	2	2	1	5	11
Females					
Dose (mg/kg bw/d)	**0**	**0.7**	**6.8**	**167.9**	**331.3**
N. of animals (week 104)	50	50	50	50	50
Hepatocellular adenoma Hepatocellular carcinoma	2 0	0 0	1 0	1 0	2 0
Combined	2	0	1	1	2

***Table**: RAC overview of kidney tumours in the long-term rat study (Anonymous, 1996)*

Males					
Dose (mg/kg bw/d)	**0**	**0.5**	**5.4**	**136.3**	**274.8**
N. of animals (week 104)	48	50	50	50	50
Tubule cell adenoma	0	0	0	0	**2**
Large free pelvic calculi Small renal pelvic epithelial calculi	3 16	1 23	4 22	37 5	47 1
Females					
Dose (mg/kg bw/d)	**0**	**0.7**	**6.8**	**167.9**	**331.3**
N. of animals (week 104)	50	50	50	50	50
Tubule cell adenoma	0	0	0	0	0
Large free pelvic calculi Small renal pelvic epithelial calculi	6 39	7 31	8 38	30 18	26 15

B6C3F1 Mouse 2-year dietary toxicity/oncogenicity study

Benfluralin was also assessed in a mouse GLP- and OECD TG 451 (1981) partially compliant but acceptable chronic toxicity and carcinogenicity dietary study (Anonymous, 1988). For logistic reasons, the study was conducted as two replicates (30 mice/sex/dose in each replicate), with initiation dates separated by a 15-day interval. The replicate results were combined for the final report.

Unlike the animals in the rat study, survival was not significantly impacted by treatment. Apart from colouration of the urine (chromaturia), a common finding in animals given high doses of dinitroanaline compounds, no other clinical signs of significance were noted. Small statistically significant decreases in body weight were observed from the lowest dose on throughout the study. At termination, results indicated only slight changes at 36 mg/kg bw/d (males) and above (males, females).

***Table**: Mean dose of benfluralin (purity 95.25-96.15%) received (mg/kg bw/d)*

Dietary concentration of benfluralin (M/F ppm)	**0**	**50**	**300**	**1500**
Males	0	6.0	36.4	184.7
Females	0	6.9	41.8	223.5

The target organs in the mouse were the kidney and the liver. Haematology was unremarkable at termination. Clinical chemistry showed increases in alkaline phosphatase (top dose) and alanine aminotransferase along with blood urea nitrogen levels (42 mg/kg bw/d and above) in females. No other renal involvement was noted. Treatment-related non-neoplastic lesions were essentially confined to the liver. At the top dose, liver weights were increased in both sexes (mean relative liver weight in males was approximately +9.5% and in females about +30% greater than in controls) and the incidence of hepatocellular hyperplasia was notably increased in males and females in the top dose group. The increased incidence of focal hyperplasia was accompanied by an increase in severity of the plastic response and a more

frequent occurrence of multiple foci of hyperplasia (following a dose response) within affected livers of both sexes.

Neoplastic findings

According to the CLH report there was one main tumour type of concern following benfluralin treatment:

1. Hepatocellular adenoma and carcinoma in males and females

Increased incidences of adenoma (224 mg/kg bw/d) and of carcinoma (≥ 7 mg/kg bw/d) were especially noted in females due to low or no incidence in concurrent controls (table below). The combined incidence was also increased at 42 mg/kg bw/d onwards (females), and a statistically significant trend was detected when the Peto's survival adjusted trend test was applied (p = 0.018). These incidences were compared to the in-house contemporary historical control incidence of liver neoplasia in females, based on studies conducted after 1980 (the Anonymous, 1988 report was based on an in-life study period from 1985–1987). RAC notes that the individual incidences of adenomas and carcinomas (5.1%), and the combined incidence (10%), in the top dose group were outside the in-house HCD range for adenomas and combined adenomas & carcinomas only (0-3.4% and 0-6.9%, respectively). HCD were only reported for females. In the males, a high number of combined adenomas and carcinomas was observed (12/60, top dose group, 185 mg/kg bw/d), but this was not statistically increased relative to the concurrent controls due to the high number of hepatocellular carcinomas in the control group (7/60 animals).

***Table**: RAC overview of liver tumours in the long-term mouse study (Anonymous, 1988)*

Males				
Dose (mg/kg bw/d)	**0**	**6.0**	**36.4**	**184.7**
N. of animals	60	60	60	60
Hepatocellular adenoma: Hepatocellular carcinoma:	2 7	1 7	3 5	5 8
Combined	9	8	8	13
Females				
Dose (mg/kg bw/d)	**0**	**6.9**	**41.8**	**223.5**
N. of animals	60	60	60	59
Hepatocellular adenoma: Hepatocellular carcinoma:	1 0	1 2	1 3	3 3
Combined	1	3	4	**6**

There were some deviations from OECD TG 451 (1981) but the DS considered the study acceptable for regulatory purposes. The DS noted the mouse study was conducted with a batch of benfluralin containing a higher level of the impurity EBNA (0.31 mg/kg) than that tested in the mutagenicity studies (0.085 mg/kg). The DS concluded that benfluralin should be considered carcinogenic in the mouse liver based on the incidence of hepatocellular carcinomas in female mice.

MoA for benfluralin-induced tumours in F344/DuCrl rats

The DS presented a summary of a 14-day dietary study (Anonymous, 2010; RAR, section B.6.8.2), designed to examine the possible MoA for benfluralin-induced tumours in F344/DuCrl rats. Male rats (6/dose/time period) were exposed to 0 and a mean of 440 mg/kg bw/d (5000 ppm) benfluralin in the diet for 7 or 14 days. The dose levels were above the known tumorigenic dose following data from the 2-year dietary rat study.

The main endpoints examined provided support for liver and thyroid tumour induction via the constitutive androstane receptor (CAR) and UDP-glucuronyltransferase (UGT)-mediated MoAs:

i. Thyroid function analysis (T_4, T_3, TSH, histopathology)
ii. Liver and thyroid organ weight increases
iii. Liver and thyroid follicular cell proliferation
iv. Targeted gene-expression
v. Liver metabolic enzyme activities

i. Thyroid function analysis

According to the DS, the available mechanistic data proposes a rodent specific explanation for the observed tumours in the thyroid. A decrease in T4 was demonstrated in the MoA study (table below). However, the DS did not take note of the compensatory increase in TSH at 7 days, instead proposing no effect on TSH based on the 14-day result alone. The DS provided evidence from further studies with benfluralin that strengthened the argument that the decrease in T4 stimulates the pituitary to release more TSH that drives the thyroid to make more T4. In the SD rat, 14-day oral gavage pubertal assays conducted with benfluralin (RAR, section B.6.8.3.4), decreased T4 levels corresponded with increases in TSH levels. These studies were GLP- and USEPA OPPTS 890.1450 (2009) guideline compliant. In the female pubertal assay (Anonymous, 2012), decreased T4 levels were demonstrated in the mid (75 mg/kg bw/d) and high-dose (300 mg/kg bw/d) groups with corresponding increases in TSH levels in the highest dose group. In males, T4 levels were reduced in all dose groups (25, 100 and 400 mg/kg bw/d) with corresponding increases in TSH levels at the two highest dose groups and significantly increased thyroid weights and altered thyroid histopathology in the top dose group. The DS outlined a conservative opinion stating that the MoA for thyroid tumours had not been clearly demonstrated.

***Table**: RAC overview of thyroid function and organ weights (Anonymous, 2010)*

Exposure length	**7 days**		**14 days**	
Dose (mg/kg bw/d)	**0**	**449**	**0**	**436**
N. of animals	6	6	6	6
T3 (ng/dL) T4 (µg/dL) TSH	115 5.6 3.6	**94*** (- 18.3%) **2.2***(- 60.7%) 5.3 (+ 47%)	118 5.0 4.8	106 (- 10%) **2.6 (- 48%)*** 4.6
Liver weight absolute relative		 + 25%* + 30%*		 +30%* +35%*
Thyroid weight absolute relative		 + 17%* + 21%*		 +16%* +22%*

ii. Liver and thyroid organ weights

All repeat dose studies show that benfluralin is a strong growth promoter of the liver. At 7 and 14 days, absolute and relative liver weights were elevated. A similar effect was noted for the thyroid (table above).

iii. Liver and thyroid follicular cell proliferation

Histopathological changes were observed in the liver and thyroid. Benfluralin-induced liver effects consisted of very slight hypertrophy of hepatocytes in the centrilobular and midzonal regions of the hepatic lobule. In the thyroid gland, treatment-related microscopic changes were minimal. Very slight follicular cell hypertrophy was seen in 3 out of 6 treated males after 7 days of benfluralin exposure and 4 out of 6 treated males following 14 days of exposure.

Incorporation of 5-bromo-2'-deoxyuridine (BrdU; a structural analogue of thymidine) into nuclear DNA was used as a surrogate marker for cell proliferation. Rats were continuously infused with BrdU via implanted osmotic pumps. At 7 days, hepatic proliferation was significantly elevated only in the periportal region (1.7-fold higher), whereas by 14 days, proliferation was significantly elevated in all regions of the liver (centrilobular, midzonal, and periportal; 1.4-fold, 1.2-fold and 1.6-fold, respectively). Thyroid follicular cell proliferation was significantly elevated at 7 days (1.6-fold), however, this returned to near control levels by day 14 (1.2-fold, non-significant).

iv. Targeted gene-expression

A summary of targeted gene expression is presented in the table below. The CAR-related transcripts, Cyp2b1 and Cyp2b2 were significantly elevated at both 7 and 14 days. The PXR-related transcript, Cyp3a3 was also significantly elevated at these two time points. The PPARα-related transcript, Cyp4a1 was not significantly altered at 7 or 14 days. The two T4-specific UGT genes, Ugt1a6 and Ugt2b17, were significantly elevated at both 7 and 14 days.

***Table**: RAC overview of targeted gene expression (at the RNA level) expressed as fold-change relative to concurrent controls (Anonymous, 2010)*

Exposure length	**7 days**		**14 days**	
Dose (mg/kg bw/d)	**0**	**449**	**0**	**436**
N. of animals	6	6	6	6
CAR: Cyp2b1	1	**475***	1	**344***
Cyp2b2	1	**11***	1	**12***
PXR: Cyp3a3	1	**9.4***	1	**7.5***
PPARα: Cyp4a1	1	0.9	1	1
CAR (T4 specific):				
Ugt1a6	1	**7.4***	1	**6.9***
Ugt2b17	1	**7.3***	1	**8.3***

* Criteria for substantial induction of the gene: significantly different at p = 0.05 and ≥ 1.5-fold-change in mRNA expression

v. Liver metabolic enzyme activities

Liver EROD (AhR), PROD (CAR), and UGT-related enzyme activities were all significantly elevated at 7 and 14 days (table below).

***Table**: RAC overview of liver enzyme activity (at the protein level) expressed as fold-change relative to concurrent controls (Anonymous, 2010)*

Exposure length		**7 days**		**14 days**	
Dose (mg/kg bw/d)		**0**	**449**	**0**	**436**
N. of animals		6	6	6	6
AhR/CAR:	EROD	1	**3.5***	1	**3.3***
CAR:	PROD	1	**6.6***	1	**4.5***
	UGT	1	**2.5***	1	**2.7***

* Criteria for substantial induction of the gene: significantly different at p = 0.05 and ≥ 1.5-fold-change in mRNA expression

Summary

There were important indicators that CAR may be involved as a mechanism for benfluralin-induced liver tumours. There were elevated levels of the CAR-related gene transcripts Cyp2b1, Cyp2b2, and the two T4-specific UGT genes, Ugt1a6 and Ugt2b17. There were elevated activities of the hepatic enzymes PROD, UGT, and crosstalk from CAR that may explain the elevated EROD activity (rather than activation of the Ah-receptor), as well as clear evidence for hepatocellular proliferation. The PPARα-related transcript, Cyp4a1 was not altered at 7 or 14 days.The DS concluded that the available mechanistic data supported a rodent specific explanation for the observed tumours (CAR) but due to a lack of further investigation (i.e. stimulation of hepatocyte cell division and use of CAR-knock out models) the DS remained unconvinced that the relevance to humans had been sufficiently explored.

With respect to the possible mechanism for thyroid tumours, greater expression of UGT mRNA transcripts and increased UGT enzymatic activity along with a decrease in T4 was demonstrated. Decreased T4 levels corresponded with increases in TSH levels in the pubertal assays conducted with benfluralin, but this was not corroborated by other studies. The effects on thyroid hormone homeostasis could be due secondary to liver enzyme induction, and may be responsible for the thyroid tumours in male Fischer 344 rats following chronic exposure to benfluralin. However, no testing strategies for alternative MoAs were employed in the Anonymous (2010) study. The DS concluded that there were no effect on TSH from this study while at the same time providing evidence for such an effect in other studies (pubertal assays, Anonymous, 2012).

Overall, the DS considered there was enough evidence to establish a positive association between exposure and tumours and to suspect that benfluralin was carcinogenic and proposed classification for benfluralin as Carc. 2; H351.

Comments received during consultation

In the response to comments document following the consultation of the CLH report, there were two comments provided, one from an MSCA supporting the DS with classification for carcinogenicity (Carc. 2; H351) and one comment from industry supporting no classification.

The MSCA outlined similar reasons to those given by the DS in supporting Carc. 2:

- Increased incidence of (mostly benign) liver and (benign as well as malignant) thyroid tumours in the long-term study in the rat.
- Increase in liver cell adenoma and carcinoma in female mice.
- Mechanistic studies do not exclude human relevance.

- Concluded there was a multi-site response in two species from which human relevance could not be excluded.
- Acknowledged that effects occurred at high dose levels resulting in significant non-neoplastic pathological findings, organ weight changes, reduced survival, and a flat dose response in mice for the liver tumours and supported a Category 2 classification.

Industry, however, provided new mechanistic data to address the concerns over the human relevance of the tumours in liver and thyroid. A set of new studies were provided that have not been assessed prior to this opinion document and were not available at the time of drafting the RAR or the CLH report. The DS has not evaluated these studies. Industry concluded that a CAR/PXR driven MoA for liver tumours was demonstrated and that this is not relevant for humans. Similarly, further studies on the thyroid were provided to support the rodent-specific UDPGT-driven thyroid tumorigenesis observed in the chronic rat study.

Additional studies

Introduction

New data from a series of additional studies or published reports were made available to address the human relevance of liver and thyroid tumours seen in long-term studies with benfluralin in rats and mice. The new information may be broadly defined into two categories:

1. *In vitro* hepatocellular proliferation studies comprising;
   i. cultured hepatocytes from wildtype Sprague-Dawley rats,
   ii. cultured hepatocytes from CAR/PXR-double knockout SD rats,
   iii. cultured hepatocytes from F344 rats,
   iv. cultured hepatocytes from B6C3F1 mice,
   v. cultured hepatocytes from human donors.

2. Thyroid MoA studies comprising;
   i. an *in vivo* 90-day study in male and female F344 rats with oral administration of benfluralin via diet (Anonymous, 2019),
   ii. an investigation on a direct effect on the thyroid by inhibition of the Thyroid Peroxidase enzyme in rat thyroid microsomes (Anonymous, 2019),
   iii. peer reviewed publication from ToxCast data with information about the lack of benfluralin inhibition on the sodium iodide symporter.

Points of note:

- In the *in vitro* studies there was precipitation of compound at both 100 µM (slight) and 300 µM benfluralin.
- Batch 1919 used in the *in vitro* studies had a high content of ethyl-butyl nitrosamine (0.18 mg/kg EBNA).
- The number of animals used in the cultured hepatocyte tests was not stated in the original study reports whereas a single animal was used to harvest cells for the cytotoxicity range-finding studies.
- Independent tests for hepatocellular proliferation were conducted on hepatocytes cultured from 3 human donors (2 males, 1 female).
- A 4th donor (male) was also tested but categorised the results as abnormal.

In vitro studies

The objective of these studies was to investigate the potential of benfluralin to stimulate cell proliferation in the hepatocytes of wildtype rats and mice in comparison with human hepatocytes to assess the relevance of the liver effects in the rodents to humans. Furthermore, proliferation was also measured in CAR^{KO}/PXR^{KO} (Constitutive androstane receptor-knockout/ pregnane X receptor-knockout) rat hepatocytes to evaluate the relevance of CAR and PXR for the liver tumour MoA.

Prior to conducting the proliferation studies, cytotoxicity was determined by measuring adenosine 5´-triphosphate (ATP) levels relative to those levels in concurrent control samples. Concentrations up to the limit of cytotoxicity were used in the main investigations. Epidermal growth factor (EGF) and phenobarbital (PB) served as positive mitogenic controls.

In vitro mouse studies

Treatment with benfluralin stimulated cell proliferation in cultures of primary hepatocytes isolated from male and female B6C3F1 mice in a dose dependent manner to a maximum of 2.6-fold at 100 µM (table below). This was similar to or greater than the effect produced by the positive control PB.

***Table**: summary of the mouse in vitro studies*

Study	Result	Test System	Reference
***In vitro* mouse studies:**			
#1. Benfluralin - cytotoxicity range finding study in cultured female B6C3F1 mouse hepatocytes	Benfluralin concentrations in the main study were 3, 10, 30 and 100 µM. Exposure to 300 µM led to an ATP reduction (~ 33%).	GLP, non-guideline 1° hepatocytes → one female B6C3F1 mouse Batch 1919; 96.4% [EBNA] = 0.18 mg/kg	Chatham, 2019
#2. Benfluralin - induction of DNA-synthesis in cultured female B6C3F1 mouse hepatocytes	Benfluralin was tested at 3, 10, 30 and 100 µM. PB at 1 mM and EGF at 25 ng/mL. No cytotoxicity. Treatment of cultured B6C3F1 hepatocytes with benfluralin resulted in increases in cell proliferation (BrdU) at ≥ 10 µM. Increases above controls of 1.2; **1.2, 1.9, 1.8, 2.6** and 4.1-fold were observed for PB, **benfluralin** and EGF, respectively. PB and EGF positive controls performed well.	GLP, non-guideline 1° hepatocytes → number of female B6C3F1 mice not reported Batch 1919; 96.4% [EBNA] = 0.18 mg/kg	Chatham, 2019
#3. Benfluralin - cytotoxicity range finding study in cultured male B6C3F1 mouse hepatocytes	Benfluralin concentration used in the main study were set at 10, 30, 100 and 300 µM. Exposure to 300 µM led to an ATP reduction (~ 13%).	GLP, non-guideline 1° hepatocytes → one male B6C3F1 mouse Batch 1919; 96.4% [EBNA] = 0.18 mg/kg	Chatham, 2019
#4. Benfluralin - induction of DNA-synthesis in cultured male B6C3F1 mouse hepatocytes	Benfluralin was tested at 10, 30, 100 and 300 µM. PB at 1 mM and EGF at 25 ng/mL. Significant **cytotoxicity** at ≥ 100 µM benfluralin. Treatment of cultured B6C3F1 hepatocytes with benfluralin resulted in increases in cell proliferation (BrdU) at 10 µM and 30 µM (max concentration tested).	GLP, non-guideline 1° hepatocytes → number of male B6C3F1 mice not reported Batch 1919; 96.4% [EBNA] = 0.18 mg/kg	Chatham, 2019

	Observed increase above controls were 1.5, **1.7 to 2.4,** and 9.4-fold for PB, **benfluralin** and EGF respectively. PB and EGF positive controls performed well.		

In vitro rat studies

Wild-type Sprague Dawley Rats

Treatment with benfluralin stimulated cell proliferation in cultures of primary hepatocytes isolated from Sprague Dawley rats (strain from whose genetic background CAR/PXR knockout rats are available), in a dose dependent manner to a maximum of 1.8-fold at 300 µM (table below). This was similar to or greater than the effect produced by the positive control PB.

Wild-type Fischer 344 Rats

Treatment with benfluralin tested at 3, 10, 30 and 100 µM was not very convincing from a dose response point of view. Benfluralin stimulated cell proliferation in cultures of primary hepatocytes isolated from F344 rats at 10 µM only with a maximum induction of 1.4-fold. Significant cytotoxicity was evident at 100 µM.

***Table**: summary of the wild-type rat in vitro studies*

Study	Result	Test System	Reference
***In vitro* rat studies**			
#5. Benfluralin - cytotoxicity range finding study in cultured male SD rat hepatocytes	Benfluralin concertation used in the main study were set at 10, 30, 100 and 300 µM. Exposure to 300 µM led to an ATP reduction relative to controls (~ 13%).	GLP, non-guideline 1° hepatocytes → one male SD rat Batch 1919; 96.4% [EBNA] = 0.18 mg/kg	Chatham, 2019
#6. Benfluralin - induction of DNA-synthesis in cultured male SD rat hepatocytes	Benfluralin was tested at 10, 30, 100 and 300 µM. PB at 1 mM and EGF at 25 ng/mL. Some cytotoxicity at 300 µM benfluralin (ATP reduction ~ 20%). Treatment of cultured SD rat hepatocytes with benfluralin resulted in dose related increases in cell proliferation (BrdU) at < 300 µM. Observed increase above controls were 1.4; **1.1, 1.4, 1.8, 1.8** and 3.4-fold for for PB, **benfluralin** and EGF. PB and EGF positive controls performed well.	GLP, non-guideline 1° hepatocytes → number of male SD rat donors not reported. Batch 1919; 96.4% [EBNA] = 0.18 mg/kg	Chatham, 2019
#7. Benfluralin - cytotoxicity range finding study in cultured male F344 rat hepatocytes	Benfluralin concentration in the main study were set at 3, 10, 30, and 100 µM. Exposure to 300 µM led to an ATP reduction relative to controls (~ 36%).	GLP, non-guideline 1° hepatocytes → one male F344 rat Batch 1919; 96.4% [EBNA] = 0.18 mg/kg	Chatham, 2019
#8. Benfluralin - induction of DNA-synthesis in cultured male F344 rat hepatocytes	Benfluralin was tested at 3, **10**, 30 and 100 µM. PB at 1 mM and EGF at 25 ng/mL. Significant **cytotoxicity** at 100 µM benfluralin (ATP reduction ~ 31%). Treatment of cultured F344 rat hepatocytes with benfluralin (max 100 µM) did not result in a clear	GLP, non-guideline 1° hepatocytes → number of male F344 rat donors not reported. Batch 1919; 96.4% [EBNA] = 0.18 mg/kg	Chatham, 2019

	proliferative response (BrdU) with dose. Observed increase above controls were 1.3, **1.1, 1.4, 1.1, 1.1** and, 4.8-fold increase for PB, **benfluralin** and EGF respectively. PB and EGF positive controls performed well.		

CAR^{KO}/PXR^{KO} double knockout rats

This study investigated the potential effect of benfluralin to stimulate cell proliferation in cultures of primary hepatocytes isolated from male Sprague Dawley rats lacking a functional constitutive androstane receptor and pregnane X receptor (CAR^{KO}/PXR^{KO} rats). Benfluralin concentrations ≤ 300 µM did not result in cell proliferation in cultured hepatocytes from CAR^{KO}/PXR^{KO} rats which supports the proposed liver tumour MoA via CAR/PXR induction (table below).

***Table**: summary of the transgenic rat in vitro studies*

Study	Result	Test System	Reference
***In vitro* CAR^{KO}/PXR^{KO} double knockout rat studies:**			
#9. Benfluralin - cytotoxicity range finding study in cultured male CAR^{KO}/PXR^{KO} SD rat hepatocytes	Benfluralin concentration were 10, 30, 100 and 300 µM. Exposure up to 300 µM → no cytotoxicity → no ATP reduction.	GLP, non-guideline 1° hepatocytes → one male CAR^{KO}/PXR^{KO} SD rat Batch 1919; 96.4% [EBNA] = 0.18 mg/kg	Chatham, 2019
#10. Benfluralin - induction of DNA-synthesis in cultured male CAR^{KO}/PXR^{KO} SD rat hepatocytes	Benfluralin was tested at 10, 30, 100 and 300 µM. PB at 1 mM and EGF at 25 ng/mL. No evidence of cytotoxicity at any concentration of benfluralin. Treatment of CAR/PXR double knockout rat hepatocytes with benfluralin or PB resulted in **no** increases in cell proliferation. A change/increase of 0.9, **0.9, 0.9, 0.9, 0.8** and 3.5-fold, relative to control were observed for PB, **benfluralin** and EGF respectively. PB → no cell proliferation EGF → enhanced cell proliferation.	GLP, non-guideline 1° hepatocytes → number of male CAR/PXR double knockout SD rat donors not reported. Batch 1919; 96.4% [EBNA] = 0.18 mg/kg	Chatham, 2019

In vitro cultured human hepatocyte studies

In human hepatocytes no proliferation occurred after treatment with benfluralin at concentrations of up to 30 µM, based on the data from three donor hepatocyte studies (table below). A 4th donor (male, 385), was described as an outlier by the Notifier because of an increase in the relative S-phase labelling index. What fails to be noticed is that reliance on the relative change of hepatocyte proliferation as a general index of the mitogenic strength of a substance ignores the absolute measure of the S-phase labelling index itself. The results from all 4 donors indicate just how low the basal rate of hepatocyte proliferation is in humans (0.1-0.2) relative to wild-type rats (5.5-6.0). The mitogenic stimulus is far stronger in all the rat studies compared with the response by any of the hepatocytes from the human donors. The

basal rate of hepatocyte proliferation in the double knockout rats is also very high (4.5) but they are refractory to a mitogenic stimulus from benfluralin.

***Table**: summary of the human donor hepatocyte in vitro studies*

Study	Result	Test System	Reference
***In vitro* Human hepatocyte studies:**			
#11. Benfluralin - cytotoxicity range finding study in cultured <u>human</u> hepatocytes	Benfluralin concentration used in the main study were set at 1, 3, 10, 30 and up to 100 µM for 1 donor. Cytotoxicity was evident at 100 µM for 2 donors (ATP ↓55%) and at 300 µM for 1 male donor (ATP ↓24%).	GLP, non-guideline 1° hepatocytes → 3 human donors (2M, 1F) Batch 1919; 96.4% [EBNA] = 0.18 mg/kg	Chatham, 2019
#12. Benfluralin - induction of DNA-synthesis in cultured <u>human</u> hepatocytes	Benfluralin was tested at 1, 3, 10, 30 and 100 µM. PB at 1 mM and EGF at 25 ng/mL. No evidence of cytotoxicity at ≤ 10 µM benfluralin. Donor 8210 (M) cytotoxicity at 30 µM benfluralin (ATP ↓40%). Donor 8219 (M) cytotoxicity at 100 µM benfluralin (ATP ↓45%). Donor 8239 (F) no significant cytotoxicity up to 30 µM benfluralin (ATP ↓15%). Treatment of human hepatocytes with benfluralin resulted in **<u>no</u>** increases in cell proliferation (tested up to 30 µM). PB → no cell proliferation in 2 donors (8210, 8219). PB → proliferation in 1 donor (8239, highly variable). EGF → enhanced cell proliferation of 4.5, 5.4 and 8.9-fold increase relative to controls for donors 8210, 8219 and 8239 respectively.	GLP, non-guideline 1° hepatocytes → 3 human donors (2M, 1F) Batch 1919; 96.4% [EBNA] = 0.18 mg/kg ---------- <u>*Note:*</u> a fourth donor (#385) was also tested in an independent 2018 study. The results were categorised as outliers because increases in cell proliferation of 2.1, 1.5 and 2.3-fold relative to controls wre observed at 30, 100 and 300 µM benfluralin. There was little to no cytotoxicity up to 300 µM benfluralin. PB → no cell proliferation EGF → enhanced cell proliferation (5.3-fold induction).	Chatham, 2019

<u>Summary of the *in vitro* cultured hepatocyte studies</u>

No proliferation was seen in human hepatocytes from 3 donors. An increase in the proliferation of hepatocytes was only observed in hepatocytes from wild-type rats and mice, but not in CAR/PXR double knockout rats which provides support for a CAR/PXR-mediated mechanism responsible for the liver tumour induction of benfluralin. There were four main weaknesses associated with these studies:

1. It is unclear how many rats were used to harvest hepatocytes for each study. A single animal would not constitute a representative sample of the rat strain population.
2. The results from the Fischer 344 rat study were not particularly convincing from a dose response point of view. They indicate a mitogenic response at 10 µM benfluralin only but not at higher concentrations (30 or 100 µM). Cytotoxicity may explain the low response at 100 µM but not at 30 µM. Essentially this is weak support for a similarity of response between the F344 rat and the SD rat strains. The responses by the positive controls were in line with expected results.

3. The results of a test in another (4th), human donor (male, 385), which was performed separately from the other 3 donors were not in line with the toxicity and proliferation data of the other donors, i.e. there was evidence of hepatocyte proliferation at 30, 100 and 300 µM benfluralin albeit with a large degree of variability. The original study report noted that the control S-phase fold-induction data for PB and EGF were consistent with the long-term HCD for male human hepatocyte cultures in the test facility. Yet, it must be noted that this response in terms of the absolute S-phase labelling index remains greatly reduced relative to the responses in the rat. The PB stimulus was minimal as expected and the EGF response was moderately strong, also as expected. It is uncertain why the results for this individual should have any less biological relevance than those of the other donors and it may indicate that a large number of donors is crucial for interpreting these kinds of *in vitro* assays to allow for normal variation in the tested population.
4. It is unclear why the B6C3F1 mouse strain was used: this strain has a very high and variable background incidence in liver adenomas and carcinomas in both sexes that makes it unsuitable as a platform for testing liver tumour susceptibility to exogenous compounds. In any case, the results indicate that hepatocyte proliferation may be stimulated by benfluralin, PB and EGF.

Thyroid mode of action studies

Since in rats and mice the liver was a main target, increased metabolic enzyme induction as an underlying mechanism for the thyroid tumours may be likely. The data made available to RAC sought to provide clarity with respect to perturbations in T4 metabolism due to induction of liver phase II UDPGT conjugating enzymes and also test if there were any direct actions on the thyroid itself that could explain changes to this gland.

90-day study in male and female F344 rats with oral administration of benfluralin via diet (Anonymous, 2019)

One group of Fischer 344 rats (10/sex) was administered the test item, benfluralin (batch 16/2018, purity 96.4%, [EBNA] not stated), via a dietary admixture at a concentration of 10000 ppm for 13 weeks (table below). Another group of 10 males and 10 females received only the untreated diet under the same experimental conditions and acted as a control group.

Thyroid hormone measurements were performed on day 29 and at the end of the treatment period (day 89). At necropsy, liver, thyroids with parathyroids, and pituitary gland were weighed and preserved for microscopic examination.

There were no unscheduled deaths. Several clinical signs such as thin appearance, piloerection, soiled urogenital region and hunched posture were reported. No behavioural or neurological abnormalities were observed during the functional observation battery tests for any animal. Small decreases in body weight were observed at the end of the treatment period (-5% and -9% compared to controls for males and females, respectively).

***Table**: Mean dose of benfluralin received (mg/kg bw/d)*

Dietary concentration of benfluralin (ppm)	0	10000 (M)	10000 (F)
Achieved dose (mg/kg bw/d)	0	557	595

Increased liver weights in animals given the top dose of benfluralin were noted in both sexes and correlated with a centrilobular or diffuse hepatocellular hypertrophy in both sexes.

Significantly increased thyroid weights were noted in males and a similar trend was noted in females. These changes correlated with the minimal to slight follicular cell hypertrophy/hyperplasia noted in the thyroid gland. Significantly lower mean T4 concentrations ranging from -57 to -77% compared to control for the males and from -52 to -65% compared to control for the females after 4 and 13 weeks of treatment were reported concomitantly to an increase in TSH levels (1.9/1.5- and 1.75/2.8-fold induction in week 4 and 13 for males and females respectively compared to control values) while T3 hormone levels were slightly increased (table below).

***Table**: Overview of thyroid hormone and organ weight changes with treatment (Anonymous, 2019)*

Exposure length	**Day 29**				**Day 89**			
Dose (mg/kg bw/d)	**0**	**M (557)**	**0**	**F (595)**	**0**	**M (557)**	**0**	**F (595)**
N. of animals	10	10	10	10	10	10	10	10
T3 (ng/mL) T4 (ng/mL) TSH (pg/mL)	0.27 35.8 2534	**0.38**** **8.1**** **4923****	0.52 23.9 1058	0.52 **8.3**** **1585***	0.44 39.4 2425	**0.58**** **16.7**** **4252****	0.47 25.2 713	**0.60**** **12.2**** **2016****
Liver weight absolute relative						**+53%**** **+61%****		**+45%**** **+61%****
Thyroid weight absolute relative						**+20%*** **+27%****		+1% +12%

Statistically significantly different: * p < 0.05, ** p < 0.01

Conclusion

These results support the proposed liver enzyme induction MoA on the thyroid via increased T4 metabolism and clearance with subsequently lowered T4 levels and a corresponding feedback-initiated mechanism that increases TSH levels. After prolonged exposure in rodents, this leads to follicular cell hypertrophy/hyperplasia and finally could give rise to thyroid tumours.

Inhibition of rat microsomal thyroid peroxidase by benfluralin (Anonymous *et al*., 2019)

To investigate whether a direct effect on the thyroid could have been involved in the promotion of tumours, the potential of benfluralin (batch 16/2018, purity 96.4%, [EBNA] not stated), to inhibit the thyroid peroxidase (TPO) enzyme was investigated in rat thyroid microsomes. Thyroid homogenates were prepared from 5 rats, strain not specified. The thyroid peroxidase activity determination was performed using the fluorogenic substrate Amplex UltraRed (AUR) which is converted to fluorescent Amplex UltroRex by TPO in the presence of excess H_2O_2. A convincing inhibition curve was generated for 6-N-Propyl-2-Thiouracil (PTU).

TPO was inhibited 76% by the positive control PTU with an IC_{50} of 0.79 µM. Benfluralin did not show any inhibition of TPO activity up to its highest soluble concentration in the incubation medium of 100 µM.

Conclusion

These results suggest the promotion of thyroid tumours by benfluralin is not via the mechanism of TPO inhibition.

Other studies investigating thyroid effects

Thyroid uptake of iodide via the sodium-iodide symporter (NIS) is the first step in the biosynthesis of thyroid hormones that are critical for health and development. Studies on NIS inhibition performed with a variety of compounds, including benfluralin, were described in a publication (Wang *et al.*, 2018)[1]. This study applied a previously validated high-throughput approach to screen for NIS inhibitors in the ToxCast phase I library. Benfluralin was negative in this test so, based on this result, no evidence of a direct thyroid effect via NIS inhibition was detected.

Assessment and comparison with the classification criteria

Introduction

There were increased incidences of 4 main types of tumours seen in long-term studies with benfluralin in rats and mice:

1. Hepatocellular adenoma and carcinoma in male Fischer 344 rats.
2. Thyroid follicular adenoma and carcinoma in both sexes in Fischer 344 rats.
3. Renal tubular cell adenomas in 2 males from the top dose group in Fischer 344 rats.
4. Hepatocellular adenomas and carcinomas at the highest dose in both sexes of B6C3F1 mice.

Fischer 344 rat - Hepatocellular adenoma and carcinoma

The benign and malignant liver tumours in the male F344 rat (above table 'RAC overview of liver tumours […]') are the key reasons for consideration of classification of benfluralin as a carcinogenic substance.

The liver is a key target organ for benfluralin. Dose-related increased liver weights (males, females) at 136/168 mg/kg bw/d and higher were observed both at the interim and terminal sacrifice. Hepatocellular hypertrophy was also seen within 14 days (prepubertal studies, Anonymous, 2012). In the subchronic toxicity studies in rats, hepatocellular hypertrophy was seen at the end of the 90-day studies (including the new 90-day thyroid toxicity study, Anonymous, 2019). Neoplastic liver findings were only seen in the long-term studies, there was no indication of reduced tumour latency.

Proposed MoA for the liver tumours

The postulated MoA is that the activation of CAR and PXR nuclear receptors in male rats results in the altered expression of several genes as well as an increase in hepatic cell proliferation leading to hepatocellular tumours.

The human relevance framework has been used to assess the human relevance of the rodent tumours. The DS could not exclude the relevance of these tumours and, based on insufficient data from existing mechanistic studies and a lack of more in-depth mechanistic studies geared

[1] High-Throughput Screening and Quantitative Chemical Ranking for Sodium-Iodide Symporter Inhibitors in ToxCast Phase I Chemical Library. Jun Wang, Daniel R. Hallinger, Ashley S. Murr, Angela R. Buckalew, Steven O. Simmons, Susan C. Laws, and Tammy E. Stoker. *Environmental Science & Technology* **2018** *52* (9), 5417-5426

towards investigating the involvement of CAR as a MoA, concluded benfluralin was carcinogenic in the rat liver.

Is the weight of evidence (WoE) provided sufficient to establish the MoA in animals in the case of benfluralin?

In addition to the studies already presented in both the RAR and CLH report, new *in vitro* mechanistic data has become available since 2019. Both phenobarbital and EGF were utilised as positive control mitogens. This data is considered in this assessment.

Three key events have been considered: the activation of CAR/PXR nuclear receptors, hepatocellular proliferation and expression of key genes and enhanced transcription of liver enzymes.

Activation of CAR and PXR nuclear receptors

Note: there were no *in vivo* mechanistic studies that utilised CAR/PXR knockout animals. Hepatocellular proliferation was briefly investigated over 7 and 14 days in the Anonymous (2010) mechanistic study. Several CAR/PXR-related effects were observed:

- All repeat dose studies show that benfluralin is a strong growth promoter of the liver. At 7 and 14 days, absolute and relative liver weights were elevated (Anonymous, 2010), similarly after 90 days (Anonymous, 2019) and 2-years (Anonymous, 1996).
- *In vivo* hepatic proliferation was significantly elevated throughout all areas of the liver after 14 days of treatment with benfluralin (Anonymous, 2010).
- Elevated CAR/PXR-related mRNA transcripts from target genes, table 'RAC overview of liver enzyme activity […]' (Cyp2b1, Cyp2b2, Cyp3a3, Ugt1a6, Ugt2b17).
- Elevated CAR/PXR-related liver enzyme activities, table 'RAC overview of liver enzyme activity […]' (EROD, PROD, UGT).
- New *in vitro* mechanistic studies using rat hepatocytes showed increased hepatic proliferation in both SD and F344 strains though the response in the F344 rat was not robust nor especially convincing. In addition, a robust response was observed in mice hepatocytes.
- *In vitro* mechanistic studies with CAR^{KO}/PXR^{KO} double knock out SD rats do not show hepatic proliferation upon treatment with benfluralin.
- *In vitro* mechanistic studies with human hepatocytes from several donors show (1) low basal levels of proliferation relative to rats, and (2) no enhanced proliferation with benfluralin treatment. An extra 4^{th} donor gave some curious results, but the levels of proliferation remained much lower than in the rat.

Enzyme markers for CAR mediated gene induction were increased as shown by enhanced activity of PROD and UGT. The increase in EROD activity must be interpreted with care as there can be significant overlap between CAR and AhR mediated induction of this enzymatic activity. The liver induction profile of benfluralin was thus consistent with CAR/PXR activation. Although no comparison with a positive control was performed in the Anonymous (2010) study, Cyp2b1 induction was higher than Cyp3a3 induction as observed with a primary CAR rather than a PXR activator. Hepatocellular proliferation as shown by BrDU labelling of

hepatocytes was statistically significantly increased in both *in vivo* and *in vitro* studies. Hepatocellular proliferation was not investigated in longer term studies.

Exclusion of alternative MoA

- Genotoxicity as a potential MoA for liver tumours can be excluded based on a complete data package *in vitro* and *in vivo* addressing mutagenicity and clastogenicity.
- The presence of the genotoxic impurity EBNA has been documented in most studies and the levels in the rat long term study (0.04 mg/kg), were much lower than in many other studies (including those in the mutagenicity data pack). Therefore, it is unlikely to have contributed to the tumorigenic profile of the rat.
- No evidence of activation of PPARα was noted in the 14-day study. Therefore, peroxisomal proliferation can be ruled out (Anonymous, 2010).
- The *in vitro* rat CAR^{KO}/PXR^{KO} double knockout study (Anonymous, 2019) showed that the presence of functional CAR and/or PXR appeared essential for the initial hepatic proliferative response from both benfluralin and phenobarbital. Indeed, in contrast with the results observed in the *in vitro* study performed with wild-type rat hepatocytes, no cell proliferation was observed at non-cytotoxic concentrations either with benfluralin or phenobarbital.
- Cyp1a1 was not tested, thus AhR activation cannot be ruled out even though EROD activity was increased and there can be some overlap in the expression profiles from CAR and AhR ligands.
- There was some evidence of liver cytotoxicity, an increase in the incidence of single cell necrosis was observed in a dose related manner in males from the 2-year rat study [2, 6, 5, 15*, 27* at 0, 0.5, 5.4, 136 and 275 mg/kg bw/d respectively]. Cytotoxicity may not be ruled out completely, but it does not appear to constitute a major MoA for rat liver tumours in this case. See further discussion below.
- There is no data suggesting that other MoA such as porphyria, statins/altered cholesterol synthesis, oestrogenic activity and immunosuppression could be responsible.

<u>Cytotoxicity</u>

Cytotoxicity, followed by regenerative cell proliferation, is a widely recognised, well characterised nongenotoxic MoA. In the case of benfluralin, some cytotoxicity was evident, but the effects were not convincing to support this as a major MoA for the observed liver tumours. The hepatocellular necrosis was described as predominantly individual cell necrosis with occasional areas of coagulation necrosis (table 'RAC overview of liver histopathology […]' above). There was no evidence of a substance related increase in preneoplastic lesions (cellular alteration, clear/eosinophilic/basophilic) or inflammation. No centrilobular degeneration was noted.

Evidence for cytotoxicity:

- There was a treatment related increase in individual cell necrosis.

- There was a treatment related increase in vacuolization.
- There was a treatment related increase in hepatocellular pigment (unknown relevance).

Evidence against cytotoxicity as a major MoA:

- Clinical chemistry does not support a cytotoxic MoA. Significant decreases were measured in serum alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase activities. Substantial liver cytotoxicity is associated with increases in these enzymes.
- No evidence of liver atrophy. Absolute increases in mean liver weights for the mid upper dose group (136/168 mg/kg bw/d M/F) and the top dose group (275/331 mg/kg bw/d M/F) were 19 and 27% for males and 22 and 43% for females respectively. There was a treatment related increase in hypertrophy but not hyperplasia.
- Hepatocellular necrosis was not observed by week 53, only after 2 years, it was increased in males (where it was graded minimal to slight), but not in females.

***Table**: RAC overview of liver histopathology in the long-term rat study (Anonymous, 1996, unscheduled deaths and terminal sacrifice)*

Males					
Dose (mg/kg bw/d)	**0**	**0.5**	**5.4**	**136.3**	**274.8**
N. of animals (week 104)	48	50	48	50	50
Hepatocellular necrosis Vacuolisation Hepatocellular pigment	2 4 0	6 2 0	5 2 0	15 3 18	25 10 30
Females					
Dose (mg/kg bw/d)	**0**	**0.7**	**6.8**	**167.9**	**331.3**
N. of animals (week 104)	50	50	50	50	50
Hepatocellular necrosis Vacuolisation Hepatocellular pigment	7 4 5	5 2 6	11 3 24	5 0 44	9 1 41

On balance and considering all the available data, RAC agrees that the proposed MoA is plausible in male rats. Nevertheless, significant uncertainties remain:

1. A general absence of dose-response data for surrogates of CAR/PXR activation
2. No data for apoptosis because of alterations in gene expression
3. No *in vivo* studies using CAR/PXR knock out or humanised CAR/PXR animals were performed to confirm the *in vitro* results
4. No exclusion of AhR activation or investigation into the contribution of cytotoxicity in the liver to the development of tumours
5. Sex differences in tumour induction have not been investigated

6. Similarities in response between the two main rat strains were not adequately explored. *In vivo* studies were not performed. Details of the numbers of animals used to harvest hepatocytes in *in vitro* studies were not reported

The new *in vitro* studies (Anonymous, 2019) showed that there were quantitative differences in the activation of CAR by benfluralin in rat and human hepatocytes *in vitro*. Indeed, the background activation of human CAR is lower than rat CAR, and cell proliferation was not detected in human hepatocytes in 3 out of 4 donors while the opposite was true in the rat. These two differences are broadly consistent with the lack of relevance of the CAR activation mechanism in humans as observed in the rat which leads to carcinogenesis in this species. RAC agrees with the DS that the sum total of the data supports the plausibility of the CAR/PXR MoA; however, there are several key uncertainties (as explained above), that prevent RAC from assuming human relevance can be excluded. The most important ones are the lack of *in vivo* investigations and that similarities in response between the two main rat strains (SD and F344) were not adequately explored. Accordingly, RAC supports the proposal of the DS to classify as Carc. 2 based on liver tumours in this case.

Fischer 344 Rat - Thyroid follicular adenoma and carcinoma in both sexes

In rats treated for 2 years with benfluralin, both males and females (treated with ≥ 136/168 mg/kg bw/d), had higher incidences of follicular cell adenoma and carcinoma of the thyroid (table 'RAC overview of thyroid tumours […]' above). There was a dose-related increase of the combined incidence of benign and malignant tumours on study conclusion. The findings were supported by an increased occurrence of focal hyperplasia at these doses.

Proposed MoA for the thyroid tumours:

The postulated MoA is that the activation of CAR and PXR nuclear receptors enables a liver enzyme-mediated mechanism to act on the thyroid via increased T4 metabolism due to induction of UDPGT. The subsequently lower T4 levels trigger a corresponding increase of TSH levels which lead to follicular cell hypertrophy/hyperplasia and finally thyroid tumours in rodents after lifetime exposure to benfluralin.

The human relevance framework has been used to assess the human relevance of these rodent tumours. The DS could not exclude the relevance of these tumours to humans and supported classification in Category 2 for carcinogenicity.

Is the WoE provided sufficient to establish the MoA in animals in the case of benfluralin?

In addition to the studies already presented in both the RAR and CLH report, new *in vivo and in vitro* mechanistic data has become available since 2019. This data is considered in this assessment.

Several key events have been considered: there is no direct effect on the thyroid; there is liver enzyme induction that is specific to the metabolism of thyroxine (T4) which results in enhanced clearance from the blood; there is a compensatory increase of TSH levels resulting in thyroid stimulation, follicular hypertrophy, hyperplasia, and growth; continued stimulation leads to tumours.

What is the weight of evidence for this thyroid MoA?

A new 90-day study in male and female F344 rats with oral administration of benfluralin via diet with a focus on thyroid function and hormonal measurements was conducted (Anonymous, 2019). In addition, the potential of benfluralin to inhibit the thyroid peroxidase

(TPO) enzyme was investigated in rat thyroid microsomes. A recent published study on compounds that might interfere with the sodium iodide symporter (NIS) was also provided (Wang *et al.*, 2018).

- Thyroid findings were limited to rodents; no comparable effects were observed in dogs.
- An amphibian metamorphosis assay was also available in the RAR (B.9.2.3.1/01 Anonymous, 2011). This was GLP and OECD TG 231 (2009) compliant. Following 21 days flow through with a mean measured concentration up to 74.4 µg/L there was no effect on thyroid activity in benfluralin-exposed tadpoles. This lends further support to the specificity of the thyroid effects in rodents and a general lack of a direct effect on the thyroid during metamorphosis.
- The dietary 14-day mechanistic study in rats (Anonymous, 2010) indicated increased thyroid weight with treatment, increased expression of T4-specific genes (Ugt1a6, Ugt2b17) responsible for glucuronide conjugation and increased UGT enzyme activity in the liver.
- In the male and female pubertal assays, a decrease of T4 along with a corresponding increase of TSH was observed following 14 days treatment with benfluralin (Anonymous, 2012).
- In the new 90-day study (Anonymous, 2019), significantly lower mean T4 concentrations (already observed in the existing 14-day study), were recorded after 4 and 13 weeks of treatment. In addition, TSH levels were increased by factors of 1.5 up to 2.8.
- Significantly increased thyroid weights were noted in males with a similar trend in females which correlated with the minimal to slight follicular cell hypertrophy/hyperplasia noted in the thyroid gland.
- An investigation into a direct effect on the thyroid by inhibition of the TPO enzyme in rat thyroid microsomes did not show any inhibition of TPO activity by benfluralin (Anonymous, 2019).
- According to a publication investigating many compounds, including benfluralin on NIS inhibition, benfluralin was found to be negative (Wang, 2018).
- Genotoxicity as a MoA could be excluded since the genotoxicity studies did not provide evidence of a genotoxic potential of benfluralin.

On balance and considering all the available data, RAC agrees that the proposed MoA is plausible and likely responsible for the thyroid tumours in F344 rats. In the CLP guidance one of the mechanisms of tumour formation considered not relevant for humans is thyroid tumours in rodents mediated by UGT induction (see CLP guidance, v5 (2017) section 3.6.2.3.2). Accordingly, RAC does not propose classification based on these tumours in this case.

Fischer 344 Rat - Renal tubular cell adenomas

In the kidney the DS noted a low incidence of tubule cell adenoma (table 'RAC overview of kidney tumours […]' above), in males only at the top dose of 275 mg/kg bw/d (2/50, 4%). The kidney was also a clear target for benfluralin. Many renal lesions occurred at ≥ 5/7 mg/kg

bw/d (100ppm) benfluralin (a level which could in principle, support classification of STOT RE 2 for renal toxicity). The effects exhibiting a clear increase in a dose-dependent response included:

- Hyaline droplets in the renal tubule lining cells.
- Tubule cell karyomegaly.
- Transitional cell hyperplasia of the renal papilla.
- Large pelvic calculi.
- Small calculi in the pelvic epithelium.

At 5.4 mg/kg bw/d (100 ppm) and above, large, free kidney calculi were observed, which were believed to be the primary cause of both an exacerbation of nephropathy (CPN) and of transitional cell hyperplasia, possibly leading to the tubular cell adenoma (males) at the top-dose (275 mg/kg bw/d). The development of these kidney tumours in association with chemically induced α2u globulin nephropathy was not shown in this study. RAC agrees with the DS that these tumours were of low relevance to humans and most likely secondary to the observed CPN and associated tubule cell hyperplasia. RAC does not propose classification for carcinogenicity based on these renal adenomas.

B6C3F1 mouse - Hepatocellular adenomas and carcinomas

Increased incidences of adenoma (224 mg/kg bw/d) and of carcinoma (≥ 7 mg/kg bw/d) were especially noted in females due to low or no incidence in concurrent controls (table 'RAC overview of liver tumours […]' above). The DS concluded that benfluralin should be considered carcinogenic in the mouse liver based on the incidence of hepatocellular carcinomas in female mice. RAC disagrees with this assessment based on the strain of mouse used in this study.

In the early 1960's the National Cancer Institute adopted the B6C3F1 mouse, the F1 hybrid of the C57BL/6 female and C3H male, as the mouse for use in the cancer bioassay program. When using specifically susceptible mouse strains such as the B6C3F1 hybrid, relatively high and variable incidences of liver tumours can occur in the untreated or vehicle control mice. In such cases the tumour incidence in the treated group may be significantly above the concurrent control but could still be within the historical incidence range for that tumour type in that species and therefore may not be providing reliable evidence of treatment related carcinogenicity.

Usually, for assessment of liver tumour data in cancer bioassays, laboratory specific HCD are extremely valuable in putting unusual high or low tumour responses into perspective. However, in this case there are some limited HCD with very low incidence of liver tumours. This is not the situation for the majority of published data where the B6C3F1 mouse strain is notorious for high background levels of liver tumours, both benign and malignant in both sexes. RAC notes that in the CLP guidance, liver tumours in B6C3F1 mice are recognised to occur with a high frequency. On this premise, RAC does not consider the tumour profile of benfluralin in this particular strain of mouse to present sufficient or robust evidence for classification.

Comparison with the criteria

Based on the mechanistic data available, a CAR/PXR mediated effect for the development of tumours is plausible although uncertainties have been noted by RAC.

Classification into category 1A

There is no information from epidemiological studies available to inform on carcinogenic potential and so classification in category 1A is not supported.

Classification into category 1B

Category 1B depends on strength of evidence, which consists of animal experiments for which there is sufficient evidence to demonstrate animal carcinogenicity together with additional considerations (part of an extended weight of evidence approach). This means a causal relationship has been established between the chemical agent and an increased incidence of malignant neoplasms *or* of an appropriate combination of benign and malignant neoplasms in:

(a) two or more species of animals *or* in two or more independent studies in one species carried out at different times (or in different laboratories or under different protocols);

(b) a single, well conducted study involving both sexes of a single species;

(c) a single study in one species and sex with the occurrence of malignant neoplasms to an unusual degree with regard to the incidence, site, type of tumour or age at onset, or when there are strong findings of tumours at multiple sites.

The criteria may initially seem to be fulfilled for benfluralin. Two species are affected by chemically induced tumours; rats have treatment-related malignant tumours of the liver (one sex) and thyroid (both sexes); mice have malignant tumours of the liver in females. However, liver tumours in the mouse are set against a very high background level in the particular strain that was used in the 2-year bioassay. There are convincing reasons to consider the thyroid tumours in both sexes of the rat to arise as a consequence of a secondary effect mediated by increased liver enzyme induction.

In conclusion, these criteria are not fully met with benfluralin. Classification as Category 1B is not supported, since there are significant additional considerations that lessen the strength of evidence.

Classification into category 2 or no classification

There is evidence of carcinogenicity in rats and mice. RAC recognises that the B6C3F1 hybrid is a specifically susceptible mouse strain for both benign and malignant neoplasms in the liver and for this reason cautions against interpreting the results in the Anonymous (1988) as being only treatment related. RAC considers the results from the B6C3F1 mouse study do not provide reliable evidence of treatment related carcinogenicity.

RAC initially considered the liver and thyroid tumours in the rat to be of primary concern for the purposes of classification (positive dose-response, incidences above historical controls, both sexes affected, statistical significance).

However, there are significant additional considerations that must be evaluated in a weight of evidence approach in order to decide if classification in Category 2 or no classification is appropriate for benfluralin.

***Table**: Weight of Evidence – consideration of main points*

Key factors to consider	Overall level of concern Classification
#01. Tumour type and background.	**Rat:** Benign and malignant liver/thyroid tumours → high concern. **Mouse:** High background liver tumours → no concern. **Classification:** **Cat. 1**
#02. Multi-site responses.	**Rat:** Yes, liver, thyroid → high concern. **Mouse:** No, liver only → no concern. **Classification:** **Cat. 2**
#03. Progression of lesions to malignancy.	**Rat:** Yes, liver (limited), thyroid → mild concern. **Mouse:** Yes, but high background incidence → no concern. **Classification:** **Cat. 2**
#04. Reduced tumour latency.	No reduced latency → no concern. **Classification:** **none**
#05. Responses in single or several species?	Two species → rat (high concern), mouse (low concern). **Classification:** **Cat. 2**
#06. Responses in single or both sexes.	**Rat:** liver → single sex (M), thyroid → both sexes → high concern. **Classification:** → **Cat. 2**
#07. Structural similarity to carcinogenic substance.	Insufficient data → no conclusions can be drawn. **Classification:** **none**
#08. Routes of exposure.	Directly relevant for humans → high concern.
#09. Comparison of ADME between animals and humans.	Very similar metabolic profile → high concern.
#10. Confounding effect of excessive toxicity at test doses.	Limited evidence, some histopathology but not supported with clinical chemistry, AhR response genes not investigated → low concern. **Classification:** **borderline**
#11. MoA of tumour formation and its relevance to humans.	Non-genotoxic. **Thyroid:** secondary to T4 metabolism → no concern. **Liver:** CAR/PXR activation → overall data package → mild concern. **Classification:** relevance for humans not fully explored → **Cat. 2**.

Tumours in experimental animal cancer studies that are associated with species-specific mechanisms or modes of action may not be considered predictive of a similar hazard to humans. If a MoA can be demonstrated to not operate in humans, experimental animal responses are not considered relevant for humans.

The weight of evidence for benfluralin suggests that the thyroid tumours in the F344 rat arise as a secondary effect rather than a direct effect on the thyroid. They are a consequence of liver enzyme induction, especially of UGT, which in rodents leads to increased thyroid hormone metabolism with subsequently lower T4 levels and increased TSH feedback. This leads to thyroid tumour induction after lifetime exposure. In the CLP guidance, one of the mechanisms of tumour formation considered not relevant for humans is thyroid tumours in rodents mediated by UGT induction (see CLP guidance, v5 (2017) section 3.6.2.3.2).

The liver tumours are considered to be the prime evidence on which to base classification. Based on the complete data package for benfluralin, RAC recognises that there are certain data deficiencies that would have made the assessment more robust such as *in vivo* studies with CAR^{KO}/PXR^{KO} double knock out animals or a wider scope of investigation into mRNA transcripts and liver enzyme activities with an emphasis on eliminating other possible MoAs. RAC concludes that while there is sufficient evidence to presume the CAR/PXR MoA is plausible in this case, significant uncertainties and a lack of *in vivo* investigations promote caution. The human relevance of the rat liver tumours cannot be excluded. **RAC considers that classification as Carc. 2; H351, is warranted.**

10.10 Reproductive toxicity

The reproductive toxicity of benfluralin has been investigated in a two-generation study in rats and in one supplementary multi-generation study in rats, as well as several developmental studies in rats and rabbits.

10.10.1 Adverse effects on sexual function and fertility

A multi-generation study and a two-generation study are available to assess the effect of benfluralin on sexual function and fertility (table 33).

Table 33: Summary table of animal studies on adverse effects on sexual function and fertility

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
Oral (dietary) 5 generations Non-GLP and guideline compliant Not acceptable; considered supplementary Rat Harlan 30M&40F /dose	Benfluralin (95.6%) Batch No: X-11424 0, 1000, 5000 ppm equivalent to 0, 50 , 250 mg/kg bw/d	**Effects at lowest dose tested (1000 ppm)** ***Parental toxicity:*** ↓body weight gain ↑mortality ↑liver fatty metamorphosis ***Offspring toxicity:*** ↓postnatal survival ↓pup weight ***Reproductive:*** ↓implantations ↓live foetuses ↓postnatal survival index	Author (1973b) Report No. R-0305, R-0795, R-0316, R-0057 and R-0657/ CA 5.6.1/01
2-generation reproductive study Rat Sprague-Dawley Crl:CD®BR 30 rats/sex/ dose OECD 416 (with several deviations from current guideline) Supplementary	Benfluralin (95.8%) Batch No: ACD 13683 0, 100, 1000, 5000 ppm equivalent to 0; 5.5; 52.6 or 278.3 mg/kg bw/day in males and 0; 7.4; 69.5 or 334.4 mg/kg bw/day in females (premating period)	***Parental toxicity*** <u>Mortality</u> F0: one non-pregnant F died GD 23 and one F died wk 18 (pyelonephritis present) at 5000 ppm. F1: one control F1 M died wk 14 (pyelonephritis present) while one F1 M at 1000 ppm died wk 3 (cause of death unknown). One F died d6 (pyelonephritis and urinary tract inflammation present) at 5000 ppm.	Author (1995) Report No. HWA 174-136/ CA 5.6.1/02

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
		5000 ppm ↓food consumption (F0/F1) ↓body weight* (F0/F1) ↓terminal body weight (F0/F1) ↓body weight gain (F0/F1) ↑liver weight (F0/F1) ↑hepatocellular hypertrophy (F0/F1) ↑kidney weight in M (F0/F1) ↑nephropathy in M (F0/F1) & F (F1) ↑tubular hyaline droplets in M (F0/F1) ↑yellow abdominal fat (F1) ↑uterus fluid filled lumen (F0/F1) ↑uterus dilatation (F0/F1) *1000 ppm* ↓food consumption in F (F0) ↓terminal body weight in F (F1) ↓body weight gain (F0/F1) ↑liver weight (F0/F1) ↑hepatocellular hypertophy (F0/F1) ↑kidney weight in M (F0) ↑nephropathy in M (F0/F1) & F (F1) ↑tubular hyaline droplets in M (F0/F1) *100 ppm* No treatment related effects ***Offspring toxicity*** *5000 ppm – pup data* ↓birth weight (F1) ↓pup weight d4-21 (F1/F2) *1000 ppm* ↓pup weight d4-21 (F1/F2) *100 ppm* No treatment related effects ***Reproductive toxicity*** *5000 ppm* ↑duration of gestation (F1) ↓number of pups delivered per litter (F0/F1) ↓live pups/litter d4 pre-cull (F1) ↓weaning index (F1) 1000 ppm No treatment related effects *body weight through pre-mating, gestation and/or lactation	

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

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

Multi-generation study

The multi-generation study is not guideline- or GLP-compliant and the results from this study is included for supportive information only.

Deviations: only 2 doses investigated; 10 ♀/dose group examined for copulatory plugs; 10/40 and 10/30 F0♀ on gestation d18-21 were sacrificed to investigate foetal anomalies; except for F0, insufficient n° of ♀ treated (20 treated, <20 gravid); except for reproductive data, incomplete raw data (weights, food consumption);

Following endpoints were not or insufficiently investigated: no clinical signs; litter weight lacking on d1 and d7; litter weight on d12 instead of d14; no necropsy or histopathology of F1, F2 and F3 survivors; no oestrus or sperm parameters investigated; no organ weights.

In short, 40♀ rats and 30♂ rats/dose (Harlan) received benfluralin during 60 days before mating and thereafter until sacrifice (reproduction phase) at the dose levels of 0, 1000 or 5000 ppm in the diet, corresponding to 50 and 250 mg/kg b.w./d, taking into account a theoretical conversion-factor of 20.

The study was subdivided into 5 sections: (i) study R-0305: F0 parents and F1 offspring, (ii) study R-0795: F1 parents and F2 offspring, (iii) study R-0316: F2 parents and F3 offspring, (iv) study R-0057: F3 parents and F4 offspring and (v) study R-0657: the F4 terminal generation adults.

Mortality

In F_0, a tendency towards increased substance-related mortality was observed in the treated groups; in the subsequent generations, isolated cases of mortality were observed, but except for the high-dose ♀ in F_3, relationship with treatment was uncertain. The cause of death was unknown, and except for F_0, no further histologic examination was performed.

Table 34: Multigeneration study of benfluralin in rats: mortality (parents)

Dose (ppm)		0		1000		5000	
	G	♂	♀	♂	♀	♂	♀
Mortality	F_0 (d0-63)	0	1	1	0	3	2
	F_0 (>d63)	4	2	6	4	7	7
	F_0 (total)	4	3	**7**	**4**	**10**	**9**
	F_1 (d0-60)	4	2	0	0	2	1
	F_1 (>d60)	0	1	0	1	1	3
	F_1 (total)	4	3	0	1	3	4
	F_2	-	-	1 (m.t. 2)	-	-	1 (d13 pp)
	F_3	-	-	-	1 (g.d. ?)	-	1 (m.t. 2) 2 (m.t. 3)

G: generation n°; m.t.: mating trial; g.d.: gestation day; pp: post-partum.

Food consumption, body weight, test article intake during growth phase

In each generation up to and including F_3, a reduced food consumption was observed in the top-dose ♂ (in the ♀ only in F_1), and a slight (♂) to moderate (♀) drop of body weight gain was also recorded. In F_4, treatment was without effect on these endpoints. The test article intake was relatively constant over the different generations.

Table 35: Multigeneration study of benfluralin in rats: food consumption, body weight, test article intake during growth phase

Dose (ppm)		**0**		**1000**		**5000**	
	G	♂	♀	♂	♀	♂	♀
Food consumption	**F_1**					↓11%	↓15%
	F_2					↓13%	-
	F_3					↓9%	-
	F_4					-	-
Body weight gain	**F_1**					↓5%*	↓24%*
	F_2					↓9%*	↓19%*
	F_3					↓16%*	↓11%*
	F_4					-	↓8%
Test article intake (mg)	**F_1**	-	-	22.2	19.5	108.0	83.0
	F_2	-	-	23.0	20.7	100.5	95.0
	F_3	-	-	22.8	19.3	101.5	98.5
	F_4	-	-	21.3	16.3	100.5	88.0

G: generation n°; Statistically significant modification: Dunnett's t-test *p<0.05.

Reproductive parameters

The treatment was without effect on the ratio of pregnant ♀, and on the proportion of live newborn pups. The ♂ fertility index (total number of matings resulting in pregnancy) was not adversely affected, and was actually higher in the dosed animals (0.66 at 1000 ppm and 0.72 at 5000 ppm) compared to the controls (0.56), as control ♀ showed less inclination to mate due to their increased body weights. However, postnatal survival was reduced at the top-dose in most mating trials of each generation. Occasionally, a reduced survival was observed in the animals treated at 1000 ppm (m.t. 4 of F_0, m.t. 1 of F_2, and m.t. 2 of F_3, on post-natal days 4, 12 or 21). In most cases, survival showed a dose-dependent decrease, and the effect was thus considered treatment-related.

Table 36: Multigeneration study of benfluralin in rats: reproductive and survival parameters

G	Dose (ppm)	m.t.	Fertility index	Gestation survival index	Survival index		
					PND 4	PND 12	PND 21
F0	**0**	1	0.90	0.98	0.97	0.93	0.93
	1000		0.90	0.97	0.98	0.97	0.96
	5000		0.97	0.98	0.94	**0.87**	**0.83**
	0	2	0.83	0.90	0.97	0.84	0.82
	1000		0.91	0.94	0.97	0.90	0.84
	5000		1.00	0.94	0.94	0.87	0.85
	0	3	0.79	0.96	0.97	0.96	0.96
	1000		0.80	0.94	0.96	0.92	0.91
	5000		0.85	0.97	**0.87**	**0.72**	**0.70**
	0	4	0.39	0.92	0.96	0.90	0.90
	1000		0.68	0.81	**0.84**	**0.70**	**0.69**
	5000		0.60	0.88	**0.87**	**0.68**	**0.68**
	0	5	0.24	0.98	0.93	0.85	0.85
	1000		0.63	0.69	0.92	0.87	0.87
	5000		0.74	0.96	**0.83**	0.82	0.81
F1	**0**	1	0.94	0.94	0.96	0.88	0.88
	1000		0.95	0.96	0.95	0.92	0.92
	5000		0.89	0.97	**0.80**	**0.77**	**0.77**
	0	2	1.00	0.84	0.96	0.92	0.92
	1000		0.89	0.90	0.98	0.94	0.94
	5000		0.94	0.95	**0.88**	**0.87**	**0.82**
F2	**0**	1	0.80	0.97	0.98	0.98	0.96
	1000		0.95	0.99	0.94	**0.91**	0.91
	5000		0.80	0.93	**0.78**	**0.78**	**0.78**
	0	2	0.95	0.94	0.87	0.86	0.81
	1000		0.90	0.97	0.91	0.87	0.83
	5000		0.95	0.99	0.85	**0.80**	**0.73**
F3	**0**	1	0.95	0.91	0.94	0.93	0.87
	1000		1.00	0.90	**0.87**	**0.82**	**0.81**
	5000		1.00	0.97	**0.79**	**0.66**	**0.64**
	0	2	0.90	0.92	0.97	0.94	0.92
	1000		1.00	0.96	0.80	0.77	**0.74**
	5000		0.85	0.92	0.88	0.85	**0.55**
	0	3	0.72	0.92	0.96	0.95	0.95
	1000		0.74	0.99	0.90	0.88	0.88
	5000		0.79	0.98	0.97	0.92	0.85

G: generation n°; m.t.: mating trial (in the table restricted to 5 for F_0, as no litters were present in control group from m.t. 6 to 8); ♀Fertility index: ratio of ♀pregnant / mated♀ (including CS ♀); Gestation survival index =ratio of live pups/total pups; PND: post-natal day; boldface: difference ≥5% of control and considered treatment-related.

Litter data

In generation F_0, F_1 and F_2, the mean number of liveborn pups per litter was slightly decreased (statistical significance was only attained in F_1) at the top-dose. A dose-dependent decrease of mean progeny weight was observed at the top-dose in each generation, and at 1000 ppm on occasions (however, the decreased pup weight on mating trials 4 and 5 of F_0 should be interpreted with caution, as the number of pregnant control dams was low, rendering dubious any statistical calculation). The sex-ratio on PND 21 was unaltered by the treatment in any generation.

Table 37: Multigeneration study of benfluralin in rats: litter data

G	Dose (ppm)	m.t.	N° pregnant	N° liveborn/ litter	pup weight (g)			%♂
					PND 4	PND 12	PND 21	
F0	**0**	1	26	11.3	9.5	22.0	36.3	51
	1000		28	10.7	9.5	20.9	35.3	51
	5000		31	**9.9**	**7.6**	**15.1**	**24.3**	47
	0	2	15	10.5	9.8	21.4	38.1	55
	1000		20	11.8	9.6	19.5	**33.7**	55
	5000		26	10.6	**8.0**	**16.5**	**25.3**	47
	0	3	15	11.3	9.2	22.0	35.9	47
	1000		16	11.1	8.7	21.0	33.2	50
	5000		17	**9.3**	**7.4**	**16.8**	**25.8**	49
	0	4	7	6.4	9.3	23.3	41.5	46
	1000		13	6.6	8.8	22.3	**37.3**	54
	5000		12	6.3	**7.6**	**16.9**	**29.6**	54
	0	5	4	10.0	9.5	26.9	45.0	38
	1000		12	5.1	9.9	**23.6**	**38.5**	38
	5000		14	5.5	8.9	**20.5**	**33.8**	48
F1	**0**	1	16	11.6	10.3	22.4	38.7	52
	1000		19	10.5	**9.3**	21.0	35.6	51
	5000		16	**8.8***	**8.6**	**18.6**	**31.1**	50
	0	2	19	10.2	9.6	21.2	34.1	45
	1000		18	11.3	9.2	19.5	32.3	60
	5000		19	10.9	**7.8**	**15.8**	**24.2**	56
F2	**0**	1	19	9.8	9.2	21.6	36.2	44
	1000		20	9.0	9.4	22.4	36.2	48
	5000		20	9.7	8.6	**19.4**	**33.2**	46
	0	2	18	9.4	10.0	23.6	37.8	50
	1000		20	9.7	9.7	22.4	35.4	49
	5000		17	**8.5**	**8.7**	**19.0**	**29.2**	49
	0	3	14	9.9	10.2	24.1	41.2	54
	1000		14	10.0	9.5	23.2	39.5	51
	5000		15	**8.1**	**8.8**	**18.8**	**30.7**	51

G: generation n°; m.t.: mating trial (restricted to 5 for F_0, as no litters in control group from m.t. 6 to 8); PND: post-natal day; Statistically significant modification: Dunnett's t-test *p<0.05; boldface result of pup weight: difference ≥10% of control.

Caesarian section data

The number of implantation sites per dam was dose-dependently decreased, but as the number of corpora lutea in the dams was not determined, it was impossible to tell if pre-implantation loss was subsequent to the exposure to the substance. The resorption index was altered at the top-dose, and also slightly at 1000 ppm, but as the the mean number of resorptions per dam, or the number of dams showing at least one resorption was not increased (the value was rather decreased), the relationship with treatment was questionable.

The data suggest that the treatment was without effect on the foetal weights, but the data are hardly interpretable due to the different number of foetuses obtained on d18, 19 and 20 respectively (despite the attempt to normalise average foetal weights by means of the development of exponential growth curves). The results are also inconsistent with those obtained after delivery (reduced pup weight at the top-dose).

Visceral examination: Hydronephrosis was detected as the sole visceral anomaly at all doses, including controls. In the absence of a proper dose-response, and as the incidence of the controls (bilateral hydronephrosis) was outside the HCD, the relevance of the finding is questionable.

No treatment-related external or skeletal defects were observed. One stillborn pup at 1000 ppm showed micrognathia, but the finding did not appear at the top-dose.

Table 38: Multigeneration study of benfluralin in rats: caesarian section data (F0: combined data of mating trial 1 and 2)

Dose (ppm)		**0**	**1000**	**5000**
N° pregnant / mated		17 / 19	16 / 17	11 / 13
N° foetuses	**live (mean / dam)**	209 (12.3)	182 (**11.4**)	118 (**10.7**)
	Stillborn	0	0	0
N° implantation sites (mean / dam)		223 (13.1)	190 (**11.9**)	121 (**11.0**)
N° resorptions (mean / dam)		14 (0.82)	8 (0.50)	3 (0.27)
Dams with ≥1 resorption		9 / 17 (53%)	6 / 16 (38%)	2 / 11 (18%)
Resorption index (%)		6.3	4.2	**2.5**
% ♂		47	51	48
Foetal weight data: N° examined	GD 18	11	0	10
	GD19	25	33	9
	GD 20	125	144	74
	total[§]	161	177	93
Actual foetal weight (g)	GD 18	1.447	-	1.454
	GD19	2.185	2.159	1.965
	GD 20	3.022	3.115	3.317
Theoretical d20-foetal weight[§§]	GD 18	2.971	-	2.985
	GD19	3.131	3.094	**2.816**
	GD 20	3.022	3.715	3.317
Theoretical d20-foetal weight (average GD18-21)		3.035	3.599	3.233
Foetal anomalies: N° examined	external	195	182	105
	visceral	137	126	73
	skeletal	58	56	32
Hydronephrosis[§§§]	unilateral	6/137 (4.3%)	14/126 (11.1%)	4/73 (5.5%)
	bilateral	11/137 (8.0%)	2/126 (1.6%)	8/73 (11.0%)

Resorption index=N° resorptions / N° implantations; GD: Gestation day;
[§]: total different from n° of live fetuses, because weight data of 4 – 1 – 2 litters (at 0 – 1000 – 5000 ppm) were discarded, as the stage of fetal development did not correspond to the calculated day of gestation (statement of notifier);
[§§]: values adjusted by the means of exponential curves foetal weight vs. age (d18-20), in order to obtain theoretical d20-values for comparability purposes.
[§§§]: *In-house historical control incidence* *unilateral hydronephrosis: 134/2348 (5.7%)*
bilateral hydronephrosis: 129/2348 (5.5%)

In general, the study is regarded as non-accepable and cannot be used for comparison with the CLP criteria. It does, however, provide information with regard to reproductive endpoints at the lowest dose tested 1000 ppm; ↓ implantations, ↓ live foetuses and ↓postnatal survival index.

Two-generation study

The two generation study is the main reproductive study. The study is GLP compliant, but has several critical reproductive endpoints missing from the guideline (see table 40). Benfluralin was administred continuously in the diet of Sprague-Dawley Crl:CD®BR (30 rats/sex/dose) at the dose levels of 0; 100; 1000 or 5000 ppm, from 10 weeks (F_0) or 12 weeks (F_1) prior to pairing up to termination. Achieved test article concentrations in mg/kg bw (high to low-end values week 0-10 pre-mating treatment period) are shown in table 39.

Table 39: Intake of benfluralin in mg/kg bodyweight/day in different phases of the study

Dose level (ppm)	**Pre-mating phase weeks 1-10**[#]		**Females**	
	Males	**Females**	**Gestation**[#]	**Lactation**[#]
100	10.0 -5.5	11.2 -7.4	7.4 – 6.3	9.1 – 19.9
1000	94.6 – 52.6	97.2 – 69.5	73.9 – 65.3	90.8 – 198.1
5000	444.5 – 278.3	502.1 – 334.4	338.6 – 310.8	394.1 – 854.1

[#] The range of mean values are presented in terms of the direction of change over time.

After pre-mating growth phase (10-weeks' exposure), F_0 animals were mated at 16 weeks of age to produce the F_1 generation. After weaning (3 weeks), the F_1 parental animals were selected and similarly mated after pre-mating growth phase (12-weeks' exposure) to produce the F_2 generation (the non-selected F_1-pups were maintained until all selected F_1-pups had successfully initiated the maturation phase). The study was terminated when the F_2 generation pups were 3 weeks old, after weaning.

Table 40: Two-generation study: summary table of deviations from current guideline

Deviations from current OECD guideline 416 (2001)
The housing conditions were not ideal; the temperature range was from 16 to 27°C (should be 22±3°C) and the relative humidity was 32 to 84% (should be 30 to 70%, preferably 50 to 60%). Organ weights were only measured for liver and kidneys (target organs) in F_0-F_1 adults. The weights of testis, epididymis, uterus, ovaries, prostate and seminal vesicles with coagulating glands and their fluids, and brain, spleen, pituitary, thyroid and adrenal grands were not measured and no sperm analysis was undertaken for the F_0 and F_1 adult animals. Histopathological evaluation of the liver and kidneys were done on all F_0 and F_1 animals, but for the tissues pituitary, thyroid, parathyroid, testis, epididymis, prostate, seminal vesicle, coagulating gland, ovary, oviduct, cervix, vagina, mammary gland, glandular stomach, abdominal cavity, ureter and urinary bladder only animals in the control and high dose group were examined. Daily vaginal smear analysis was not performed for evaluation of the oestrus cycle. Histopathological investigations on ovaries were not performed. The uteri were not examined for the presence and number of implantation sites. Developmental landmarks were not assessed. Brain, spleen and thymus from F_1 and F_2 pups were not weighed. Animals were not tested for sensory functions. Complete gross necropsy was performed on only 10 weanlings/sex/group from F_1 and F_2. Gross necropsy and full histopathological characterization of preserved tissues should have been performed on at least one pup/sex/litter. Due to an increased post-weaning mortality in the high-dose litters in F_0, the initially non-selected F_1-pups were maintained until all selected F1-pups had successfully initiated the maturation phase. Historical control data as delivered are of limited value since only few of the studies overlap with the 1993 ± 2 years as the year of initiation of 2-generation studies. Also the control data from the benfluralin- study has lower values than the other control data from this period. It thus seems that the HCD data are not suited for comparisons with the results in this study.

Parental toxicity

F_0 parental generation

In top-dose animals, one non-pregnant F_0-♀died on gestation day 23, following thin and unkempt appearance the day before. No dams died during lactation, and one ♀ died during week 18 (1 week after weaning), but no adverse clinical signs were seen before. The two unscheduled deaths exhibited both pyelonephritis and papilla necrosis. Ureter hyperplasia, renal pelvic dilation (calculus present in pelvis and ureter) and irregularly shaped kidneys were noted in the first reported incidence. In the other, necropsy revealed distended ureters and roughened renal cortex with raised areas in the kidneys, along with bladder calculi. In both cases, the pyelonephritis was associated with urinary calculi observed at necropsy which compromised the urothelium and probably led to an ascending infection and subsequent pyelonephritis.

From week 10 onwards, a dose-related occurrence of bright (mid-dose and above in ♂ and ♀) and/or dark (top-dose in ♂) urine was observed, which was related to the excretion of the substance. During gestation and lactation, three ♀ animals showed alopecia (mainly paws and limbs) at the top-dose.

During pre-mating (growth phase week 0-10), body weights of top-dose animals were significantly decreased at all intervals. In the ♂, body weights remained 9% lower than controls up to week 19 at this dose. Throughout the growth phase period (and mainly during the first 5 weeks), body weight gains were significantly decreased at the top-dose in both sexes, whereas a slightly diminished growth rate was also observed in the ♀ treated at 1000 ppm. During gestation and lactation, the decreases in body weights at the top-dose ranged from 11-20%. The body weight gain was impaired during the entire gestation period, whereas during lactation, the growth rate decrease was particularly marked during the first week, and mainly during days 0-4, where top-dose animals lost weight (–0.96 g), compared to the control group (+15.8 g). From d4 on, the weight loss was compensated in the treated groups. The body weight effects were explained by diminished food intakes, which

were consistently low at the top-dose (♂,♀), and in the ♀ at the mid-dose (especially in weeks 0-1 and 7-9, where differences were >10%) and above during pre-mating. Also during gestation and lactation, food consumption was low over the entire period at the top-dose, and occasionally at the mid dose (significant decreases up to 10% on lactation days 7-10 and 10-16).

The body weights effects in ♀ were, however, not explained by the number of pups or by the weight of the pups. When total weight of each litter was subtracted from the weight of the animal at gestational day 20, the decrease in body weights at the top-dose was comparable to the effects in body weights in the pregnant animal (see table 43). The mean pup weights were slightly reduced from 6 grams in control animals to 5.7 grams in the top-dose. There was a reduction in total number of liveborn pups at the top-dose and the mean number of pups were decreased from 13.5 pups per litter in control ♀ to 11.1 pups per litter in top-dose ♀ (significant reduction, see table 47, litter performance). It was of note that total litter loss was observed in one top-dose F_0-♀ (lactation day (LD) 8).

Table 41: Two generation reproduction toxicity of benfluralin in rats: ADULT data: mortality and clinical signs

Dose (ppm)	**0**		**100**		**1000**		**5000**	
	F0	***F1***	***F0***	***F1***	***F0***	***F1***	***F0***	***F1***
♂ **Mortality** (time of death)	-	§1(wk 14)	-	-	-	*1(wk 3)	-	-
♀	-	-	-	-	-	-	§§2(wk 18-gd 23)	$1(d 6)
Clinical signs (♀)								
n° examined (gestation/lactation)	27/28	27/25	30/29	28/25	28/29	28/25	29/28	29/26
bright **coloured urine**	0/0	0/0	2/0	0/0	9/7	2/0	16/12	0/8
Dark	0/0	0/0	0/0	0/0	0/0	0/0	0/18	5/13
Alopecia	0		0		0		3/3	

§Pyelonephritis, calculus and urinary tract inflammation present. *Cause of death unknown. §§Pyelonephritis and calculus present. $Pyelonephritis, calculus, urinary tract inflammation and CPN (minimal) present.

F_1 parental generation

One top-dosed ♀ was found dead on day 6 (with urine stains and opaque eyes as the sole clinical signs). The unscheduled death (♀) exhibited CPN (minimal), renal pelvic dilation, microconcreted tubuli in the renal cortex, pyelonephritis, bladder calculi , and urinary tract inflammation. In addition, the death of one ♂ during week 3 showed soft brain stem and cranial cavity fluid (microscopic evidence to account for the death were not found). One control ♂animal died during week 14 (with renal pelvic dilation, kidney/ureter/urinary bladder calculi, enlarged kidneys and urinary tract inflammation present) and pyelonephritis was stated by the notifier to be the cause of death.

Discolored urine was observed at the top-dose only from 10 week onwards.

In F_1, approximately the same trend was observed as in the previous generation. During *pre-mating*, food consumption was reduced at the top-dose, and both body weights and body weight gains reduced in parallel. In the mid-dosed animals, the overall growth rate (0-12 weeks) appeared unaffected, but this was due to major fluctuations during sampling periods: significant changes in body weight gain were apparent during weeks 0-1 in ♂ and in ♀ during weeks 8-9. During gestation and lactation, top-dose dams showed a reduced food intake. Growth rate was low during gestation but not during lactation. The isolated statistically significant changes at the lowest dose, or those lacking dose-response were considered irrelevant. It was of note that mortalities,

clinical signs, and body weight of "rest phase" females (F_0-♀ and F_1-♀ kept on treatment after weaning) were comparable to those of the other discussed phases (pre-mating, gestation, lactation).

The pup weights in F_1 did not explain the effects on maternal body weight as the mean pup weights showed a minor reduction from 5.8 grams in control animals to 5.7 grams in the top-dose. Further, when total weight of each litter was subtracted from the weight of the animal at gestational day 20, the decrease in body weights for the F_1 generation was also comparable to the effects in body weights on the pregnant animal. The reduction in total number of liveborn pups at the top-dose was slight, but the numbers are not directly comparable due to fewer control animals. The mean number of liveborn pups were decreased from 13.08 pups per litter to 11.43 pups per litter (significant reduction, see table 47, litter performance). It was of note that total litter loss was observed in two F_1-♀ (LD 7 and 8) at the top-dose, and in one control F_1-♀. One top-dose ♀ had a prolonged delivery.

Table 42: Two generation reproduction toxicity of benfluralin in rats: ADULT data: food consumption, body weights and body weight gains

Dose (ppm)			100		1000		5000	
			F_0	F_1	F_0	F_1	F_0	F_1
Food consumption								
pre-mating	**wk0-10/12**	♂			-	↓5%	↓10%**	↓23%**
		♀			↓8%**	↓4%	↓15%**	↓21%**
Gestation	**d0-20**				↓4%	-	↓20%**	↓19%**
Lactation	**d0-4**				↓9%	-	↓34%**	↓16%**
	d0-21				↓5%	-	↓28%**	↓26%**
Body weight								
pre-mating	**wk 10**	♂					↓10%**	↓22%**
		♀					↓10%**	↓15%**
Gestation	**d0**						↓11%**	↓17%**
	d7						↓13%**	↓18%**
	d14						↓15%**	↓18%**
	d20/21						↓17%**	↓18%**
lactation	**d0**						↓16%**	↓16%**
	d4						↓20%**	↓20%**
	d7						↓17%**	↓17%**
	d14						↓11%**	↓11%**
	d21						↓13%**	↓13%**
Body weight gain								
pre-mating	**wk 0-1**	♂		(↑**)	-	↓10%*	↓38%**	↓37%**
		♀					↓54%**	↓14%**
	wk 1-2	♂				-	-	↓29%**
		♀					-	↑19%**
	wk 2-3	♂				-	↓17%**	↓24%**
		♀					↓37%**	↑21%*
	wk 3-4	♂				-	↓19%**	↓18%**
		♀					↓33%**	↓7%
	wk 4-5	♂		(↓**)		(↓*)	↓21%**	-
		♀					↓13%	↓8%
	wk 8-9	♀			-	↓35%*	-	↓56%**
	wk 0-10/12	♂			-	-	↓17%**	↓15%**
		♀			↓8%*	-	↓28%**	↓3%
Gestation	**d0-7**				-	↓12%	↓28%**	↓24%**
	d7-14				-	-	↓34%**	↓22%**
	d14-20				-	↓4%	↓26%**	↓20%**
	d0-20				-	↓3%	↓28%**	↓24%**
Lactation	**d0-7**						↓53%**	↓4%
	d0-21						-	(↑**)

Statistically significant modifications: Dunnett's t-test *p<0.05, **p<0.01 (figures in parentheses considered irrelevant).

Table 43: Two generation reproduction toxicity of benfluralin in rats: ADULT data and PUP data: individual body weights

Dose (ppm)	**0**		**5000**	
	F₀	***F₁***	***F₀***	***F₁***
N° pregnant (/30)	28	26	28	28
N° pregnant with individual data during gestation	26	24	28	28
N° of liveborn pups d0	378	340	312	320
N° of pups with weight data d0	378	337	307	317
N° of stillborn (no weight data available)	5	5	3	10
Mean pup weight (g)	6	5.8	5.7	5.7
Mean maternal body weight (g) GD 20	372	385	310	314
corrected with pup weight data d0*	292	309	248	249
Individual maternal body weights (GD 20)/ litter size/mean pup weights d0/n° of stillborn	350 / 14 / 6.0 / 0 354 / 14 / 5.7 / 0 415 / 15 / 5.7 / 0 404 / 14 / 5.8 / 0 390 / 13 / 6.5 / 0 336 / 13 / 5.6 / 0 408 / 16 / 6.0 / 0 353 / 11 / 6.3 / 1 379 / 14 / 6.6 / 0 350 / 11 / 6.4 / 0 363 / 13 / 6.0 / 0 376 / 11 / 6.8 / 0 398 / 15 / 5.5 / 0 367 / 12 / 5.7 / 0 380 / 16 / 5.8 / 0 391 / 14 / 5.5 / 1 379 / 14 / 6.2 / 0 354 / 14 / 5.3 / 0 410 / 14 / 5.9 / 1 367 / 12 / 6.4 / 1 361 / 15 / 6.1 / 0 356 / 14 / 5.4 / 0 343 / 12 / 5.9 / 0 348 / 13 / 6.6 / 0 350 / 13 / 5.9 / 0 390 / 12 / 6.4 / 1 -- / 15 / 5.8 / 0[#] --/ 14 / 5.9 / 0[#]	377 / 13 / 5.9 / 0 365 / 15 / 5.0 / 0 349 / 10 / 6.7 / 0 362 / 15 / 5.3 / 0 440 / 16 / 5.4 / 0 387 / 13 / 6.1 / 0 394 / 12 / 6.5 / 0 398 / 12 / 5.5 / 0 407 / 15 / 5.5 / 0 360 / 14 / 5.4 / 0 409 / 14 / 6.1 / 1 399 / 14 / 6.0 / 0 353 / 7 / 6.6 / 0 393 / 12 / 5.4 / 0 421 / 17 / 5.7 / 0 409 / 11 / 6.2 / 1 375 / 12 / 6.3 / 1 416 / 14 / 6.2 / 0 383 / 16 / 4.8 / 0 348 / 10 / 6.8 / 1 340 / 11 / 6.5 / 0 366 / 11 / 6.2 / 0 380 / 14 / 5.2 / 0 396 / 13 / 6.4 / 1 -- / 10 / 3.7 / 0[§#] -- / 16 / 5.9 / 0[#]	295 / 12 / 5.2 / 0 315 / 13 / 4.2 / 0 305 / 12 / 5.1 / 1 290 / 8 / 6.2 / 0 309 / 10 / 5.9 / 1 308 / 6 / 6.3 / 0 289 / 13 / 5.4 / 0 314 / 12 / 5.6 / 0 334 / 10 / 6.3 / 0 300 / 12 / 5.9 / 0 290 / 12 / 5.4 / 0 317 / 8 / 6.1 / 0 315 / 14 / 5.4 / 0[§] 309 / 7 / 5.8 / 1 314 / 12 / 5.7 / 0 307 / 10 / 5.9 / 0 333 / 15 / 5.0 / 0 303 / 13 / 5.7 / 0 285 / 8 / 6.1 / 0 318 / 9 / 6.2 / 0 291 / 11 / 6.2 / 0 352 / 15 / 5.5 / 0 288 / 8 / 6.6 / 0 324 / 11 / 6.0 / 0 306 / 11 / 5.3 / 0 320 / 13 / 6.1 / 0 355 / 10 / 5.3 / 0[¤] 294 / 12 / 5.9 / 0	268 / 9 / 6.4 / 0 293 / 3 / 6.7 / 3 346 / 15 / 5.5 / 0 283 / 12 / 5.3 / 0 394 / 13 / 6.8 / 0[§] 279 / 11 / 6.1 / 0 332 / 13 / 6.2 / 0 299 / 13 / 6.0 / 0 301 / 11 / 5.8 / 1 295 / 9 / 5.6 / 0 305 / 10 / 5.7 / 1 316 / 12 / 6.0 / 0 328 / 10 / 6.7 / 1 354 / 15 / 6.3 / 0[§] 318 / 10 / 5.5 / 0 347 / 14 / 4.6 / 0 277 / 12 / 5.1 / 0 337 / 14 / 5.7 / 0 300 / 10 / 6.0 / 0 308 / 12 / 5.2 / 0 315 / 10 / 5.6 / 0 304 / 7 / 5.6 / 0 317 / 11 / 5.5 / 3 286 / 11 / 5.3 / 0 295 / 11 / 6.0 / 0 297 / 12 / 5.7 / 0 374 / 14 / 5.0 / 0 311 / 13 / 5.0 / 1
Median	367 / 14 / 6.0 / 1	394 / 13/ 6.1 / 1	308 / 11 / 5.9 / 1	307 / 11.5 / 5.7 /1

[*]total weight of each litter is subtracted from the weight of the pregnant animal (maternal body weight) on gestation day (GD) 20; [#]pup weights excluded in corrected mean maternal body weights; [§]total litter death d5-21; [¤]prolonged delivery (delivered 13 pups). Data are shown for informational purpose only (statistics is not run).

Organ weights

Terminal body weights were marginally (1000 ppm, ♀) or markedly (5000 ppm, ♂,♀) decreased in both generations. Liver weights were increased at 1000 ppm and above, and a histological correlate was found. The increased kidney weights in the ♂ at 1000 ppm and higher was also considered relevant, in view of the kidney lesions.

Table 44: Two generation reproduction toxicity of benfluralin in rats: ADULT data: organ weights and macropathology

Dose (ppm)		**0**		**100**		**1000**		**5000**	
		F0	***F1***	***F0***	***F1***	***F0***	***F1***	***F0***	***F1***
Terminal body weights	♂					-	-	↓9%**	↓17%**
	♀					↓5%	↓7%*	↓15%**	↓21%**
Absolute liver weight	♂					↑17%**	↑13%**	↑35%**	↑21%**
	♀					↑13%**	↑9%**	↑40%**	↑39%**
Relative liver weight	♂					↑15%**	↑16%**	↑48%**	↑45%**
	♀					↑18%**	↑18%**	↑63%**	↑76%**
Absolute kidney weight	♂					↑13%**	-	↑7%*	-
	♀					-	-	-	↓16%**
Relative kidney weight	♂					↑10%**	↑11%**	↑17%**	↑25%**
	♀					(↑7%)	-	(↑7%)	-
Gross pathology									
LIVER; n° examined	♂	*29*	*29*	*30*	*30*	*26*	*29*	*27*	*30*
	♀	*30*	*30*	*29*	*30*	*29*	*30*	*25*	*29*
prominent reticular pattern	♂	0		0		**2**		**2**	
	♀	0		0		0		0	
pale area	♂	0	1	0	0	1	1	1	0
	♀	0	1	0	1	0	1	0	**4**
Enlarged	♂	0	0	0	0	0	0	0	**3**
	♀	0	0	0	0	0	0	**2**	**4**
UTERUS; n° examined		*30*	*28*	*30*	*25*	*30*	*25*	*28*	*28*
fluid filled lumen		1	2	0	1	1	1	**3**	**3**
ABDOMINAL FAT; n° examined	♂	*30*	*29*	*30*	*30*	*30*	*29*	*30*	*28*
	♀	*30*	*30*	*30*	*30*	*30*	*30*	*28*	*29*
dark/orange/yellow appearance	♂	0	0	0	0	0	1	0	**25**
	♀	0	0	0	0	0	1	**2**	**20**

Statistically significant modifications Dunnett's t-test *p<0.05, **p<0.01.

Gross pathology and histopathology

Most effects in both generations were restricted to the liver and the kidneys. During necropsy, some animals exhibited enlarged livers and pale areas (F_1♀) at the top-dose. Kidney findings were observed in isolated cases in the treated animals (F_0) and along all groups including controls (F_1), and comprised renal pallor, cyst, dilated pelvis with or without calculi. Animals reported with pyelonephritis and calculi all died before terminated sacrifice. In 3 top-dose ♀, uterus lumen was filled with fluid. Most F_1 animals exhibited a discolored (dark) adipose tissue in the abdominal cavity, while the finding was not marked in F_0. The finding was considered to reflect the deposition of test article in the adipose tissue, as no histomorphologic correlate was observed.

Histologically, a clear dose-dependent increase of hepatocellular hypertrophy (of centrilobular to midzonal character in F_1) was observed at 1000 ppm and 5000 ppm. The treatment caused an exacerbation of CPN from 1000 ppm onwards, in the ♂ (F_0/F_1) with increase in both incidence and severity. In ♀ (F_1) there was an increase in incidence of animals observed with minimal CPN. An increased incidence of kidney tubular hyaline droplets was observed in the ♂, and it was assumed that this finding was caused by the accumulation of α2-microglobulin, which is typical for the ♂ ageing rat. In addition, a compound-related increased incidence of uterus dilatation was observed in the top-dose ♀, and corroborated the necropsy observations. As the increase was slight, it was assumed that the low dose (100 ppm) could be regarded an NOAEL for this effect (intermediate doses were not examined).

Table 45: ADULT data: histopathology

Dose (ppm)		**0**		**100**		**1000**		**5000**	
		F0	***F1***	***F0***	***F1***	***F0***	***F1***	***F0***	***F1***
n° examined	♂	*30*	*29*	*30*	*30*	*30*	*29*	*30*	*30*
	♀	*30*	*30*	*30*	*30*	*30*	*30*	*28*	*29*
LIVER: hepatocellular hypertrophy	♂	0	0	0	0	0	**25**	**30**	**30**
severity (minimal/slight/moderate)							**13/12/0**	**4/22/4**	**2/16/12**
	♀	0	0	0	0	**8**	**16**	**28**	**29**
severity (minimal/slight/moderate)						**8/0/0**	**13/3/0**	**2/15/11**	**3/18/8**
KIDNEY: nephropathy (CPN)	♂	18	22	15	21	15	**27**	**25**	**30**
severity (minimal/slight/moderate)		**17/1/0**	**21/1/0**	**13/2/0**	**19/2/0**	**11/4/0**	**23/5/0**	**19/5/1**	**8/21/1**
	♀	5	14	5	11	**8**	**21**	**10**	**29**
severity (minimal/slight/moderate)		**4/1/0**	**14/0/0**	**5/0/0**	**11/0/0**	**7/1/0**	**20/1/0**	**7/3/0**	**26/3/0**
KIDNEY: tubular hyaline droplets	♂	2	1	0	0	**19**	**22**	**17**	**29**
severity (minimal/slight/moderate)		**2/0/0**	**1/0/0**			**19/0/0**	**15/7/0**	**17/0/0**	**8/20/1**
	♀	0	0	0	0	0	0	0	0
KIDNEY: pyelonephritis[§]	♂	0	1	0	0	0	0	0	0
calculus collected by necropsy			1						
urinary tract inflammation			1						
	♀	0	0	0	0	0	0	2	1
						1		2	1
									1
UTERUS: dilatation		2	8	0	n.e	0	n.e	**4**	**16**

[§]not included in n° examined; sacrifice status was unscheduled for animals observed with pyelonephritis. n.e.: not examined.

Offspring toxicity

Litters

Body weights: following delivery, a slight body weight decrease was restricted to the top-dose animals in F_1. At the top-dose, a time-dependent decrease of body weight was observed up to weaning in both generations. At the next-lower dose, a moderate but significant decrease was observed in F_1, and to a lesser extent in F_2. Contrarily to the top-dose F_1-pups, the F_2-pup body weight on d0 was not different from the controls, which was interpreted as a lack of an effect *in-utero* on this parameter in F_2.

Table 46**:** two generation reproduction toxicity of benfluralin in rats: PUP data: body weights

Dose (ppm)	100				1000				5000			
	F1		***F2***		***F1***		***F2***		***F1***		***F2***	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
d0					-	-	-	-	↓8%**	↓9%**	-	-
d4 pre-cull					↓13%**	↓10%**	-	-	↓27%**	↓29%**	↓26%**	↓27%**
d4 post-cull					↓12%**	↓10%**	-	-	↓26%**	↓28%**	↓26%**	↓27%**
d7					↓13%**	↓10%**	↓8%**	↓10%**	↓36%**	↓36%**	↓35%**	↓34%**
d14					↓10%**	↓8%**	↓11%**	↓11%**	↓39%**	↓38%**	↓40%**	↓40%**
d21					↓8%**	↓6%**	↓10%**	↓10%**	↓40%**	↓49%**	↓41%**	↓40%**

Statistically significant modifications (obtained on covariate adjusted means): Dunnett's t-test **p<0.01. 100 ppm; no significant differences were found.

Litter data

In both F_0 and F_1, the treatment was without effect on the pre-coital interval or on the ♂ and ♀ fertility indices. The treatment did not affect the sex ratio at any sampling time, and at any generation.

On the contrary, the duration of the gestation was slightly high in the top-dose animals, and the effect was more marked when the incidence of animals showing a gestation time ≥23d (mostly =23d) was considered. The effect was considered relevant, as one top-dose dam (#B39079) was reported to have a prolonged delivery during the observation of the clinical signs. The top-dose dam (#B39079) delivered 13 pups in total, but foetal weights were reported for 10 pups only on d0, suggesting that three of the pups were delivered after the others (see table above). Only five pups survived to precull day 4. On the other hand, the treatment did not affect the gestation index (n° of ♀ with live pups /n° of pregnant ♀) in neither F_0 nor F_1, as it was actually 100% in all dose-groups. The number of stillborn pups was slightly increased in the top-dose in F_2, with 3% incidence compared to the control (1.3%). Overall, the total number of pups, and the number of live pups pups delivered per litter was decreased in both generations at the top-dose, and a slight effect was also visible in F_0 at 1000 ppm. It appeared that pup mortality occurred in the period up to postnatal day 4, at 1000 ppm (F_0) and above ($F_{0/1}$). Top-dose litters were more affected by mortality up to weaning, especially in F_1, where litter sizes decreased on postnatal days 14 and 21. Again, a subtle decrease of litter size was also noted at 1000 ppm in F_0. Two additional top-dose pups died post-weaning, on days 22-25. The historical control data provided was regarded as not relevant as only one study was within the time frame of ± 2 years of the time of conduction of this study. It is not known if the reduced survival of the pups is caused by effects on the pups in utero, effects via the milk, or effects on the rearing of the pups.

Table 47: Two generation reproduction toxicity of benfluralin in rats: PUP data: litter performance

Dose (ppm)		0		100		1000		5000	
		F0	***F1***	***F0***	***F1***	***F0***	***F1***	***F0***	***F1***
n° of ♀ pregnant (/30)		28	26	29	25	29	25	28	28
duration of gestation (d)		22.0	21.9	22.0	22.1	22.3	22.1	22.2	22.3**
n° ♀ ≥23d gestation[§]		1/26	1/24	1/29	4/25	4/28	2/25	6/28	7/28
(%)		3.8	4.2	3.4	16.0	14.3	8.0	21.4	25.0
Group mean litter sizes									
n° of ♀ with liveborn pups		28	26	29	25	29	25	28	28
total pups d0	Total	383	345	392	316	372	347	315	330
	liveborn	378	340	387	309	370	341	312	320
	stillborn	5	5	5	5	2	6	3	10
mean total born d0		13.68	13.27	13.52	12.64	12.83	13.88	11.25**	11.79**
mean liveborn d0		13.50	13.08	13.34	12.36	12.76	13.64	11.14**	11.43**
Live birth index (%)		99	99	99	98	99	98	99	96
live pups/litter d4 pre-cull		13.14	12.46	13.10	12.28	12.03	13.32	10.21	10.43**
Viability index (%)		97	94	98	99	95	98	93	92
live pups/litter d4 post-cull		8.00	7.73	7.86	7.92	7.66	8.00	7.75	7.71
live pups/litter d7		7.93	7.65	7.83	7.92	7.52	8.00	7.61	7.22
live pups/litter d14		7.89	7.88	7.83	7.92	7.38	8.00	7.67	6.88**
live pups/litter d21		7.89	7.88	7.83	7.88	7.34	8.00	7.56	6.58**
Weaning index (%)		99	96	100	100	96	100	94	79[# #]
Pup loss[§§]	**d0-4**	10	16	7	2	21	8	26	28
	d5-21	3	4	1	1	9	0	13	45
	d22-28	0	0	2	0	0	0	19	2
Entire litter loss[§§]	**d0-4**	0	0	0	0	0	0	0	0
	d5-21	0	1	0	0	0	0	1	2

Statistically significant modifications: Dunnett's t-test*p<0.05, **p<0.01; [# #]: significant trend (p<0.01). Life birth index= n° liveborn /total born; Viability index= n° alive d4 pre-cull /n° liveborn; Weaning index= n° alive d21 /n° alive d4 post-cull; [§]: females with no recording of individual data during gestation were excluded from the calculation; [§§]: loss means dying, killed, missing or cannibalized.

10.10.3 Comparison with the CLP criteria

According to the CLP criteria, adverse effects on sexual function and fertility include those that interfere with the reproductive system, onset of puberty, gamete production/transport, reproductive cycle, sexual behaviour, fertility, parturition, pregnancy outcome, reproductive senescence or any other function that is dependent on the reproductive system. Not all of these effects have been sufficiently investigated, as several critical reproductive endpoints were not addressed in the available studies. The available data from the two-generation study give, however, an indication that exposure to benfluralin has no adverse effects on sexual function or fertility. Impaired maternal health status (decreased body weight, liver and kidney toxicity) was observed at the top-dose, with effects on the organs appearing from the mid-dose. At the top-dose, the treatment did not affect the gestation index, but a slight increase in the duration of the gestation, and a decreased number of live pups delivered per litter in both generations were seen. In the multigeneration study, the mean number of liveborn pups per litter was also slightly decreased at the top-dose in the first three generations. The number of implantation sites per dam was dose-dependently decreased, but it was not possible to determine if the pre-implantation loss was subsequent to the exposure to the substance (the number of corpora lutea in the dams was not determined).

It is to be noted that in the female pubertal assay (refer to Annex I, section B.6.8.3) evidence of endocrine activity was observed at the top-dose (300 mg/kg bw/day) characterized by increase in the mean age vaginal opening, increase in age at first estrus cycle, decrease in ovary weight, decrease in blotted uterus weight and decrease in pituitary weight together with reduction on the overall body weight gain (5.5%) and an increase in creatinine (44%). In addition, decreased prostate weight, decreased seminal vesicle plus coagulating glands weight, decreased epididymal weight and decreased testosterone levels were observed in the male pubertal assay at the top-dose (400 mg/kg bw/day) together with reduction in overall body weight gain at 9.5%. In the two-generation study, no sperm analysis, histopathological examination on the ovaries or weight of other

accessory organs in the reproductive system were undertaken, and it is not known whether the observed effects are due to inteference with the reproductive system through endocrine activity or through other mechanisms. Further, the observed effects in the two-generation study are not supporting adversity. Therefore classification on sexual function and fertility is not proposed.

10.10.4 Adverse effects on development

The potential of benfluralin to adversely affect development has been assessed in one study in rats and one study in rabbits using the same batch of benfluralin. There are two more studies available (one range-finding study in rats and rabbit teratology study) in section in Annex 1 (see B.6.6.1) not considered fit for the purpose to assess effects on development of offspring. The developmental toxicity findings from the two-generation study are also included in this section.

Table 48: Summary table of animal studies on adverse effects on development

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
EPA 40 CFR 158 GLP Rat Crl:CD®(SD)BR Female 25 dams/dose, Teratology	Benfluralin (97.3%) Batch No.: 231EF4 0, 50, 225, 475 and 1000 mg/kg bw/day	***Maternal toxicity*** 1000 mg/kg bw/d 2 females with alopecia ↓body weight gain (-15% d0-20) 475 mg/kg bw/d 1 female with alopecia ↓body weight gain (-13% d0-20) 225 mg/kg bw/d No treatment related effects ***Developmental toxicity*** 1000 mg/kg bw/d ↑litter incidence of variation of the vertebrae/sternebrae 475 mg/kg bw/d No treatment related effects	Author (1985b) Report No. 6180-101/CA 5.6.2/02
Guideline: EPA FIFRA 83-3 GLP Rabbit (NZW) Female 20 dams/dose	Benfluralin (97.3%) Batch No.: 231EF4 0, 25, 50, 100 and 225 mg/kg bw/day	***Maternal toxicity*** 225 mg/kg bw/d 1 female found dead (d18) 3 abortions (2; d20, 1; d23) 5 females with alopecia 3 females with discoloured urine ↓voided faeces (6 females with scant and 3 with no faeces) ↓food consumption (-10% d0-29, -43% d12-15)	Author (1991) Report No. 3130.9/ CA 5.6.2/04

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		↓body weight gain (-24%) 100 mg/kg bw/d 1 female with discoloured urine ↓voided faeces (6 females with scant and 1 with no faeces) ↓food consumption (-6% d0-29, -24% d12-15) ↓body weight gain (-29% d0-29) 50 mg/kg bw/d ↓body weight gain (-13% d0-29) No other treatment related effects 25 mg/kg bw/d No treatment related effects ***Developmental toxicity*** 225 mg/kg bw/d ↓ viable litters (3 abortions, 1 maternal death) ↑ incidence of accessory skull bones 100 mg/kg bw/d No treatment related effects	

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

Developmental toxicity in rats

Twenty five mated ♀rats/dose (Sprague-Dawley Crl:CD®BR) received benfluralin dissolved in a 10% w:v acacia oil by gavage at the dose level of 0; 50; 225; 475 or 1000 mg/kg bw/day, during a 20 days period. The results of the test substance analysis indicated a test substance purity of 92.5%, slightly lower than initial 97.3%.

Maternal data

There were no deaths in control or in treated groups during the study. Few cases of alopecia were detected at the top-dose. In addition, urine stains were observed on the pan papers from the lowest dose on up to and including the top-dose, and varied from light yellow to orange (reflecting excretion of the test substance). Some maternal toxicity was evident at the two highest doses, characterised by markedly lower food consumption during the first day of gestation, and remained low up to d16 at 475 mg/kg bw/day. The body weights were marginally impaired at any measurement, leading to weight changes which were only significantly reduced between gestation days 6 and 11. As uterus weights were only slightly lower at 475 mg/kg bw/day but not at the top-dose, corrected body weight changes showed some dose-dependent decrease

at the two highest doses. The apparent (non-significant) effects at 225 mg/kg bw/day were considered irrelevant, as decreased b.w. changes were already observed during the pre-treatment period. There were no relevant gross pathology findings; the observed renal and hepatic effects showed no dose-dependency, and could thus be regarded incidental. No organ weight data was collected for the dams in this study.

Foetal data

There was no foetal mortality, and the number of corpora lutea and of implants was unaffected by the treatment. The number of resorptions was not increased by the treatment (in contrast to the results of the range-finding experiment), and foetal weights were unaltered.

Table 49: Oral teratogenicity study of benfluralin in rats (main study)

Dose (mg/kg bw/d)		**0**	**50**	**225**	**475**	**1000**
MATERNAL DATA						
Clinical signs: alopecia		0	0	0	1	2
Food consumption[§]	**gd 6-11**				↓17%*	↓20%*
	gd 11-16				↓8%*	↓7%*
	gd 16-20				-	-
	gd 6-20				↓6%	↓9%
Body weight	**gd 6**				-	-
	gd 11				↓4%	↓5%
	gd 16				↓4%	↓4%
	gd 20				↓4%	↓3%
Uterus weight					↓8%	-
Body weight change	**(d 0-6)**			(↓13%)	(↓7%)	(↓3%)
	gd 6-11			(↓19%)	↓52%*	↓48%*
	gd 0-20			(↓5%)	↓10%	↓5%
Body weight change (d0-20, corrected)				(↓9%)	↓13%	↓15%
Gross pathology						
dilated renal pelvis		2	1	1	1	2
enlarged hepatic lobes		0	4	1	1	3
FOETAL DATA						
Number of pregnant ♀		24	24	24	24	25
Corpora lutea/dam		16	16	18	15	17
Implantations/dam		16	15	15	14	16
Resorptions/litter		0.8	1.0	0.8	0.8	0.5
	%	*5.2*	*6.7*	*5.0*	*5.5*	*3.3*
Implantation loss (%)	pre–	3.2	2.9	11.3	7.5	8.4
	post–	5.2	6.7	5.0	5.5	3.3
live foetuses/litter		14.7	14.5	14.7	13.6	15.4
sex ratio (♂/♂+♀,%)		51	51	48	47	52
foetal weight (g)	♂	3.6±0.26	3.5±0.31	3.5±0.37	3.5±0.25	3.5±0.24
	♀	3.3±0.21	3.3±0.28	3.3±0.31	3.3±0.27	3.3±0.25

Values are group average litter data; Statistically significant modifications: Dunnett's -test *p<0.05; **§:**significances based on the daily food consumptions; data between parentheses considered biologically irrelevant.

External observations: Isolated incidences of tail absence (lowest dose) and of thread-like tail/no anus (at 225 mg/kg bw/day) were considered incidental.

Visceral observations: Diffuse haemorragic areas on the liver were detected at 225 and 1000 mg/kg bw/day, and considered of questional toxicological significance in the absence of a proper dose-effect relationship.

Skeletal malformations: Findings which could be regarded treatment-related included the slightly increased incidence of vertebral anomalies or ossification delays, and unossified sternebrae at the highest dose. The litter incidence of unossified sternabrae increased from 14 (58%) in the control to 21 (88%) at the top-dose (24 litters examined), while the incidence in foetuses increased from 18.4% to 28.3%. When compared to the in-house historical control data, the incidences were outside the range, stated being 57% (litter incidence) and 17.5% (foetus incidence). The in-house historical control data consist of 11 studies conducted from 1970 to 1985 and are therefore not fully contemporary to the current study, but the reported incidences were however in the same range as in the current control suggesting that incidences of unossified sternebrae in the control animals were stable during this specific time-period.

The effect in the dams (reduced food consumption and body weight gain) at the two highest doses were not considered to be relevant for the observed developmental findings in the pups as no effects were observed at 475 mg/kg bw/day. Other findings occurred without any dose-relationship and were therefore considered irrelevant.

Table 50: Foetal alterations in the main teratogenicity study of benfluralin in rats: PUPS: visceral and skeletal observations (selected findings)

Dose (mg/kg bw/d)	**0**	**50**	**225**	**475**	**1000**
Visceral observations: n° examined	*167 (24)*	*168 (24)*	*171 (24)*	*157 (24)*	*187 (25)*
Liver: dark red/brown areas	0	0	3/2 (5*)	1/0 (1)	2/2 (4)
Skeletal observations: n° examined	*185 (24)*	*179 (24)*	*182 (24)*	*167 (24)*	*198 (24)*
Centra abnormalities of the vertebrae **V**	5/5 (7)	5/1 (5)	2/3 (4)	6/5 (7)	6/12 (**12**)
%	*5.4 (29)*	*3.4 (21)*	*2.7 (17)*	*6.6 (29)*	*9.1 (50)*
Unossified vertebrae/centra **V**	0/1 (1)	0	1/3 (1)	0	3/1 (2)
%	*0.5 (4.2)*	*0*	*2.2 (4.2)*	*0*	*2.0 (8.3)*
Unossified sternebrae **V**	10/24 (14)	17/23 (16)	21*/26 (15)	12/25 (15)	22/34 (**21**)
%	*18.4 (58)*	*22.3 (67)*	*25.8 (63)*	*22.2 (63)*	*28.3 (88)*
Rudimentary ribs **M**	4/1 (4)	5/2 (6)	1/1 (2)	3/4 (5)	0/5 (5)
%	*2.7 (17)*	*3.9 (25)*	*1.1 (8.3)*	*4.2 (21)*	*2.5 (21)*

M :Malformation, **V** :variation; foetal incidences (♂/♀), litter incidence between parentheses;
Statistical significant modifications: Dunnett's test *p<0.05.

In-house historical control data (11 studies, 10/1970-02/1985):
Anomalies of torso (visceral: N=1967 foetuses, 262 litters; skeletal: N=2340 foetuses, 256 litters)

	Foetuses	*Litters*
Vertebrae centra anomalies **V**	*62 (2.6%)*	*58 (22.7%)*
Unossified vertebrae/centra **V**	*4 (0.17%)*	*4 (1.6%)*
Unossified sternebrae **V**	*409 (17.5%)*	*146 (57.0%)*
Rudimentary ribs **M**	*184 (7.9%)*	*82 (32.0%)*

Two-generation study

In the two-generation rat study, effects on reduced viability and weaning indices were seen at the top-dose. During cage-side observations, some pups had a weak appearance, and there was no visible milk in the stomach at the mid-dose (F_0) and above ($F_{0/1}$), and some top dose pups felt cold to touch or showed a pale appearance. Cyanosis or partially cannibalisation was also observed in isolation at the top-dose (F_1).The total number of pups, and the number of pups delivered per litter was decreased in both generations at the top-dose, and a slight effect was visible in F_0 at the mid-dose. As pre- and post implantion loss endpoints were not investigated the cause of the reduced litter size is not known.

Pup mortality together with decreased body weights occurred in the period up to postnatal day 4, at the mid-dose (F_0) and above ($F_{0/1}$). Top-dose litters were more affected by decreases in body weights and by mortality up to weaning, especially in F_1, where litter sizes decreased on postnatal days 14 and 21. Hence, the weaning index for the F_1-pups (n° alive d21/ n°d4 post-cull) was significantly reduced. A subtle decrease of litter size was also noted at 1000 ppm in F_0. Two additional pups died post-weaning, on days 22-25. The weight of the liveborn F_0 pups was slightly reduced, but no foetal weight reduction was evident in F_1-pups. Therefore, the reduced maternal body weight and body weight gain through pre-mating and gestation did not adversely effect the foetal weight on d0.

Table 51: Two generation reproduction toxicity of benfluralin in rats: PUP data: clinical signs

Dose (ppm)	**0**		**100**		**1000**		**5000**	
	F0	***F1***	***F0***	***F1***	***F0***	***F1***	***F0***	***F1***
pups: weak	0	1	0	1	2	0	5	9
pups: milk absent in stomach	0	1	0	1	2	1	4	5
pups: cold to touch	0	0	0	1	0	0	3	6
pups: pale	0	0	0	1	0	0	0	7

The higher mortality and decreased body weight observed in pups were observed together with a slight impaired maternal health status at the mid-dose. There was a slight reduction in food consumption in F_0 during pre-mating, but major fluctations in body weight gain (reductions in weeks 0-1 and 8-9) was observed in F_1. The effect on the liver was increase in weight with a clear dose-dependent increase of hepatocellular hypertrophy (of centrilobular to midzonal in F_1). On the kidney the treatment caused an exacerbation of F_1 females reported with minimal CPN from the mid-dose onwards. At the top-dose, effects on food consumption, body weights and body weight gain were reduced and the decreases were almost consistently in parallel through all phases (pre-mating, gestation and lactation). All animals had further increase in liver weight with corresponding hepatocellular hypertrophy and there was an increase in both incidence and severity of CPN.

It should be noted that strong weight loss in the top-dose mothers during lactation was only evident in F_0 from day 0-7, and not in the F_1 generation suggesting that the reduced survival might be linked to effects on lactation, e.g. transfer of the substance through the milk, that the pups were too weak to nurse or low milk production in the mothers, effects that could be relevant to the reduced pup body weights throughout the postnatal days. The maternal body weights during lactation were however reduced in both top-dose generations. It is to be noted that most F_1 animals exhibited a discolored (dark) adipose tissue in the abdominal cavity, while the finding was not marked in F_0. The finding reflected the deposition of test article in the adipose tissue. Further, extended necropsy on 10 pups/sex revealed the accumulation of test substance in the abdominal fat of 7/20 top-dose animals (only examined in the F_1-pups). The confirmed accumulation of the test article in F_1 animals and F_1-pups strongly suggests that F_1-pups were exposed in the uterus and/or via the milk. Still, it is not known if the reduced survival of the pups could be caused by effects on the pups in utero, via the milk, effects on the rearing on the pups, or by effects due to liver and kidney toxicity (e.g. maternal toxicity).

Some of the effects reported in the two-generation study were also observed in the multigeneration study as the mean number of liveborn pups were slightly decreased (F_0, F_1 and F_2). Effects on postnatal survival was also reduced at the top-dose in most mating trials of each generation and a dose-dependent decrease of mean progeny weight was observed at the top-dose in each generation, and at 1000 ppm on occasions. In contrast to the two-generation study, implantation sites per dam were investigated. The number of implantation sites per dam was dose-dependently decreased, but as the number of corpora lutea in the dams was not determined, it was impossible to tell if pre-implantation loss was subsequent to the exposure to the substance.

Developmental toxicity in rabbits

Twenty artificially inseminated rabbits/dose (NZW) received Benfluralin (purity 97.3%) dissolved in 10% w:v aqueous acacia oil by gavage at the dose level of 0; 25; 50; 100 or 225 mg/kg bw/day (dosing volume 5 mL/kg) from gestation day d6 to d19 included.

Maternal data

One ♀ was found dead on d18, following a history of thrashing (violent movements), convulsions, dark eyes, rapid respiration and hypoactivity immediately after dosing on the day prior to death. Necropsy revealed foamy tracheal contents, mottled lungs, dark areas on the thymus and dark red mammary tissue. Part of these findings were consistent with an intubation error as the death cause. Three ♀ aborted, two on d20 and one on d23. Prior to abortion, the ♀ were visibly anorexic and showed marked weight loss.

A marked decrease of food consumption was observed from d9 of gestation onwards, at the two highest doses. The effect was unremarkable from d19 on up to termination. In contrast, body weights were barely affected; even at the top-dose, the difference with controls was insignificant, both on d19 and on d29 (uncorrected or corrected weight).

The body weight gains were affected in the early stages of the gestation at 100 mg/kg b.w./d and above (up to d12) and at the top-dose (from d12 to termination). Overall, body weight gains were decreased at the two highest doses during treatment, but rebounded when animals were given normal feed (up to d29). According to the CLP criteria, the body weight gain may not be useful indicators of maternal toxicity because of normal fluctuations in body weight during pregnancy in rabbits. However, the corrected body weight gains were low at 50 mg/kg b.w./d (–13%) and above (–29% and –24% respectively for the doses of 100 and 225 mg/kg bw/day), demonstrating that uterine weight gain was impeded during the gestation.

During gestation days 0-20, isolated cases (maximally 4) of alopecia were detected in all treated groups, but dose-dependency was not evident, neither in the incidence nor in the frequency of observation. However, an increased incidence of hairloss was also observed at necropsy at the top-dose. Diminished defaecation was observed at 100 mg/kg bw/day and above, which was related to the decreased food intake. Discoloured urine (dark yellow/light orange) was indicative of substance excretion at the two highest doses.

Table 52: Oral teratogenicity study of benfluralin in rabbits: MATERNAL DATA

Dose (mg/kg bw/d)		**0**	**25**	**50**	**100**	**225**
Mortality (n/20, *day of death*)						
Disposition of animals	♀ inseminated	20	20	20	20	20
Abortion (day of abortion)		0	0	0	0	**3** (d20, d23)
Mortality (day of death)		0	0	0	0	**1** (d18)
Dams at necropsy		20	20	20	20	16
Dams gravid	with viable young	17	16	15	18	11
	with only resorptions	0	0	0	0	1
Food consumption[§]	d6-9				-	↓8%
	d9-12				↓10%	↓26%**
	d12-15				↓24%*	↓43%**
	d15-19				↓14%	↓41%**
	d6-19				↓14%	↓30%**
	d0-29				↓6%	↓10%
Body weight[§]	d19					(↓4%)
Body weight gain[§] (g)	d6-9	38	31	43	**20**	**8**
	d9-12	51	57	45	**19**	**4***
	d12-15	85	64	91	66	**13***
	d15-19	57	45	66	54	**–18**
	d6-19	232	198	245	**158**	**14****
	d19-24	128	147	135	122	207
	d24-29	61	41	66	40	68
	d0-29	679	722	751	631	656
	d0-29 (corrected)	316	290	**274**	**223**	**241**
Clinical signs (/20)						
scant/no faeces		0/0	3/0	0/0	**6/1**	**6/3**
discolored urine		0	0	0	**1**	**3**
Necropsy	n° examined	20	20	20	20	16
Alopecia		1	3	6	5	**5**
urine stain		0	0	1	1	2

[§]: non-pregnant ♀ excluded; Statistical significant modifications: Dunnett's test *p<0.05, **p<0.01

Foetal data

As 3 abortions and one maternal death occurred, the number of viable litters was decreased at the top-dose. Other parameters were unaffected by the treatment.

External, visceral and skeletal observations:

The only visceral finding was the presence of 2 foetuses in one litter showing pale spleen.

There was an apparent increased incidence of some skeletal variations at the top-dose (13th rudimentary rib and misaligned sternebrae), but the dose-response of these findings was not evident, as the incidence at the next-lower dose was nearly comparable to the controls. The increased incidence of rib anomalies at 100 mg/kg bw/day was also of questionable relevance, in the absence of findings at the highest dose.

The increased foetal incidence of the 7th cervical rib, and increased litter incidence of bent hyoid arches at 50 mg/kg bw/day was irrelevant, as incidences were unaltered at the higher doses. The only possible meaningful change concerned the accessory skull bone, which was dose-dependently increased at the top-dose. When compared to the in-house historical control data, foetal incidence was slightly ouside the historical control incidence, while there was no increase in litter incidence (17 studies were conducted within a time range of two years, in a time period of up to three years of the current study. The term in-house is interpreted to mean

both the same laboratory and animal strain as it is not stated in the report). As this was a variation, occurring at a dose which was also maternotoxic, this finding was considered to not warrant classification.

Table 53: Oral teratogenicity study of benfluralin in rabbits: FOETAL DATA

Dose (mg/kg bw/d)		**0**	**25**	**50**	**100**	**225**
Number of pregnant ♀		17	16	15	18	17
Number of viable litters		17	16	15	18	11
Corpora lutea/dam		10.8	9.9	11.1	10.9	9.9
Implantations/dam		7.2	7.2	8.3	6.9	7.6
Resorptions	Early	1.4	0.4	0.3	0.1	0.8
	Late	0.1	0.1	0.2	0.3	0.1
Implantation loss (%)	pre–	3.6	2.8	2.8	4.0	2.3
	post–	1.4	0.4	0.5	0.4	0.9
live foetuses/litter		5.8	6.8	7.8	6.6	6.7
pregnant uterine weight		363.6	432.4	477.4	408.1	414.9
foetal weight (g)		45.9	46.3	43.8	46.3	45.4
sex ratio (♂/♂+♀,%)		53	46	58	41	53
Foetal examination: n° foetuses (litters)		*99 (17)*	*108 (16)*	*117 (15)*	*118 (18)*	*80 (11)*
Visceral examination: pale spleen		0	0	0	0	2 (1)
Skeletal examination						
rib anomaly [M]		0	1 (1)	2 (2)	2 (2)	0
	%	*0*	*0.93 (6.3)*	*1.7 (13)*	*1.7 (11)*	*0*
13th rudimentary rib [V]		18 (10)	20 (9)	30 (12)	22 (12)	13 (9)
	%	*18 (59)*	*19 (38)*	*26 (80)*	*19 (67)*	*16 (82)*
misaligned sternebrae [V]		15 (7)	13 (9)	21 (9)	16 (7)	15 (9)
	%	*15 (41)*	*12 (56)*	*18 (60)*	*14 (39)*	*19 (82)*
accessory skull bones [V]		1 (1)	1 (1)	2 (2)	2 (2)	**4 (3)**
	%	*1 (5.4)*	*0.9 (6.3)*	*1.7 (13)*	*1.7 (11)*	***5.0 (27)***

[M]: malformation; [V]: variation

In-house historical control data (17 studies, 03/1988-12/1989: N=2120 foetuses, 294 litters)

	Foetuses	*Litters*
Rib anomalies [M]	*mean: 10 (0.47%), range: 0.0-1.8%*	*mean: 10 (3.4%), range: 0.0-12.5%*
Accessory skull bones [V]	*mean: 19 (0.90%), range: 0.0-3.5%*	*mean: 18 (6.1%), range: 0.0-29%*

In summary, the available studies in rats indicate that benfluralin may have adverse effects on development. In the developmental rat study, benfluralin caused an increased incidence of vertebral centra changes (reduced ossification) at the top-dose. Effects on pup survival was evident in the two-generation study, as reduced viability and weaning indices were seen at the top-dose in addition to higher pup mortality and reduced body weight at the mid-dose and above. The total number of pups and the number of pups delivered per litter was also decreased in both generations at the top-dose. In the rabbit study, benfluralin caused an increased incidence of skull variations (accessory bones in the suture line) at the top-dose, which was clearly maternotoxic (decreased food consumption, reduced body weight gain, and increased abortion rate) and benfluralin was not considered to have adverse developmental effects in rabbits.

10.10.6 Comparison with the CLP criteria

According to the CLP criteria, developmental toxicity includes any effect which interferes with normal development of the conceptus, either before or after birth, and resulting from exposure of either parent prior to conception, or exposure of the developing offspring during pre-natal development, or postnatally, to the time of sexual maturation. The major manifestations of developmental toxicity include death of the developing organism, structural abnormality, altered growth and functional deficiency. Substances are either classified in Category 1 (1A or 1B; known or presumed human reproductive toxicant) or Category 2 (suspected human reproductive toxicant). A substance known to have produced an adverse effect on development in humans is classified in Category 1A and the data are mainly based on evidence from humans. If the data are largely derived from animal studies, a substance is either classified Category 1B or Category 2 based on the strength of the evidence and the relevance of the effect for humans.

There are no relevant data on adverse effect on development in humans, hence classification for Category 1A is not proposed.

Classification in Category 1B is largey based on data from animal studies. According to the CLP criteria, such data shall provide clear evidence on development in the absence of other toxic effects. If the effects occur together with other toxic effects, the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effects. When there is mechanistic information that raises doubt about the relevance for humans, classification in Category 2 may be more appropiate. Substances are classified in Category 2 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.

For benfluralin, the available information is based on data from animal studies. There was an increase in the incidence of vertebral centra changes (reduced ossification) at the top-dose in the rat developmental study. Maternal toxicity (lower food consumption and reduced body weight gain) was comparably evident at the two highest doses, but the developmental effects were only produced at the top-dose. According to the CLP criteria, developmental effects that occur in the presence of maternal toxicity are considered to be evidence of developmental toxicity, unless it can be unequivocally demonstrated on a case-by-case basis that the developmental effects are secondary to maternal toxicity. No other severe results (e.g. death or severe inanition results) were reported in the dams. The developmental toxicity findings should therefore be considered as treatment-related effect which have not been demonstrated to be secondary to maternal toxicity. In the study conducted in rabbits, the increased incidence of skull variations (accessory bones in the suture line) at the top-dose was not considered to be adverse, and the effect was observed together with a reduced maternal health status (decreased food consumption, reduced body weight gain and increased abortion rate).

In the two-generation study, the total number of pups, and the number of live pups pups delivered per litter was decreased in both generations at the top-dose, and a slight effect was also visible in F_0 at 1000 ppm. Reduced viability and weaning indices were seen at the top-dose in addition to higher pup mortality and reduced body weight at the mid-dose and above. Notably, the weaning index in the F_1-pups was significantly reduced. During cage-side observations, some pups had a weak appearance, and there was no visible milk in the stomach at the mid-dose (F_0) and above ($F_{0/1}$), and some top dose pups felt cold to touch or showed a pale appearance. Maternal toxicity was less marked at the mid-dose through liver and kidney toxicity, while further effects on body weight and body weight gain was evident at the top-dose. Some F_0 animals showed alopecia on paws and limbs. It is not known whether a reduced maternal health status affected the rearing of the pups, but their health status did not considerably affect the foetal weight on d0 (a slight body weight decrease was restricted to the top-dose in F_1). Further, the reduced survival and the weight reductions in the pups could be due to that the pups were too weak to drink milk or that the dams were in bad conditions and unable to feed the pups.

The multigeneration study supports the findings in the two-generation study, as effects on the number of live pups pups delivered per litter, reduced body weight and postnatal survival were also evident.

The results from the 90 day dietary studies in the rodents, show that the target organs in the rat are the blood, the liver and the kidney. Slight regenerative anaemia was suspected at 5000 ppm and above, and clinical chemistry modifications confirmed effects on the liver and the kindney (please refer to section 10.12, STOT-

RE). Further, effects on thyroid hormones were reported in the MoA study and the pubertal assays (please refer to section 10.9, Carcinogenicity). Whether one of these effects alone or in combination may have influenced the survial of the pups and/ or the reduction in litter size is not known.

A significant finding that should not be disregarded was that the test article was found to accumulate in the abdominal cavity in F_1 animals and in the abdominal fat in F_1-pups (revealed by extended necropsy in F_1-pups only). Since benfluralin was detected in the abdominal fat in the F_2 generation it is highly likely that the pups were exposed in the uterus and/or via the milk.

The available information shows that benfluralin has adverse effect on the development in rats. The effects occur together with, but not evidently secondary to reduced maternal health. Importantly, there are no available data to exclude that the reduced survival of the pups and the reduced litter size could be due to effects via the uterus. On the other hand, there is no information regarding the rearing of the pups and it is not known if maternal toxicity contributed to the effects in the pups.

Since developmental effects were observed in the rat developmental study through increased incidence of vertebral centra changes, and through death of the developing organism and reduced growth in the two-generation study, classification on adverse effects on development of the offspring is proposed. Taking into account the considerations whether the effects are solely treatment-related or could be a result of one or several other factors stated above, classification for Caterogy 2 is considered appropiate.

10.10.7 Adverse effects on or via lactation

Information relevant to any potential adverse effects on or via lactation after the administration of benfluralin can be derived from the two-generation study in rats and from a metabolism study in cow.

Table 54: Summary table of animal studies on effects on or via lactation

Test substance, dose levels duration of exposure	**Guideline, Material and Methods**	**Results**	**Reference**
Benfluralin Lot No. 553-VN6-257 Radiochemical Purity 99.7% Test concentration: Equivalent to a dietary concentration of 10 mg/kg in gelatine capsules	OECD 503, FIFRA Guideline No.171-4, GLP Nature of [^{14}C] benefin residues in bovine tissues and milk **Test animal:** One lactating Holstein dairy cow: 1 cow/dose **Test system:** One lactating Holstein cow was administered gelatine capsules containing [U-phenyl-^{14}C] and unlabelled benfluralin (both dissolved in dichloromethane) at a nominal dose level of 10 mg/kg for 3 days. The cow was fed with alfalfa hay and water during the acclimation, dosing and withdrawal periods. Additionally, one cow was maintained as a control. **Sample collection and storage:** Samples of milk were collected at approximately 12-hour intervals after the administration of the first dose, throughout the dosing and up to one day after the final dose. All milk samples were stored frozen. Urine and faeces were collected during the 12-hour period after administration of the third dose. The cow was sacrificed 23 hours after administration of the third dose and samples of liver, kidney, muscle, gall bladder and fat were collected for analysis. All tissue samples were stored frozen. It is recorded in the study report that the experimental start date was February 13th 1991 and the experimental termination date was August 9th 1991. Further details on storage conditions including storage time until analysis and storage temperature were not included in the study report.	Radioactive residues in tissues: from 0.004 to 0.32 mg benfluralin equivalents/kg (highest level occurred in liver). Radioactive residues in milk: from 0.003 to 0.006 mg benfluralin equivalents/kg. Maximum residue concentration of 0.006 mg benfluralin equivalents/kg was reached approximately 2 days after initiation of dosing.	Author (1991) Report No.: Dow AgroSciences, unpublished report No. T2A FMBOVI AM 3-70 Dow Chemical Company Study ID: MET91002

Test substance, dose levels duration of exposure	Guideline, Material and Methods	Results	Reference
	Extraction: Milk samples were mixed with acetone to precipitate protein, which was separated by filtration. Acetone was removed from the filtrate under vacuum and the remaining aqueous fraction was extracted with ethyl acetate. The ethyl acetate was removed under vacuum and the oily residue remaining was re-dissolved in hexane and partitioned with acetonitrile. Ground fat tissue was dissolved in hexane and the solution (minus connective tissue and water) was partitioned with acetonitrile. Finely ground kidney and liver were separately refluxed with methanol/water (7:3), centrifuged and the pellet re-suspended in methanol. After filtration and removal of the solvent, the aqueous solution was partitioned with ethyl acetate (ethyl acetate-1), made acid and partitioned with ethyl acetate (ethyl acetate-2). The remaining aqueous phase (aqueous-1) was incubated overnight at 37ºC with a β-glucuronidase solution with aryl sulphatase activity, then acidified and partitioned with ethyl acetate (ethyl acetate-3). The aqueous phase was made 1.0M with respect to hydrochloric acid, hydrolysed by reflux and extracted with ethyl acetate (ethyl acetate-4). Extracted tissue was refluxed with 1.0M hydrochloric acid, centrifuged and the supernatant partitioned with ethyl acetate at pH 2 (ethyl acetate-5) and pH 7 (ethyl acetate-6). Extracted tissue was re-suspended in methanol, centrifuged and re-suspended in water. Solvent was removed from the methanol solution under vacuum and residual methanol was removed from the aqueous tissue suspension under nitrogen. The tissue suspension was neutralised and incubated with protease overnight at 37˚C, partitioned with ethyl acetate at neutral pH (ethyl acetate-7) and then at pH 2 (ethyl acetate-8). Urine was mixed with water, neutralised with hydrochloric acid and partitioned with ethyl acetate (ethyl acetate-1) then re-partitioned at pH 2 (ethyl acetate-2). The extracted aqueous solution was hydrolysed and incubated overnight at 37˚C with a β glucuronidase solution with aryl sulphatase activity, then acidified and partitioned with ethyl acetate (ethyl acetate-3). **Method of analysis:** Radioactivity in urine, milk and bile was measured directly by LSC. Radioactivity in faeces and tissues was determined by radio combustion analysis and LSC. The extracts were assayed by TLC, silica gel column chromatography using a range of solvents and column chromatography using XAD 2 resin. **Limit of quantification:** Not stated **Method validation:** No information provided **Reference standards:** Reference compounds were synthesized at the laboratories		

Metabolism study in cow

The metabolism study in cow is the only study with data confirming the presence of benfluralin in milk. The study was submitted and evaluated in Annex B.7 Residue data of the RAR (not included in Annex 1), and the full assessment of the study is presented here.

Results

Total radioactive residues in tissues ranged from 0.004 to 0.32 mg benfluralin equivalents/kg, and the highest level occurred in liver. Radioactive residues in milk ranged from 0.003 to 0.006 mg benfluralin equivalents/kg and a maximum residue concentration of 0.006 mg benfluralin equivalents/kg was reached approximately 2 days after initiation of dosing (see table 46).

Combined extractions with different solvents and liquid/liquid partitioning with ethyl acetate and acetonitrile allowed the solubilisation of most of the radioactivity in all the tissues. No parent compound was recovered in any of the matrices.

In milk, 50% of the total radioactive residues (TRR) was extractable into ethyl acetate, 33.3% remained in the extracted aqueous and 16.7% in the protein precipitate. The acetonitrile fraction was shown to be multi-component using silica-gel column chromatography, with the largest two components containing up to 0.001 mg benfluralin equivalents/kg. Thin layer chromatography (TLC) of one of these fractions demonstrated the presence of multiple radioactive components, with none exceeding 5% (0.0003 mg/kg) of the TRR.

In fat, after extraction with hexane, 16.7% (0.001 mg/kg) of the TRR remained with the connective tissue and 50% (0.003 mg/kg) was partitioned into acetonitrile. Silica-gel column chromatography of the acetonitrile extract demonstrated it was multi-component with each of the eight components present consisting of 1.2 to 20.9% of the TRR. The levels of radioactive residues in muscle and fat were very low suggesting that there was no accumulation of residues in fatty tissues despite of the liposoluble character of the parent compound. Therefore, no further metabolites characterization in muscle and fat was performed.

In kidney, the radioactivity present in the ethyl acetate organosoluble phases did not chromatograph with any of the reference compounds. They were multi-component with individual metabolites occurring at concentrations not exceeding 0.002 mg/kg. Therefore, no further attempts for metabolite identification were made.

In liver, chromatographic analysis of the ethyl acetate organosoluble extracts and the aqueous solutions showed that they were multi components with individual metabolites occurring at concentrations not greater than 0.002 mg/kg and that the radioactive components did not co-chromatograph with any reference standards. The parent compound and the other non-polar metabolites were not present in liver, but the radioactive residues were also composed of numerous components.

Urine samples were collected as a potential source for metabolites characterization/identification. Chromatographic analysis of the ethyl acetate extracts showed that benfluralin and the related non-polar metabolites were not present in urine and further analysis in a more polar solvent mixture demonstrated the numerous compounds recovered in these extracts. Individual metabolites represented less than 4% of the TRR (0.08 mg/kg) in urine.

Table 55: Metabolites distribution of the residues of benfluralin in milk and tissues of lactating cow following administration of benfluralin at dietary concentration of 10 mg/kg - residues expressed in% of the TRR and in (mg benfluralin equivalents/kg)

	Muscle	**Fat**	**Kidney**	**Liver**	**Milk**	**Urine**
Total radioactive residues in% of TRR – *(mg benfluralin equivalent/kg)*						
	100 *(0.004)*	100 *(0.006)*	100 *(0.073)*	100 *(0.320)*	100 *(0.006)*[*]	100 *(2.004)*
Extractability of the total radioactive residues in% of TRR – *(mg benfluralin equivalent/kg)*						
Acetone extraction phase	Na	na	Na	Na	83.3 (*0.005*)	Na
Hexane extraction phase		np				
Methanol/water (70:30; v/v) extraction phase			68.5 (*0.050*)	37.8 (*0.121*)		
EtOAc organosoluble partitioned phase			32.9 (*0.024*)	14.4 (*0.046*)	50.0 (*0.003*)	10.57 (*0.212*)
Acetonitrile organosoluble partitioned phase		50 (*0.003*)			50.0 (*0.003*)	
Aqueous soluble partitioned phase		33.3 (*0.002*)	35.6 (*0.026*)	23.4 (*0.075*)	33.3 (*0.*002)	89.4 (*1.792*)
Unextracted radioactive residues		16.7 (*0.001*)	31.5 (*0.023*)	62.2 (*0.199*)	16.7 (*0.001*)	
HCl extracted phase by reflux		na	12.3 (*0.009*)	20.3 (*0.065*)	na	Na
EtOAc organosoluble partitioned phase			1.4 (*0.001*)	3.4 (*0.011*)		
Aqueous soluble partitioned phase			11.0 (*0.008*)	16.9 (*0.054*)		
Residual radioactive residues			19.2 (*0.014*)	41.6 (*0.133*)		
Methanol extraction phase			9.6 (*0.007*)	20.0 (*0.064*)		
Post extracted residues (bound residues)			8.2 (*0.006*)	21.6 (*0.069*)		
EtOAc organosoluble partitioned phase				0.3 (*0.001*)		
Aqueous soluble partitioned phase				21.6 (*0.069*)		
Accountability (extracted phases + unextracted radioactive residues)		100 (*0.006*)	100 (*0.073*)	100 (*0.320*)	100 (*0.006*)	100 *(2.004)*

[*] The highest residue level of 0.006 mg benfluralin equivalents/kg was reached approximately 2 days after initiation of dosing.
na: not applicable
np: not provided
Remark: No extraction step and no metabolites characterization were performed in muscle due to the very low level of radioactivity recovered in the matrix.

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

Two-generation study

During gestation and lactation in the rat study, some F_0 animals showed alopecia (mainly on paws and limbs) at the top dose. Further during cage side observations, some pups had a weak appearance, and there was no visible milk in the stomach at 1000 ppm (F_0) and above ($F_{0/1}$), and some top dose pups felt cold to touch or showed a pale appearance (please refer to section 10.10.5). Cyanosis or partially cannibalisation was also observed in isolation at the top-dose (F_1). It was unclear whether the dose-dependent increased incidence of pups with empty stomachs was due to a possible transfer of the substance into the mother's milk, with either (i) milk avoidance subsequent to unpalatability or other toxic effects, or (ii) inability to feed the pups due to the bad condition of the dams. As body weight decreases and pup mortality was already increased in the first days of lactation (up to d4, where no autonomic feeding of the pups was expected), it was not excluded that the substance was transferred via the milk. As body weights further decreased time-dependently from d4 on up to d21, the contribution via normal feeding was probable and the effects in the pups may be explained by

the impaired maternal health status (although maternal toxicity was less marked and less consistent at 1000 ppm).

Gross pathology revealed few cases at the top-dose with gaseous distention of the GIT, distended urinary bladders and pale kidneys in either F_0 or F_1. In addition, the extended necropsy on 10 pups/sex revealed the accumulation of test substance in the abdominal fat in 7/20 top-dose animals (only examined in the F_2-pups), appearing as yellow abdominal fat. The accumulation of benfluralin in the adipose tissue is also confirmed by the toxicokinetic data available on distribution (please refer to section 9), and it should be noted that tissue residues of benfluralin were mostly higher in females than in the males.

The potential transfer via the mother milk could not be confirmed or infirmed as no data concerning the presence of substance in the milk were present in the two-generation rat study.

Metabolism study in cow

According to the current guidance for metabolism in livestock (OECD Guideline 503), ruminants should be dosed daily for at least five days instead of three days as in this study. Whether this deviation would affect the results of the study is unknown. In fact, the parent compound constituted 34% (0.090 mg/kg) of the TRR in skin/fat and 4% (0.09 mg/kg) of the TRR in eggs following administration of benfluralin for 10 days at dietary concentration of 15 mg/kg in a poultry metabolism study. This may indicate that accumulation of benfluralin in fat and milk may occur if a cow is dosed for a longer period. It should be noted that radioactive residues in fat was 0.006 mg benfluralin equivalents/kg, the same amount as the maximum residue concentration in milk (table 46), suggesting that the substance has the same affinity for fat and milk.

In the study report, it is stated that *the cow produced a mean of 16.9 kg of milk per day during the collection period*. Since the cow produced a mean of 17.4 kg of milk per day during the last four days of the acclimation period, the milk production seems to be unaffected by the treatment. However, data on feed consumption, body weight and animal health throughout the study are not available.

The low levels of the total radioactive residues in tissues and milk indicated that the parent compound was poorly absorbed by the ruminants.

No parent compound was recovered in any of the matrices suggesting that benfluralin was rapidly and extensively metabolised. Due to the low radioactive residue levels in the tissues and the numerous compounds constituting the total radioactive residues, no further metabolites characterization was attempted. No metabolic pathway of benfluralin in lactating ruminants has therefore been depicted.

The study is not in compliance with the current test guidelines and reliable residue definitions in animal matrices cannot be derived, but the study shows that benfluralin is present in milk.

10.10.9 Comparison with the CLP criteria

According to the CLP criteria, classification for effects on or via lactation are assigned if there is human evidence that indicates a hazard to babies during lactation and/or clear evidence from animals that a substance causes adverse effects in offspring because of transfer in the milk or adverse effects on the quality of the milk and/or ADME studies indicate that the substance is present in the milk at potentially toxic concentrations. No human evidence is available for benfluralin, but one two-generation study in rats and one metabolism study in cow are available.

In the two-generation reproductive toxicity study there was a dose-dependent increased incidence of pups with empty stomachs. From d4-d21 the decreased body weight could be attributable to maternal toxicity (but maternal toxicity was less marked and less consistent at 1000 ppm). It cannot be excluded, however, that the substance was transferred via the milk since body weight decreases and pup mortality was already increased in the first days of lactation before the pups started eating the pelleted feed.

In the metabolism study in cow, benfluralin or its metabolites was detected in the milk. The data from the study shows that the same amount of benfluralin is present in body fat and milk and it was stated that accumulation may occur in fat and the milk if the cow had been dosed for a longer period than three days. This assumption

is supported by the provided toxicokinetic information (ADME studies), that benfluralin has affinity towards the adipose tisse, and accumulation of the substance in the adipose tissue was also evident by the yellow/orange/dark coloured abdominal cavity in F_1 generation and in abdominal fat in F_2 pups at the top dose in the two-generation study. In addition, the log Pow of benfluralin is greater than the trigger value of 3 (log Pow = 5.3). Thus, the available data indicate that benfluralin could be present in potentially toxic levels in milk. Consequently it cannot be exluded that the empty stomach and the decreased body weight in pups in the two-generation study were due to transfer of the substance via the milk.

Classification for effects on or via lactation is therefore proposed.

10.10.10 Conclusion on classification and labelling for reproductive toxicity

Classifications for adverse effects on development of the offspring 'Rep. 2'(H361d) and adverse effects on or via lactation 'Lact.' (H362) are considered appropriate

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

Adverse effects on sexual function and fertility

The DS described two studies in its evaluation of benfluralin for effects on sexual function and fertility. The first study was an old (Anonymous, 1973), non-guideline, non-GLP-compliant generation study that was included for supportive information only. However, the study was considered unacceptable by the DS for regulatory purposes and is not considered further in this opinion.

The DS based its evaluation on a 2-generation reproductive toxicity study in SD rats (GLP-compliant, based on OECD TG 416) from 1995. The DS noted that several critical reproductive endpoints were missing (table 40, CLH report) and this made a reliable assessment of reproductive toxicity with regard to classification and labelling difficult. Developmental landmarks were not assessed.

The MTD was exceeded, clear maternal toxicity was evident in both generations from significant reductions in food consumption (-10-34% relative to controls), body weight (-10-22%) and body weight gain (-4-54%) at the top dose. There were two unscheduled female deaths in the F0 high dose group and 1 in the F1 high dose group. Liver weights were significantly and grossly increased at the top dose (absolute weight: ↑40%/↑39% for F0/F1; relative weight: ↑63%/↑76% for F0/F1). There was also an increase in the incidence of animals observed with CPN in the top dose group (17/29 for F0/F1 compared with controls 2/1 for F0/F1).

The calculated intake of benfluralin (purity 95.8% [EBNA] = 0.04 mg/kg) was poorly described. A range of values were presented for each phase of the study. The most notable effects occurred in the 1000 ppm and 5000 ppm dose groups.

***Table**: F0 intake of benfluralin in mg/kg bw/d throughout the study*

Females				
Dose (ppm)		**100**	**1000**	**5000**
Study Phase:	Premating:	11-7	97-70	502-334
	Gestation:	7-6	74-65	339-311
	Lactation:	9-20	91-198	394-854

F1 intakes were similar.

The most severe reproductive effects were seen at the top dose level:

- The number of females with ≥ 23-day gestation period increased substantially at the top dose in both generations [6/28 and 7/28 in F0 and F1, respectively].
- Significant reductions were observed on pup survival following birth and weaning for the top dose group in both generations with an aggravated effect for the second generation [PND0-28 pup loss, not culled: 58/75 in F0/F1, respectively vs. 13/20 observed in the control group].
- Failure to thrive: pup bw (covariate adjusted mean) was significantly and highly impacted from LD4 to LD21 in the top dose group in both generations [↓29-41% / ↓27-40% for F0/F1 pups, respectively].

The overall gestation index was unaffected. Information on implantation sites (useful in interpreting the reduced litter size and the lower number of liveborn pups) was missing, as was the timing of pubertal landmarks. It is not known if the reduced survival of the pups was caused by effects on the pups *in utero*, effects via the milk, or effects on the rearing of the pups.

The DS did not propose classification for adverse effects on sexual function and fertility.

Developmental toxicity

The DS described two studies in its evaluation of benfluralin for effects on development.

Rat developmental toxicity

In a GLP and guideline-compliant (US EPA 40 CFR 158) study from 1985, 25 mated female SD rats/dose group received benfluralin (purity 97.3%, [EBNA] = 0.09 mg/kg) dissolved in a 10% w/v acacia oil by gavage, at dose levels of 0, 50, 225, 475 and 1000 mg/kg bw/d, from gestation day (GD)6 through to GD15. There was no maternal mortality. Clinical signs were minor.

There was no foetal mortality, and the number of corpora lutea and implantations was unaffected by the treatment. The number of resorptions was not increased by treatment, and foetal weights were unaltered.

Treatment-related findings included a slightly increased incidence of vertebral anomalies or ossification delays, and unossified sternebrae at the highest dose. The centra abnormalities of the vertebrae and ossification delays were considered in the context of reversible growth retardation. There was no evidence of an increase in the incidence of malformations.

Rabbit developmental toxicity

In a GLP and guideline-compliant (US EPA 40 CFR 158) study from 1991, 20 artificially inseminated rabbits/dose group (NZW) received benfluralin (purity 97.3%, [EBNA] = 0.31 mg/kg) dissolved in 10% w/v aqueous acacia oil by gavage at dose levels of 0, 25, 50, 100 or 225 mg/kg bw/d from GD6 to GD19 inclusive.

With 3 abortions (dams were visibly anorexic) and one maternal death (intubation error), the number of viable litters was decreased at the top dose. Other parameters were unaffected by treatment. Maternal toxicity was limited to isolated effects, reduced food consumption and reduced body weight gain.

There were no treatment related increases in malformations. Various skeletal variations and rib anomalies were present but did not always follow a dose response. The only possible meaningful change was a dose-dependent increased incidence of skull variations (accessory bones in the suture line).

The DS concluded that benfluralin did not give rise to adverse developmental effects in rabbits.

Effects on or via lactation

The DS described a metabolism study with a single Holstein dairy cow. The cow was administered gelatine capsules containing [U-phenyl-14C] and unlabelled benfluralin (both dissolved in dichloromethane) at a nominal dose level of 10 mg/kg bw for 3 days.

This was the only evidence available with data confirming the presence of benfluralin residues in milk. Milk production was unaffected by the treatment. Low levels of total radioactive residues in tissues and milk indicated that the parent compound was poorly absorbed by the ruminant. This is in agreement with metabolism studies with rats. No parent compound was recovered in any of the matrices, suggesting that benfluralin was rapidly and extensively metabolised. RAC considers this study of limited use since there is no indication that parent benfluralin is available to neonates via milk. Instead, various residues at low levels may be available but there is no data with respect to their identification or toxicity.

The DS proposed classification for effects on or via lactation citing the effects seen in the rat 2-generation study on pup survival and body weight in the first days of lactation before the pups started eating the pelleted feed.

Conclusion

The DS proposed classification of Repr. 2 for development and classification for effects on or via lactation. The DS relied on results from the rat 2-generation study which included decreased body weight and survival of offspring (weaning index) during the lactation phase, a decrease in litter size and the increased incidence of vertebral centra change (reduced ossification) in foetuses of the developmental toxicity study in rats.

Comments received during consultation

In the response to comments document following the consultation of the CLH report, there were two comments provided from Industry supporting no classification, and one from an MSCA supporting the DS with classification for effects on or via lactation only.

Industry provided a position paper in which they explained that the effects were due to a combination of excessive maternal toxicity and direct toxicity of the substance toward juveniles.

The MSCA did not support classification for development and disagreed with the DS point of view that the effects should be considered as effects on development rather than fertility. The MSCA outlined its support for classification for effects on or via lactation.

Assessment and comparison with the classification criteria

There are no relevant data on adverse effect on development in humans; hence, classification in Category 1A is not proposed.

Classification in Category 1B is largely based on data from animal studies. According to the CLP criteria, such data shall provide clear evidence on fertility/development in the absence of other toxic effects. The criteria for Category 1B are not fulfilled; the study data for benfluralin suggests there may be reproductive effects but the studies either do not show any evidence (as is the case for the rat and rabbit developmental studies) or lack the detail and robustness (2-generation study).

Adverse effects on sexual function and fertility

The main effects of concern from the rat 2-generation study and pubertal developmental studies were:

1. ↑ frequency of dams with an elongation of the gestation period (≥ 23-day) in the top dose group over 2 generations [6/28 and 7/28 for the F0 and F1 dams, respectively, compared with controls; 1/26 and 1/24 for the F0 and F1 dams, respectively].

2. Neonatal survival was adversely affected (table 'Pup loss following birth for both the F0 and F1 parents' below). Significant reductions were observed on pup survival following birth and weaning for the top dose group in both generations → support classification for developmental toxicity according to the CLP Regulation.

3. Failure to thrive: pup bw (table 'Pup bw reductions […]' below) was significantly and grossly impacted from Lactation day (LD)4 to LD21 in the top dose group in both generations → support classification for developmental toxicity according to the CLP Regulation.

4. Mean age at vaginal opening was significantly increased (↑2.9 days at 300 mg/kg bw/d) → Pubertal development study (Anonymous, 2012; RAR B.6.8.3.4).

5. Serum testosterone was significantly decreased (↓69% at 400 mg/kg bw/d) relative to control testosterone levels → Pubertal development study (Anonymous, 2012; RAR table B.6.8.3.4-10).

Only two of the main effects (pup survival and pup body weight), support classification according to the CLP Regulation, Annex I, 3.7.1.4, Adverse effects on development of the offspring; these are detailed under *7.3 Development*.

The remaining effects were not considered sufficient to support classification for sexual function and fertility. RAC considers that no classification is warranted for adverse effects on sexual function and fertility.

Adverse effects on or via lactation

No data are available for the concentration of benfluralin and/or its metabolites in milk. ADME data showed poor oral absorption and a wide distribution of benfluralin including fat suggesting that transfer to milk is plausible. Nevertheless, the reduced mean weight and the mortality in post-weaning (PND 22-28) pups demonstrates that direct toxicity to pups from the diet during weaning is also plausible along with the toxicity of benfluralin or its residues in milk. The lethality among these offspring may thus be a combination of events involving maternal toxicity impacting on nurturing behaviour, exposure to benfluralin and/or residues in the mother's milk and a potential consequence of the food intake to body weight ratio and poor xenobiotic clearance mechanisms increasing systemic exposure in the immature juvenile. Cage-side observations showed an increase in effects at the top dose: weak appearance, pups felt cold to the touch or showed a pale appearance in addition, a lack of milk in the stomach suggesting inadequate nursing. RAC considers that no classification is warranted for effects on or via lactation.

Development

In both rat and rabbit developmental studies, no treatment-related malformation was induced, and benfluralin was not considered teratogenic. The increased incidence of reduced ossification sites in the vertebrae (rat) or accessory skull bones (rabbit) were not considered adverse, and the developmental NOAELs were established at the top-dose. In the rat, foetal body weight or litter parameters were not impaired. Maternal toxicity (decreased body weight change) occurred at 475 mg/kg bw/d and above. In the rabbit, the maternal toxicity was established at 50 mg/kg bw/d, based upon decreased food consumption and a concomitant decreased body weight gain at the next dose. Litter parameters or foetal weight were not affected.

It is noteworthy that the prenatal development study in rats which had less maternal toxicity after dosing up to 1000 mg/kg bw/d (approximately 7-fold higher than the 2-generational study) had no effect on embryo-foetal survival at any dose level (which would translate postnatally to smaller litter sizes). This lack of consistency in effect across studies decreases the concern for a direct effect on the maintenance of the pregnancy but does not provide an explanation for the changes in post-natal survival or subsequent growth retardation.

RAC considers the 2-generation rat study to be the key study for reproductive classification purposes. Though several deficiencies were noted, very clear effects on the retardation of pup growth (table 'Pup bw reductions […]' below) and increased pup loss (table 'Pup loss following birth for both the F0 and F1 parents' below) were observed.

With reference to ***CLP Regulation, Annex I, 3.7.1.4.*** *Adverse effects on development of the offspring:*

"Developmental toxicity… any effect which interferes with normal development... either before or after birth… resulting from exposure… of the developing offspring during prenatal development, or ***postnatally, to the time of sexual maturation****.* [Furthermore]*… These effects can be manifested at any point in the life span of the organism. The major manifestations of developmental toxicity include* ***(1) death of the developing organism,*** *(2) structural abnormality,* ***(3) altered growth****, and (4) functional deficiency."*

The CLP Regulation states that any death/growth retardation following birth up to sexual maturation is a development effect.

***Table**: Pup bw reductions relative to controls following birth for both the F0 and F1 parents. Comparison of raw mean data*

Mean dose (mg/kg bw/d)		0	15.4 / 15.3	149 / 156	651 / 664
PND F0/F1:	0	0 / 0		-- / --	↓5% / --
	4 (pre cull)	0 / 0		↓11% / ↓3%	↓24% / ↓22%
	7	0 / 0	*no treatment effect	↓12% / ↓6%	↓37% / ↓34%
	14	0 / 0		↓11% / ↓10%	↓39% / ↓42%
	21	0 / 0		↓8% / ↓10%	↓40% / ↓42%

Males and females combined. – no change or no reduction in bw
*Mean pup weights were similar or slightly greater than controls for the 15 mg/kg bw/d group, no treatment effect.

Benfluralin may be considered a reproductive toxicant. The decreased pup weights and increased post-natal mortality at >650 mg/kg bw/d (5000 ppm) were treatment-related, and maybe regarded as a failure or reduction in reproductive performance or fecundity for the rat population because of retarded growth and decreased neonatal survival over 2 generations. The reductions in body weight occurred throughout the lactation period up to PND 21 (table 'Pup bw reductions […]' below).

Benfluralin treatment resulted in a statistically significant increase in age at vaginal opening (+2.9 days) and age at first oestrus (37.2 days vs 35.4 days in controls) together with decreased weights in oestrogen-sensitive tissues (ovaries, ↓18% and blotted uterus, ↓21%). The decrease in uterine weights was not due to differences in stages of oestrous at termination.

Age and body weight at preputial separation were not affected by benfluralin treatment. Serum testosterone was significantly decreased (↓69% at 400 mg/kg bw/d) relative to control testosterone levels. However, this was secondary to enhanced liver metabolism resulting in enhanced clearance (Anonymous, 2012; Hershberger assay, ^{14}C-testosterone clearance study, RAR Table B.6.8.3.3-10). There were no effects on male or female reproductive indices in the 2-generation rat study.

***Table**: Pup loss following birth for both the F0 and F1 parents*

Mean dose (mg/kg bw/d)		0	15.4 / 15.3	149 / 156	651 / 664
PND F0/F1	0-4	10 / 16	7 / 2	21 / 8	26 / 28
	5-21	3 / 4	1 / 1	9 / 0	13 / 45
------		------	------	------	------
	22-28	0 / 0	2 / 0	0 / 0	19 / 2

Pup survival was adversely affected throughout the lactation period and beyond (table above). However, RAC notes that the effects in the pups were compounded by significant maternal toxicity (decreased body weight, liver and kidney toxicity). Lower pup body weights and survival are well recognised as a common consequence of poor maternal nurturing and care as a secondary consequence of excessive maternal toxicity. It has already been demonstrated by the DS that the body weights and food consumption of dams at the top dose was severely impacted by treatment, constituting significant maternal toxicity. Clinical signs for the dams, however, were sparce, nothing of note was documented during lactation and mating behaviour was unaffected by benfluralin treatment. It is not possible to determine the extent to which

maternal toxicity contributed to the effects in the offspring. RAC concluded that the criteria for classification as developmental toxicant are fulfilled.

Conclusion

RAC considers the 2-generation rat study to be the key study for reproductive classification purposes. Though several deficiencies were noted, very clear effects on the retardation of pup growth and increased pup loss were observed. In addition, several other effects with the potential to impact on fertility were also noted; these included a significant delay in vaginal opening, significant reductions in serum testosterone (albeit as a secondary effect to liver enzyme induction), and an increase in the frequency of dams with an elongation of the gestation period. In conclusion, RAC considers that **classification is warranted for developmental toxicity, as Repr. 2; H361d**.

10.11 Specific target organ toxicity-single exposure

Table 56: Summary table of animal studies on STOT SE

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results**	**Reference**
OECD TG 403 Deviations: 2 Rat Fischer 344 M, F 5/dose level	Benfluralin (purity 97.3%), 4hr, dust, nose-only M/F: 1.12 mg/l air, 2.16 mg/l air (technical highest attainable concentration)	LC_{50} M/F: >2.16 mg/L air (technical highest attainable concentration) MMAD: 25.88±4.06 µm and 23.72±3.59 µm Clinical signs, high dose: dyspnea, hypoactivity, poor grooming and body weight loss (recovery by Day 6) Death (necroscopy), high dose: hepatic and pulmonary congestion	Author (1986) Reoport No.R-H-048-85, CA 5.2.3/01

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

One inhalation study was performed in rats. The complete evaluation of the study is given in the RAR (section B.6.2.3). Groups of ten male and ten female fasted Fischer 344 rats were exposed nose-only for a single four hour period to solid test atmospheres of technical benefin (benfluralin) at target concentrations of 1.12 or 2.16 mg/l air (technical highest attainable concentration). The Mass Median Aerodynamic diameter was 25.88±4.06 µm and 23.72±3.59 µm. No concomitant control group was included. Clinical signs of reaction to treatment, behaviour, bodyweight changes and mortality were recorded for evaluation of toxicity during exposure and during a subsequent 14 day period of observation. Bodyweights were recorded on days –1, 0, 1, 3, 5, 7 and 14. The animals were killed on day 15 and subjected to necropsy, which included examination of external body orifices, general bodily condition and organs/tissues in the thoracic and abdominal cavities.

Two males and one female exposed to 2.16 mg/L died during the exposure. Necropsy revealed hepatic and pulmonary congestion. The remaining high dose and all low dose animals survived treatment. Clinical signs

in the high dose group included dyspnea, hypoactivity, poor grooming and body weight loss with recovery apparent by Day 6. In the low dose group, poor grooming and weight loss were also noted but the animals had recovered by Day 4. Body weight was decreased in animals exposed to 1.12 mg/L (d3) and to 2.16 mg/L (d1, 3 and 5). No abnormalities were evident during necropsy for any of the survivors.

The LC_{50} (4 hr, aerosol) for male and female rats was >2.16 mg/L air, which was the highest technically attainable concentration. According to OECD TG 403 (adopted 7 September 2009), testing at concentratrations greater than 2 mg/L should only be attempted if a respirable particle size can be achieved. If the MMAD significantly exceeds 4 µm, further efforts should be employed to reduce the test article's MMAD. The notifier brought under attention the technical difficulty to generate an inhalable dust fraction from the wet cake. According to CLP Regulation EC 1272/2008, the limit concentration for aerosols is 5 mg/L. However, considering the above, including the difficulty in generating a respirable fraction, classification for acute inhalation toxicity is not required for benfluralin.

The study was considered acceptable with the following deviations:

- The high number of animals used (10 instead of 5 animals per dose)
- The mass median equivalent aerodynamic diameter which was higher than 4µm (>20µm).

It should further be clarified that in the report of the inhalation study, the aerosol distribution was bimodal. This implicates that although the mean particle size was relatively large, approximately half of the particle mass was found in the smaller size portion of the distribution, with a modal diameter of about 6-7 µm. Pulmonary congestion can be associated with inflammation as well as heart failure. To what extent inhalation of benfluralin caused an inflammatory response, and to what extend this was caused by larger particles deposited in the upper parts of the lungs, or particles of smaller size reaching the deeper parts of the respiratory system, is uncertain, based on the reported findings. The report from the study does not contain any information about water solubility of the particles.

Considering the hepatic and pulmonary congestion observed in two males and one female which died during exposure to 2.16 mg benfluralin/L air, classification as STOT-SE 2 is proposed.

10.11.2 Comparison with the CLP criteria

According to the CLP criteria, classification for STOT-SE is appropriate when it has been demonstrated from human or animal data that specific non-lethal target organ toxicity arises from a single exposure to a substance. Category 1 and 2 cover non lethal "significant and/or severe toxic effects", and they reflect the dose level required to cause the effect. Category 3 covers "transient effects" occurring after a single exposure, specifically respiratory tract irritation (RTI) and narcotic effects (NE) for which a substance does not meet the criteria to be classified in Categories 1 or 2.

The acute rat inhalation study relates to a 4-hour experimental exposure period and a LC_{50} value of > 2.16 mg/L. At the high dose, necropsy revealed hepatic and pulmonary congestion in two males and one female which died during the exposure. However, as stated above, no information regarding the bioavailability of the dust particles was available. It is not certain whether inhalation of benfluralin caused inflammatory response, whether the larger particles was deposited in the lungs or whether the smaller particles reached the deeper parts of the respiratory system.

According to the CLP classification criteria for STOT-SE, the guidance value ranges for single-dose exposures classifies substancens in Category 1 or Category 2. For inhalation route (dust) the guidance values are $5{,}0 \geq C > 1{,}0$ for Category 2, which is in accordance with exposure to 2.16 mg benfluralin/L air.

Considering the hepatic and pulmonary congestion observed in two males and one female which died during exposure to 2.16 mg benfluralin/L air and the clinical signs of the survivors (dyspnea, hypoactivity, poor grooming and body weight loss with recovery apparent by Day 6), classification as STOT-SE 2 is proposed.

For Category 3 there are currently no validated animal tests that deal specifically with respiratory tract irritation, but animal studies may provide useful information in terms of clinical signs of toxicity (dyspnoea, rhinitis etc) and histopathology (e.g. hyperemia, edema, minimal inflammation, thickened mucous layer) which are reversible and may be reflective of the characteristic clinical symptoms described above. In the

available study, no histopathological changes were noted for any of the survivors, and the changes revealed in the animals that died during exposure were not of a transient nature. Though some clinical signs were evident, the available data do not show clear evidence for RTI nor NE as no narcotic effects were evident. Hence no classification for STOT-SE 3 according to CLP regulation is proposed.

10.11.3 Conclusion on classification and labelling for STOT SE

Classification as 'STOT-SE 2' (H371) is considered appropriate.

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

Summary of the Dossier Submitter's proposal

The DS considered the acute rat inhalation study (Anonymous, 1985) in the context of proposing classification for STOT SE. Groups of ten male and ten female fasted Fischer 344 rats were exposed nose-only for a single 4-hour period to solid test atmospheres of technical benfluralin at target concentrations of 1.12 or 2.16 mg/L air. Two males and one female exposed to 2.16 mg/L died during the exposure on day 1. Necropsy revealed hepatic and pulmonary congestion in these three animals. The remaining high dose and all low dose animals survived treatment. Clinical signs in the high dose group included dyspnoea, hypoactivity, poor grooming and body weight loss with recovery apparent by day 6. There were no abnormalities evident in the surviving animals at necropsy following scheduled termination at the end of the study.

The DS proposed classification as STOT SE 2 based on:

1. Hepatic and pulmonary congestion observed in two males and one female which died during exposure to 2.16 mg/L; this exposure was compared with the guidance values (GV) for category 2 via the inhalation route for dusts (GV: 5.0 ≥ C > 1.0 mg/L).
2. Clinical signs of the survivors (dyspnoea, hypoactivity, poor grooming and body weight loss with recovery apparent by day 6).

Comments received during consultation

Two comments were received: one from a MSCA and the other from Industry. In both cases there was no support for the DS proposal for STOT SE based on hepatic and pulmonary congestion observed in two males and one female which died during exposure in the high dose group.

The MSCA noted that congestion of the lungs or the liver was the most remarkable pathological finding in the 3 deceased animals of the top dose group. The MSCA expressed doubts over the approach taken by the DS and concluded with no support for STOT SE. They based this on several points:

1. STOT SE is intended to cover non-lethal target organ toxicity. What is not known is if the hepatic and pulmonary congestion had directly contributed to death.
2. Common transient effects in the high dose group were also used to support the classification proposal. This was not supported by the MSCA. These findings, generic in nature, do not represent specific target organ toxicity.
3. The high MMAD raises serious doubts as to whether a respirable fraction could be achieved; this makes the study unsuitable for classification purposes.

Industry's position was that the highlighted findings were considered a post-mortem artefact. The animals were not autopsied immediately after death. The animals were found dead following the inhalation exposure period (4h). Following death, the lungs and liver are vascular soft tissues particularly prone to stagnation of blood and localised pooling. With the cessation of blood flow, *in situ* clotting is eventually observed. Under normal procedures of necropsy, immediately following sacrifice, there is no time for blood to pool in tissues because the animal is immediately exsanguinated. In the case of animals found dead, blood that has clotted *in situ* in the lungs and liver is generally responsible for acute passive congestion. According to Industry, these observations were concluded to be due to the sequence of post-mortem changes naturally occurring following sudden death and should therefore not be interpreted as being a consequence of a pathological condition in the absence of other supporting data.

Assessment and comparison with the classification criteria

STOT SE 2 was proposed by the DS based on two points:

1. Three deaths (3/20; 2/10 males and 1/10 females) in the acute inhalation study in rats with congestion of the lungs or the liver being the most remarkable pathological finding.
2. Reversible clinical signs in survivors were used to further support the proposal.

STOT SE is intended to cover non-lethal target organ effects and it should be considered where there is clear evidence of toxicity to a specific organ. In the present case, the DS proposed classification of benfluralin based on a presumed impairment of liver and lungs which led to lethality. However, this interpretation can be disputed.

True congestion of the liver parenchyma is a frequent finding in chronic injury of the organ such as the case with cirrhosis. Other features would accompany the finding of congestion if it had a true pathological origin, such as atrophy of centrilobular hepatocytes and notable necrosis or fibrosis in the case of chronic conditions. In the present case, the time frame is too short for such an advanced pathological feature to be expressed. Acute passive congestion of the liver is a very common finding at autopsy in humans caused by the presence of right-sided heart failure before death causing retrograde venous congestion of the liver.

Similarly, true congestion of the lungs is a frequent finding in humans suffering from congestive heart failure with other features such as intra-alveolar haemosiderin deposition. Again, the time frames here are indicative of an underlying pathology (e.g. it takes about 2 days for haemoglobin to be converted to haemosiderin). No such features were reported in the present study. As in the case of the liver findings, acute passive congestion of the lungs seems most likely due to the sequence of post-mortem changes naturally occurring following sudden death.

The original study report was brief and did not include any in-depth discussion of the necropsy findings. There was no information regarding the basis for the morphological changes observed

in the liver and lungs of the three animals found dead following exposure. No comment can be made regarding cytotoxicity, inflammation or any other pathology.

Suggesting that the congestive changes observed in the lungs and the liver was the cause of death for the two males and one female in the top dose group is speculative without evidence of an underlying pathology due to treatment. The transient clinical signs observed in surviving rats were generic in nature and not indicative of any type of single organ toxicity.

In addition, it could be argued that STOT SE classification for narcosis (i.e. category 3) may be warranted on the basis of specific transient effects. Hypoactivity was observed in the acute studies and could potentially indicate narcosis. On the other hand, it is commonly accepted as a generic clinical sign of toxicity and without further evidence of more severe effects that impact on the overall function of an organism, such as a state of deep stupor, unconsciousness or anaesthesia, or lethargy, lack of coordination righting reflex and ataxia, hypoactivity alone is not sufficient to indicate a state of narcosis. It is reasonable to assume that if narcosis was truly present then it would have been described as such on the basis of strong evidence (i.e. accompanied by some of the signs indicated above), in the original study report. No such description was reported.

RAC does not consider the evidence indicative of an effect that supports classification with STOT SE. Hence, **classification for STOT SE is not considered to be warranted**.

10.12 Specific target organ toxicity-repeated exposure

Information regarding specific target organ toxicity of benfluralin is available for repeated oral and dermal exposure from several repeated-dose toxicity studies, conducted in rats (90-day), mice (90-day), dogs (90-day & 1-year), and two dermal studies in rabbits (21-day).

Table 57: Summary table of animal studies on STOT RE

Method, guideline, deviations (if any)	**Species, strain, sex, no/group**	**Test substance, route of exposure, dose levels, duration of exposure**	**CLP guideline value for classification (mg/kg bw/d)**	**Results**	**Author**
90-day rat oral (dietary) OECD 408 GLP Deviations: no data on sensory activity, grip strength & motor activity	Rat F344/Tac M/F Study R33989: 15 rats/sex/dose Study R33989: 15 rats/sex/dose Study R29990: 15 male rats/ /dose	Benfluralin (96.6%) Batch No.: 231EF4 Study R33989[1] 0, 250, 1100, 5000 ppm Study R44089[2] 0 and 7500 ppm Study R29990[3] 0, 50, 500 or	Cat 1 = 10 Cat 2 = 100	**No adverse effects** 250 ppm (17/20 mg/kg bw/day). **Mortality** There were no deaths in any of the three studies. **Clinical signs** Orange/yellow urine coloration, indicative of the excretion of the test article was observed at all dose levels, except the lowest dose tested (50 ppm). **Ophthalmology**	Author (1996) Report No. R33989, R44089 and R29990/ CA 5.3.2/02

Method, guideline, deviations (if any)	**Species, strain, sex, no/group**	**Test substance, route of exposure, dose levels, duration of exposure**	**CLP guideline value for classification (mg/kg bw/d)**	**Results**	**Author**
		5000 ppm		No treatment-related effects **<u>BW and FC</u>** BW minimally depressed at 7500 ppm (522/604 mg/kg bw/day). FC lower at 5000 ppm (341/395 mg/kg bw/day) during first week. **<u>Haematology</u>** Slight regenerative anaemia at ≥ 5000 ppm: ↓RBC, Hb, Hct, MCH and MCHC in M & F. **<u>Clinical chemistry</u>** Treatment relative changes at ≥ 5000 ppm: ↑BUN (1100 ppm in M), ↑total bilirubin, ↑albumin, ↑globulin (1100 ppm in F), ↑γ-GT ↑total protein (≥250 ppm in F), ↓ALP (1100 ppm) ↓ALT, ↑p-NAOD activity in M &/or F. At 7500 pm: ↓AST and ↑cholesterol in M & F. **<u>Urinalysis</u>** ↑ AST and LDH excretion (non-persistent effect) at ≥ 500 ppm (32 mg/kg bw/day) in M. **<u>Organ weights</u>** ↑ liver weight (>10%) at ≥1100 ppm (74/88 mg/kg bw/day) in M & F ↑ thyroid weights at 5000 ppm in M & F and at 7500 ppm in F. ↑kidney weight at ≤5000 ppm in F and at 7500 and 5000 ppm in M. **<u>Gross pathology</u>** Enlarged livers at 1100 ppm (74 mg/kg bw/day) in M and above in M & F. Yellowish discoloration of adipose tissue at 5000 ppm and above. **<u>Histopathology</u>** Minimal to moderate nephrosis (biletaral) from 250 ppm onwards, characterized by hyaline droplets in M and by a golden-brown pigment deposition in F. Minimal to slight centrilobular hepatocyte hypertrophy in M, minimal in F at 250 ppm to 1100 ppm onwards.	

Method, guideline, deviations (if any)	**Species, strain, sex, no/group**	**Test substance, route of exposure, dose levels, duration of exposure**	**CLP guideline value for classification (mg/kg bw/d)**	**Results**	**Author**
				Slight increase of hepatodiaphragmatic nodule incidence in F at 7500 ppm. Increased slight pigment deposit in the proximal convoluted tubule, with supporting evidence of increase in kidney weight in F at 1100 ppm (74 mg/kg bw/day). ↑incidence of hyaline droplets; positive for α2μ-globulin at 5000 ppm[3] (322 mg/kg bw/day) in M.	
90-day mice oral (dietary) OECD 408 Report and raw data were subject to a GLP-standard audit. Deviations: food consumption was not recorded; test article intake was estimated on default consumptions of 4 g/d (♂) and 3.9 g/d (♀). No data on sensory activity, grip strength & motor activity	Mouse B6C3F1/CrlBr, M/F 15 mice/sex/dose	Benfluralin (98,22%) Batch No.: X-35746 0, 100, 300, 1000, 3000, 10000 ppm	Cat 1 = 10 Cat 2 = 100	**<u>Mortality</u>** Two deaths considered non-attributable to treatment: One F on Day 63 at 1000 ppm (168.2 mg/kg bw/day). One M on Day 58 at 3000 ppm (420.8 mg/kg bw/day). **<u>Clinical signs</u>** Dose-related increase in chromaturia from slight to dark at 300-10000 ppm (40.3-1364.4 mg/kg bw/day). Behavioural changes (including controls) due to gang caging of the animals. BW and BWG unaffected. **<u>Haemotology</u>** ↓RBC, Hb and Hct slightly reduced at 10000 ppm (1364.4/1730.2 mg/kg bw/day). ↓MCV in F at 1000-10000 ppm (168.2-1730.2 mg/kg bw/day). **<u>Clinical chemistry</u>** ↑ALT slight increase (3/15 animals) at 10000 ppm (1364.4 mg/kg bw/day). ↑AP in M only 10000 ppm. ↑p-NAOD activity in F at 1000-10000 ppm (1682.2-1730.2 mg/kg bw/day) and in M at 10000 ppm. **<u>Urinalysis</u>** Not investigated. **<u>Organ weights</u>** ↑ liver weight at 3000-10000 ppm in M &	Author 1988a Report No. M00180/ CA 5.3.2/03

Method, guideline, deviations (if any)	**Species, strain, sex, no/group**	**Test substance, route of exposure, dose levels, duration of exposure**	**CLP guideline value for classification (mg/kg bw/d)**	**Results**	**Author**
				F. ↓Uterus+ovaries weight at 3000-10000 ppm in F. **Gross pathology** No changes reported. **Histopathology** Mild centrilobular hepatocyte hypertrophy was noted predominantly among M dosed at 1000 ppm and above, occasionally in F. Multifocal cell necrosis and nodular hyperplastic nodules observed in two M at 10000 ppm.	
90-day dog oral (capsules) OECD 409 GLP	Dog (Beagle purebred) M, F 4/sex/dose	Benfluralin (95.8%) Batch No.: ACD 13683 0, 5, 25, 125 mg/kg bw/day	Cat 1 = 10 Cat 2 = 100	**Mortality** There were no deaths. **Clinical signs** Food emesis at 5 mg/kg bw/day (F) and above (M & F). No differences in BW, BWG and FC. **Ophthalmology** There were no treatment-related ophthalmoscopic changes **Haemotology** RBC parameters were unaffected. Platelets marginally increased at 25 mg/kg bw/day and above (a non-significant increase, but a dose-response was present). **Clinical chemistry** ↑AP weakly increased in M at 125 mg/kg bw/day (indication of dose-related trend), slight increase in F at 5 and 25 mg/kg bw/day. **Urinalysis** There were no differences between treated and control groups. **Organ weights** ↑ liver weakly but significantly increased at 25 mg/kg bw/day and above in F. **Gross pathology** There were no findings attributable to treatment.	Author (1993) Report No. HWA 174-135/ CA5.3.2/05

Method, guideline, deviations (if any)	**Species, strain, sex, no/group**	**Test substance, route of exposure, dose levels, duration of exposure**	**CLP guideline value for classification (mg/kg bw/d)**	**Results**	**Author**
				Histopathology 125 mg/kg bw/day: ↑liver sinusoidal cell/spleen pigmentation at in M & F. ↑Centrilobular to diffuse hepatocellular hypertrophy in M. 25 mg/kg bw/day and above: ↑incidence of cell pigmentation in spleen in M & F.	
1-year dog oral (capsules) OECD 409 OECD 452 GLP Deviations: The list of organ weights did not include the epididymides, spleen and uterus	Dog (Beagle purebred) M, F 4/sex/dose	Benfluralin (95.8%) Batch No.: ACD 13683 0, 5, 25, 125 mg/kg bw/day	Cat 1 = 2.5 Cat 2 = 25	**Mortality** One F sacrificed on week 27 in the high dose group, 125 mg/kg bw/day (due to weak health condition). **Clinical signs** Thin appearance, warm to touch, hypoactivity, pale gums, emesis and lacrimation in high dose group only (125 mg/kg bw/day). **BW, BWG and FC** ↓BW in all treated F (no dose-response). ↓FC in F in the high dose group. BWG not affected at termination. **Ophthalmology** No treatment-related changes. **Haemotology** Weak modifications in RBC parameters (non-significant). Platelets marginally increased (dose-response, partly time-dependent) at 25 mg/kg bw/day. **Clinical chemistry** ↓ALT dose-dependent from 25 mg/kg onwards *125 mg/kg bw/day in F;* ↑AP and total cholesterol ↓ALT and glucose level (wk 13, 26) ↓albumin ↑glubolin (wk 13) ↓BUN and creatinin level (wk 26) **Urinalysis** There were no differences between treated and control groups.	Author (1995) Report No. CHV 174-143/ CA 5.5/04

Method, guideline, deviations (if any)	**Species, strain, sex, no/group**	**Test substance, route of exposure, dose levels, duration of exposure**	**CLP guideline value for classification (mg/kg bw/d)**	**Results**	**Author**
				Organ weights ↑liver weight at 125 mg/kg bw/day **Gross pathology** There were no findings attributable to treatment. **Histopathology** ↑liver sinusoidal cell pigmentation (golden-brown material) at 125 mg/kg bw day.	
21-days rabbit dermal OECD 410 GLP Deviations: Test article was not moistened No record of dermal changes or any indication of when dermal assessments were completed relative to time of bandage removal have been reported Recom-mended treatment of 10% of the test animals's body surface area, was able to be accomplishe d only in the mid and high	Rabbit (NZW) M, F 5/sex/dose	Benfluralin (97.3%) Batch No.: 231-EF4 0, 100, 325 or 1000 mg/kg bw/day	Cat 1 = 80 Cat 2 = 800	**Mortality** One male with pneumonia at the lowest dose (100 mg/kg bw/day) died on day 7 (histopathology revealed necrosis of the thymic lymphocytes, and slight centrilobular hepatocyte fatty vacuolation). **Clinical signs** Dose- and time-related increase of dermal irritation: *100 mg/kg bw/day*: slight progressing to severe erythema and oedema. *325 mg/kg bw/day*: moderate progressing to severe erythema and slight progressing to severe oedema *1000 mg/kg bw/day*: slight progressing to severe erythema and moderate progressing to severe oedema. **BW, BWG and FC** ↓BW in M throughout treatment at 325 mg/kg bw/day and above (not statistically significant). ↓FC in M at 1000 mg/kg bw/day (non-significant decrease at 325 mg/kg bw/day). **Ophthalmology** There were no treatment-related changes in any group. **Haemotology** ↑platelets/thrombocytes in M at 1000 mg/kg bw/day and in F at 325 mg/kg bw and above. ↑leukocyte number F at 325 mg/kg bw/day. Decrease of the lymphocyte and	Author (1986) Report No. B02185/ CA 5.3.3/01

Method, guideline, deviations (if any)	**Species, strain, sex, no/group**	**Test substance, route of exposure, dose levels, duration of exposure**	**CLP guideline value for classification (mg/kg bw/d)**	**Results**	**Author**
dose groups				concomitant increase in the neutrophil fraction (dose-dependent trend) in F. ↑dose-dependent basophil fraction in M. **Clinical chemistry** ↓AP in M & F at 1000 mg/kg bw/day. **Urinalysis** Not performed. **Organ weights** ↓Kidney, liver, thyroids and adrenal weights in M at 1000 mg/lg bw/day. **Gross pathology** No organ abnormality attributable to treatment were detected. **Histopathology** There were no treatment-related lesions.	
21-days rabbit dermal OECD 410 GLP Deviations: FC was not assessed (rabbits consumed their entire ration of 4 ounces of food each day). Dermal exposure was limited to three weeks of five dosing occasions/week. Dermal changes were assessed on a weekly basis.	Rabbit (NZW) M, F 5/sex/dose	Benfluralin (97.3%) Batch No.: ACD13683 0, 100, 500 or 1000 mg/kg bw/day	Cat 1 = 80 Cat 2 = 800	**Mortality** There were no deaths. **BW and BWG** No relevant findings. FC was not assessed. **Local irritation (day 21)** Dose-dependent increase of erythema, eschar, oedema and scaling from low dose in M & F. *100 mg/kg bw/day*: Erythema: very slight (1 M & 3 F) to well-defined (3 M & 1 F). Edema: very slight (3 M & 3 F) and moderate severe (1 F). *500 mg/kg bw/day:* Erythema: well-defined (2 M), moderate severe (3 M & 2 F) to severe (3 F). Edema: moderate-severe (1 F) to severe (5 M & 4 F). Scaling: slight (2 F) to moderate-severe (3 M & 2 F). *1000 mg/kg bw/day:* Erythema: moderate-severe (1 M & 1 F) to severe (4 M & 4 F). Edema: Severe in all animals. Scaling: slight (2 M & 1 F) to moderate-severe (1 M & 4 F). No signs of fissuring, scabs or necrosis.	Author (1993) Report No. DR-0097-3397-002/ CA 5.3.3/02

Method, guideline, deviations (if any)	**Species, strain, sex, no/group**	**Test substance, route of exposure, dose levels, duration of exposure**	**CLP guideline value for classification (mg/kg bw/d)**	**Results**	**Author**
Animals were weighed prior to first application and at termination only.				**Ophthalmology** There were no treatment-related changes. **Haematology** ↑platelets in M (34%) and F (26%) at 1000 mg/kg bw/day (not statistically significant). **Clinical chemistry** ↑globulin in M at 1000 mg/kg bw/day and in F at each dose (no increase with increasing dose and concentrations reported to be within HCD data of which the details have not been provided). **Urinalysis** Not performed. **Organ weights** ↑liver weight in all doses in M & F (no dose-respones). Above 10% at 100 mg/kg bw/day in F and at 1000 mg/kg bw/day in M only. **Gross pathology (treated skin)** ↑redness and svelling at all doses (dose-related increase) in M & F. ↑scale formation at 500 mg/kg bw/day and above in M & F. **Histopathology** *Skin* ↑dose-dependent inflammatory response, with subsequent regenerative lesions of the epidermis at all doses. Epidermal necrosis, ulcers and supurative lesions at 500 mg/kg bw/day and above in M & F (underlying tissues also affected). *Liver* Necrosis (with accompanying inflammation) in: M at 500 (2/5) and 1000 (2/5) mg/kg bw/day. F at 100 (1/5) and 500 (2/5) mg/kg bw/day (1 F in control group).	

Rats 90-days

In three studies designed to study subchronic toxicity, benfluralin was administered by dietary admixture to rats (Fisher F344/Tac) for 90 days/13 weeks. The study, included endpoints from these three integrated studies with doses ranging from 50 to 7500 ppm. Following the exposure period, 15 (Study R33989) or 10 (Study

R44089) rats/sex/dose were sacrificed, and 5 rats/sex (Study R44089) of the 0 and 7500 ppm-group were placed on control feed for another 6 weeks (recovery group). In study R29990 an interim necroscopy of 5 rats/dose occurred after 2 weeks.

In the rat, the target organs were the blood, the liver and the kidney. Slight regenerative anaemia was suspected at 5000 ppm and above. Major clinical chemistry modifications at 5000 ppm and higher included increased total bilirubin, increased γ-GT activities, and increased protein and cholesterol levels, indicating the effect on the liver, whereas the increased BUN levels (and the elevated urinary AST and LDH activities in the males) indicated an effect on the kidney. The increased hepatic CYP 1A-activities (based upon slight activity change of p-nitroanisole O-demethylase), visually enlarged and heavier livers, and increased incidence of centrilobular liver cell hypertrophy, were explained by the inductive effect of the CYP-mediated metabolic enzymes, and were thus adaptative rather than toxic events.

In the liver of male (15/15) and female rats (7/15) exposed to 250 ppm, minimal centrilobular hepatocyte hypertrophy was observed, progressing to slight centrilobular hepatocyte hypertrophy at the next dose levels. Although this effect may correspond to the increase in liver weight, identified at all doses, the increase in liver weight in rats exposed to 250 ppm was less than 10% and therefore considered not adverse. Consequently, the minimal centrilobular hepatocyte hypertrophy observed at this dose level was also considered as non-adverse.

Renal findings included the increased organ weight in the males and the females. The males developed nephrosis, characterised by hyaline droplets in the kidney tubules at 250 ppm, i.e. secondary lysosomes staining positively for α2μ-globulin, which is known to be specific for the male rat. However, an increased incidence of golden-brown pigment deposition in the kidneys of the female animals were indicative of some nephrotoxicity, which was observed as minimal at 250 ppm (15/15), progressing to slight in 1/15 animals at the next dose level (1100 ppm).

Notably, ovary cysts were observed at 250 (2/15), 1100 (7/15) and 5000 (1/15) ppm, but not at 7500 ppm. Although small increases in absolute and relative weights of the ovaries were detected at 250, 1100 and 5000 ppm, a statistically significant increase in the relative (14%), but not absolute weight of the ovaries was only detected upon exposure to 7500 ppm, were no cysts were detected.

In summary, a dose of ≤ 250 ppm (17 mg/kg bw/day) was considered as non-adverse in rats based on the increased slight pigment deposit in the proximal convoluted tubule, with supporting evidence of increase in kidney weight in the female rat at 1100 ppm (74 mg/kg bw/day).

Mice 90-days

Groups of 15 male and 15 female B6C3F1/CrlBr mice were fed a diet containing 0, 100, 300, 1000, 3000 and 10000 ppm of benfluralin (equivalent to 0, 13.5, 40.3, 132.8, 420.8 or 1364.4 mg/kg bw/day for males and 0, 17.4, 51.1, 168.2, 506.6 or 1730.2 mg/kg bw/day for females) for 13 weeks /90 days. The mice were checked daily and a detailed examination was performed once weekly. The mice were also weighed at weekly intervals. Blood samples were taken prior to necropsy, after overnight fasting. In addition to the routine clinical chemistry analyses, liver samples taken at necropsy from 5 rats/sex/dose were assayed for hepatic enzyme induction (by determination of hepatic p-nitroanisole O-demethylase).

The study is considered acceptable according to guideline OECD 408 with the following devations:

1. No ophtalmological examination was conducted.

2. No observations of sensory reactivity to stimuli of different types, but assessments of grip strength and of motor activity were conducted.

3. Food consumption was not recorded; test article intake was estimated on default consumptions of 4 g/d (males) and 3.9 g/d (females).

4. The following biochemistry determinations were not included: sodium, potassium, total cholesterol, urea, total protein, and albumin.

5. Epididymides, thymus, and brain was not weighed at necropsy, and weights were recorderd of kidneys with adrenals attached and of uterus with ovaries attached.

In the mouse, RBC parameters were slightly low at the top-dose (10000 ppm). At 1000 ppm in female mice, a decrease in MCV (1.6%), an increase in hepatic p-nitroanisole O-demethylase activity (37%), and increases in absolute (9.6%) and relative (13%) liver weights occured. In male mice, these effects occured at higher doses and liver enzymes (AP, ALT) were high at 3000 ppm and higher. However, mild centrilobular hepatocytic hypertrophy was seen in male, but not in female mice dosed 1000 ppm and above. Like in the rat, the substance induced CYP-450 mediated metabolic enzymes, with concomitant increased liver weight and hepatocellular hypertrophy.

Based upon marked (>10%) liver weight increase and increased enzyme activities, 1000 ppm (133 mg/kg bw/day) should be considered a non-adverse dose.

Dogs 90-days and 12-months

In the dog studies, 4 dog/sex/dose (Beagle purebred) were fed gelatin capsules with benfluralin at dose levels of 0, 5, 25 or 125 mg/kg bw/day for a period of 90 days (90d study) or a period of 12 months (1-year study). Samples were not checked analytically.

The effect of capsule-feeding of benfluralin to the dog did not markedly affect body weight parameters. At 25 mg/kg bw/d, marginal increases of AP-activities, of liver weight and increased sinusoidal spleen cell pigmentation was observed in the 90d or 1 yr-assay, the effects becoming more prominent at the top-dose (125 mg/kg bw/d). In an earlier 2 yr study, some effects of the substance on the erythron were reported.

90-day study: At 25 mg/kg bw/d, the observed spleen haemosiderosis was associated with an physiological adaptive response, indicating an increased RBC turnover, the effect becoming adverse at the top-dose (slight effects on the RBC, further haemosiderosis in the spleen, and effects appearing in the liver). It was confirmed a marginal decrease (6%) of RBC counts (females) at the top-dose. However, at 25 mg/kg bw/day, neither of the erythron parameters were altered, indicating that the compensatory mechanism of the haematopoiteic system was not overcome at this dose. Furthermore, a slight increase in alkaline phosphatase activity was observed at 5 and 25 mg/kg bw but not at the top-dose in the females, and the liver weight was weakly, but significantly increased at 25 mg/kg bw/d and above in the males only.

1-year study: At 25 mg /kg bw/day (males) and above (males and females), platelet levels were marginally increased, with a dose-dependent and a partly time-dependent trend. This effect was also observed in an earlier 90d study, but the etiology remained unclear. Another effect occuring from 25 mg/kg bw/day onwards (males, females), was a dose-dependent decreased alanine aminotransferase activity. However, the toxicological significance of this effect was unexplained. The altered parameters in clinical chemistry at the top-dose in females were explained by inflammation in the liver. The AP-increase was considered toxicologically relevant, as the the effect was seen in the subchronic study, and the liver was the target organ.

It was discussed in the previous DAR (2006) that an overall NOAEL for the dog studies (90d and 1-year) should be established at 25 mg/kg bw/day, based on the following evaluation: *"The effects mentioned at 25 mg/kg bw/d were relatively weak: AP activity increase was +54% control value but absent at the top-dose in the ♀. Liver weight was only increased in the ♂, and at the limit (r.w.) or in the absence (a.w.) of statistical significance. The increase of platelet level was also relatively weak (+23%), and not statistically significant. Whereas an association with the slight anemia (top-dose) was not excluded, the toxicological significance was unexplained. Finally, slight spleen pigmentation was observed at 25 mkd, but in the absence of frank haematological lesions, it was considered an adaptive rather than a toxic effect*."

Therefore, the lowest dose (25 mg/kg bw/day) should be considered non-adverse in both dog studies.

Rabbits 21-days (dermal)

The subchronic toxicity of benfluralin was also studied by the dermal route in rabbit.

In the first dermal study (1986), five rabbits/sex/dose (NZW) were exposed to benfluralin, at the dose levels of 0, 100, 325 or 1000 mg/kg bw during 6h/d for 21 days. The test article covered 10% of the body surface for

the mid- and the top-dose, but was not moistened to improve skin contact. Stability analysis had been performed on other occasions.

At termination, the area of involvement (but not severity) differed in relationship to the dose. In all treatment groups (except controls), desquamation occurred within 5-20 days of treatment, casually followed by epithelisation (without scar tissue or other indication of corrosive effects). The skin exhibited a coriaceous, cracking and bleeding appearance in addition. In one low-dose and one mid-dose female, the progression to severe oedema (day 10-13) was characterised by the appearance of 'masses': areas of local severe oedemateous lesions within a zone of slight oedema, which were considered as consecutive to the presence of non-uniformely dispersed particulates over the treatment zone.

Platelets/thrombocytes were consistently increased in both males and females. In the females, this increase amounted >20% at 325 mg/kg bw and above, and in the males, the increase was significant at the top-dose. The leukocyte number was markedly increased in the females at the mid-dose (but less pronounced at the top-dose). However, a dose-dependent trend was observed for the decrease of the lymphocyte and concomitant increase in the neutrophil fraction. The increased eosinophil and basophil fraction was not confirmed at the top-dose. In contrast, the increased basophil fraction in the males seemed to be dose-dependent. Although globally, the modifications in WBC parameters showed no dose-effect relationship, they should be considered treatment-related, as they were probably a secondary response to the marked skin inflammation.

The observed decreases of kidney, liver, thyroids and adrenal weights in the top-dose males were considered to reflect the body weight drops at necropsy time.

At 100 mg/kg bw/day, slight (not statistically significant) decreases in body weight and alkaline phosphatase level, and increase in basofils, was observed in male rabbits. Notably, these changes were part of a continous decreasing or increasing trend with increasing dose. At 325 mg/kg bw/day there was a non-significant decrease in food consumption in males only (-4%) and a significant decrease in body weight in males (-14%). Both body weight (-19%) and food consumption (-12%) were further reduced in the top-dose group (1000 mg/kg bw/day).

In the second dermal study (1993), five rabbits/sex/dose (NZW) were exposed to Benfluralin, at the dose levels of 0, 100, 500 or 1000 mg/kg bw during 6h/d for 21 days (except weekends). The neat test material was moistened with 1 mL distilled water per gram of test material, and applied to the back of the rabbits under a gauze patch. Since the material was applied in neat form, homogeneity, stability and concentration checks were not applicable. In a range-finding study, 2 rabbits/sex/dose received a dermal application of 500 or 1000 mg/kg bw/d during 6h/d for 4 consecutive days. In this probe study, evaluation was restricted to a visual inspection of the treated zone at termination.

At termination, a dose-dependent increase of erythema, eschar, oedema and scaling was observed from the lowest dose on, in both the males and the females. It was of note that, at the low- and mid-dose dose, the scores after week 2 (data not presented) were slightly higher than after week 3. No animals showed fissuring, scabs (crusts) or necrosis. The slightly increase in platelets level in the top-dose males (1000 mg/kg bw/day), was in line with the marked skin inflammation. The globulin level was slightly (+28%) increased in top-dose males (2.3 mg/dL) and was high at each dose in females. The increase in females was not dose-dependent and the observed concentrations (2.0 mg/dL) were within the reported historical control data (means ± s.d. males:2.4 ± 0.3 mg/dL; females: 2.3 ± 0.3 mg/dL) of which no details needed to assess the appropiateness have been provided.

Statistically significant liver weight increases were observed in the liver weights at all doses. However, it was unclear what the toxicological significance was of this finding in the females as dose-responsiveness was lacking, and the magnitude of the weight difference with controls was rather low. The increase in the males at 500 mg/kg bw/day was more consistent, and attained 10% at the top-dose.

The treatment-related lesions were predominantly in the treated skin. There was a dose-dependent inflammatory response to benfluralin exposure, with subsequent regenerative lesions of the epidermis at all doses. At the highest doses, necrosis, ulcers and supurative lesions occurred. Underlying tissues were also affected, as inflammation and oedema of the dermis, and sebaceous gland hyperplasia was observed at all doses.

The etiology of the liver necrosis (with accompanying inflammation) at 100 mg/kg bw/day and above was unclear, as dose-response was not evident. In the rat, liver was detected as the target organ, but lesions were mostly restricted to hypertrophy (related to enzyme induction), without marked toxicity like necrosis and inflammation. It was suggested by the notifier that liver lesions could casually be explained by a secondary reaction to septicemia (as ulcers would constitute a route of entry to the blood stream) or else a spontaneous reaction, although the former is less probable, in the absence of bacteria in the liver. In addition, it was stated that the distribution of hepatocellular necrosis in the lobule was random, not suggesting a typical chemical-induced hepatotoxic pattern. As a clear mode of action for the liver necrosis in the 2/5 top-dose males was lacking, it was considered an adverse systemic effect. Notably, in females, liver necrosis was detected at both 100 (1/5) and 500 (2/5) mg/kg bw/day, but not at the top dose.

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

A set of standard well conducted oral toxicity studies are available in a range of species (rats, mice and dogs), as well as two dermal studies (rabbits). In the rats, renal findings included nephrosis in males with hyaline droplets positive for α2μ-globulin, which was specific for the male rat, and incidence of golden-brown pigement deposition in the females. In females, there was a progress from slight to moderate deposition in 1/15 animals at 74 mg/kg bw/day while nephrotoxicity with moderate deposition was first evident in all animlas at 341 mg/kg bw/day. The effects on the liver were clinical chemistry changes, enlarged and heavier livers as well as increased CYP 1A-activities with incidences of hepatocellular hypertrophy, which were indicative of an adaptive rather than an adverse response. An adaptive response in the liver was also observed in the mice. The observed effects in the dogs, like increases in AP activites, liver weight and increased sinusoidal spleen pigmentation, were prominent at the top-dose (125 mg/kg bw/day). The observed spleen haemosiderosis at 25 mg/kg bw/day was associated with an adaptive physiological response, indicating an increased RBC turnover which became adverse at the top-dose.

The dermal application of benfluralin to the rabbit during 21 day caused dermatitis at all doses, and associated inflammatory increase of leukocytes and thrombocytes at 500 mg/kg bw/day and higher. Consequently, all doses caused local irritation. The main systemic adverse effects observed were reduction in food consumption an body weight in males only and the modifications of WBC parameters was considered as a secondary response.

10.12.2 Comparison with the CLP criteria

A substance is classified for STOT-RE if specific target organ toxicity arises after a repeated exposure. All significant health effects which are due to functional disturbance or morphological changes resulting in impaired function, both reversible and irreversible, immediate and/or delayed should be considered. For the purpose of classification, adverse findings should generally be at or below the oral guidance value of 100 mg/kg bw/day (for category 2) or 10 mg/kg bw/day (for category 1) obtained in a 90-day rat study. Equivalent guidance values for 21-day, 90-day and 1-year studies are extrapolated according to Haber's rule (i.e for 21-day study, an increase by a factor of four is appropriate). Adjusted guidance values for categories 1 and 2 are summarised in the table below.

Table 58: Adjusted guidance values for categories 1 and 2

Duration	**Adjusted guidance values (mg/kg bw/day)**
21-days (dermal)	Cat 1 = 80 Cat 2 = 800
90-days (oral)	Cat 1 = 10 Cat 2 = 100
12-months (oral)	Cat 1 = 2.5

	Cat 2 = 25

For benfluralin, all available evidence, and relevance to human health, from appropriate studies in experimental animals were taken into consideration for identification of toxic effects that can suppert classification for STOT-RE. According to the CLP criteria, effects considered to support classification for specific targey organ toxicity following repeated exposure are:

(a*) Morbidity or death resulting from repeated or long-term exposure*

(b) Significant functional changes in the central or peripheral nervous systems or other organ systems

(c) Any consistent and significant adverse changes in clinical chemistry, haematology or urinalysis parameters

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

(e) Multifocal or diffuse necrosis, fibrosis or granuloma formation in organs with regenerative capacity

(f) Morphological changes that are potentially reversible but provide clear evidence of marked organ dysfunction

(g) Evidence of appreciable cell death in vital organs incapable of regeneration

There were not identified any adverse effects for benfluralin considered relevant for humans at or below the adjusted guidance values that fulfilled these criteria.

Further, the effects that were observed after administration of benfluralin (at the relevant doses for classification) are not considered to support classification. These findings are summarised below as they relate to the CLP criteria.

(a) Clinical observations

Chromaturia was observed in rats and mice, indicative of excretion of the test article. Incidence of food emesis associated with the mode of administration (i.e bolus capsule) was noted in dogs (90-day study only). In the dermal studies, the rabbits showed dose- and time-related increase of dermal irritation.

(b) Small changes in clinical biochemistry, haematology or urinalysis parameters

Non-persistent increase in AST and LDH excretion were noted in rats. In dogs, a slight increase in AP, and platelet levels were marginally increased (unclear etiology). A dose-dependent decrease in ALT activity was noted in the 1-year dog study (unexplained).

(c) Changes in organ weights with no evidence of organ dysfunction

Small increases in liver weight and kidney weight considered non-adverse were noted in rats. Small increase in liver weight was noted in dogs, and increase above 10% increase in rabbits (in one study only).

(d) Adaptive responses that are not considered toxicologically relevant

The liver weight increases observed in rats and mice after administration of benfluralin were explained by findings indicative of an adaptive response; induction of CYP-450 mediated metabolic enzymes and incidence of hepatocellular hypertrophy were noted in both species.

(e) Substance induced species specific mechanisms of action

Nephrosis in the male rats was positive for α2μ-globulin, an unique mechanism to male rats; hence the nephropathy was not considered relevant for humans.

The evaluation of available data show that effects observed after administration of benfluralin were clinical observations, small changes in biochemistry, haemotology, urinalysis parameters and changes in organ weights. Changes in liver weight were explained by histopathological findings and induction of CYP-mediated metabolic enzymes in the rodents indicative of an adaptive response. Liver necrosis with accompanying inflammation in the rabbits lacked a clear mode of action. The finding was not consistent with lesions in the

rat, but it was confined to one single study and does not support classification. The effect on the kidney was evident as nephrosis in the male rat caused by a mechansism unique to male rats. Nephrotoxicity characterized by pigment deposition in the female rat was minimal and not considered adverse at the relevant doses for classification. In conclusion, there were no observations to support classification and it would not be appropriate to classify benfluralin for STOT-RE.

10.12.3 Conclusion on classification and labelling for STOT RE

Not classified - conclusive but not sufficient for classification

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

Summary of the Dossier Submitter's proposal

The DS included the following repeat dose studies with benfluralin in the assessment of STOT RE:

1. 3 × 90-day rat dietary studies (Anonymous, 1996)
2. 1 × 90-day mouse dietary study (Anonymous, 1988)
3. 1 × 90-day dog dietary study (Anonymous, 1993)
4. 1 × 1-year dog dietary study (Anonymous, 1995)
5. 2 × 21-day rabbit dermal application studies (Anonymous, 1986; 1993)

All studies were guideline- and GLP compliant.

Rat 90-day studies

The rat studies tested several doses from 50 ppm up to 7500 ppm (3-522/604 mg/kg bw/d) in both males and females. Both liver and kidneys were the primary affected organs; several other generic effects on body weight and blood parameters suggesting slight anaemia were also noted. Clinical chemistry changes indicated liver and kidney involvement. Increased liver weight and hepatocyte hypertrophy were significant at high dose levels and increases in alpha 2μ-globulin in male rats at high doses contributed to nephropathy. Ovary cysts were observed at 20 (2/15), 88 (7/15) and 395 (1/15) mg/kg bw/d, but not at 604 mg/kg bw/d. The lack of a dose response does not support this effect as a substance mediated one. None of these effects were considered relevant for classification.

Mouse 90-day study

Groups of 15 male and 15 female B6C3F1/CrlBr mice were fed a diet containing 0, 100, 300, 1000, 3000 and 10000 ppm of benfluralin (equivalent to 0, 13.5, 40.3, 132.8, 420.8 or 1364.4 mg/kg bw/d for males and 0, 17.4, 51.1, 168.2, 506.6 or 1730.2 mg/kg bw/d for females) for 13 weeks /90 days. The liver and blood were target organs, but effects were only of

toxicological relevance at very high doses. The NOAEL was set at 133 mg/kg bw/d, above the criterion for STOT RE 2. The DS found no evidence to support adverse effects at or below the guidance values for classification.

Dog 90-day and 1-year studies

In the dog studies, 4 animals/sex/dose (Beagle, purebred) were fed gelatine capsules with benfluralin at dose levels of 0, 5, 25 or 125 mg/kg bw/d for a period of 90 days or a period of 12 months. There was evidence of increased red blood cell turnover, slight increases in alkaline phosphatase activity and liver effects were also noted and considered adverse only at the top dose of 125 mg/kg bw/d. There were no effects considered to be toxicologically relevant at the lower doses. The DS did not propose classification based on a lack of effects in the dog studies.

Rabbit 21-day dermal studies

The subchronic toxicity of benfluralin was also studied by the dermal route in rabbit. In the first dermal study (1986), 5 rabbits/sex/dose (NZW) were exposed to benfluralin, at dose levels of 0, 100, 325 or 1000 mg/kg bw during 6h/d for 21 days. The test article was not moistened. In the second dermal study (1993), 5 rabbits/sex/dose (NZW) were exposed to benfluralin, at dose levels of 0, 100, 500 or 1000 mg/kg bw during 6h/d for 21 days (except weekends). The neat test material was moistened with 1 mL distilled water per gram of test material and applied to the back of the rabbits under a gauze patch. Benfluralin caused dermatitis at all doses. Both studies showed clear, local irritation effects and treatment-related lesions in the skin. In the second study, there were some indications of liver necrosis with accompanying inflammation, but the lesions were not consistent and did not follow a clear dose response and lacked a clear mode of action (MoA). These dermal studies were also used to support the proposal for classification for Skin Irrit. 2; H315 (Causes skin irritation).

The DS did not consider any other studies in its assessment of repeated dose toxicity. The DS concluded there were no observations to support classification and did not propose STOT RE classification for benfluralin.

Comments received during consultation

There was a single comment from an MSCA. Classification for STOT RE was not supported. Adverse effects that might warrant consideration were confined to dose levels above the guidance values or were species-specific with regard to kidney effects.

Assessment and comparison with the classification criteria

The DS concluded that no adverse effects were observed at or below the adjusted guidance values that fulfilled the criteria for classification. The DS did not assess data from other repeated dose studies for benfluralin; these included studies on carcinogenicity and fertility and development.

Assessment of data from 90-day studies, 1-year dog and 28-day dermal toxicity studies

A set of standard, well conducted oral toxicity studies were available in a range of species (rats, mice and dogs), as well as two dermal toxicity studies (rabbits).

In rats, renal findings included nephrosis in males with hyaline droplets positive for α2μ-globulin (specific for males), and an increased incidence of golden-brown pigment deposition in the females. Nephrotoxicity characterised by pigment deposition in the female rat was minimal and not considered adverse at the relevant doses for classification. Effects on the liver were indicated by clinical chemistry changes, enlarged and heavier livers as well as increased CYP1A activities with incidences of hepatocellular hypertrophy. These were considered to be indicative of an adaptive rather than an adverse response. A similar adaptive response in the liver was also observed in the 90-day mouse study.

Prominent effects in dogs included increases in alkaline phosphatase activities, liver weight and increased sinusoidal spleen pigmentation at the top-dose (125 mg/kg bw/d). The spleen haemosiderosis at 25 mg/kg bw/d was associated with an adaptive physiological response.

The dermal application of benfluralin to the rabbit in the 21-day studies caused dermatitis at all doses, and an associated inflammatory increase of leukocytes and thrombocytes at 500 mg/kg bw/d and higher. Liver necrosis with accompanying inflammation in the rabbits lacked a clear MoA. The finding was not consistent with lesions in the rat or dog and it was confined to one study only. The second rabbit study did not show this effect.

In summary, the effects observed following administration of benfluralin showed no evidence of organ dysfunction at the cut-off levels where classification could be considered. Marked effects generally occurred at levels above the guidance values for classification.

Assessment of data from other repeat dose studies, rodent carcinogenicity, rat 2-generation and rat and rabbit developmental toxicity studies

In the rat 2-year carcinogenicity study many renal lesions occurred at ≥ 5/7 mg/kg bw/d (100ppm) benfluralin (a level which could in principle, support classification of STOT RE 2 for renal toxicity). The effects exhibiting a clear increase in a dose-dependent response included:

- Hyaline droplets in the renal tubule lining cells,
- Tubule cell karyomegaly,
- Transitional cell hyperplasia of the renal papilla,
- Large pelvic calculi,
- Small calculi in the pelvic epithelium.

However, at low levels of benfluralin these effects did not occur with sufficient adversity to cause significant toxicity or organ dysfunction.

Overall, RAC considers that **classification for STOT RE is not warranted**.

10.13 Aspiration hazard

10.13.1 Short summary and overall relevance of the provided information on aspiration hazard

Benfluralin is a solid. This hazard class is not assessed in this dossier.

10.13.2 Comparison with the CLP criteria

Not assessed in this dossier.

10.13.3 Conclusion on classification and labelling for aspiration hazard

Hazard class not assessed in this dossier

11 EVALUATION OF ENVIRONMENTAL HAZARDS

11.1 Rapid degradability of organic substances

A summary of the available relevant information on the fate of benfluralin in the aquatic environment is presented in Table 59. The studies have been summarized in the subsections of this chapter. A discussion of the studies and the conclusion regarding the rapid degradability of benfluralin in the aquatic environment is presented in a separate conclusion at the end of this chapter.

The dissipation and degradation pathway of benfluralin is shown in Figure 1.

Table 59: Summary of relevant information on rapid degradability

Method	**Results**	**Remarks**	**Reference (Author, year, Report no. / study data point)**
OECD Test Guideline 301D (Closed bottle test), GLP, Benfluralin: 99.9% purity	Day 28: degradation equal to 5% of the calculated biological demand.	Valid	Lebertz and Heim, 2002 Report No. IF-101/37381-00 / CA 7.2.2.1/01
Aquatic hydrolysis, EEC C 7.3, GLP, Benfluralin: >99% purity	Stable (study temperature 50 °C)	Valid	Knoch and Heim, 2002a Report No. IF-101/25976-00 / CA 7.2.1.1/01
Aquatic hydrolysis, guideline not specified but mainly in line with EEC C 7, non-GLP, Benfluralin: purity not reported	Stable (study temperature 26 °C)	Supporting information only	Saunders et al., 1985 Report No. EWD8447 / CA 7.2.1.1/02
OECD Test guideline 309 (Aerobic mineralisation in surface water), GLP, Benfluralin 98.7% purity)	No degradation	Valid	Blüthgen-Schiller, 2016 Report No. 20150016 / CA 7.2.2.2/01
Aerobic aquatic degradation in water/sediment BBA Part IV, Section 5-1 (December 1999), GLP, Benfluralin: purity > 99%	DT50 system: 3.1 days (at 20 °C), recalculated to 6.6 days at 12 °C (Q_{10} = 2.58) 1.7-2.0% AR mineralisation after 100 days	Valid, but not used for classification purposes.	Knoch and Heim, 2002b Report No. IF-101/25543-00 / CA 7.2.2.3/01
Aquatic photolytic degradation,	DT50 2.2 hours (study conditions), equivalent to 18	Valid, but degradation rate considered less	Knoch and Heim, 2003

Method	**Results**	**Remarks**	**Reference (Author, year, Report no. / study data point)**
SETAC 1995, GLP, Benfluralin purity 98%	hours, summer sunlight at 40 ºN (25 ºC)**.**	reliable than the rate from study by Ding, 2016	Report No. IF-101/25798-00 / CA 7.2.1.2/01
Aquatic photolytic degradation, OECD Test Guideline 316, GLP, Benfluralin purity 98.7%	DT50 7.8 hours (study conditions), equivalent to 1.7 hours, summer sunlight at 40 ºN (25 ºC)	Valid, but not used for classification purposes.	Ding, 2016 Report No. 150654 / CA 7.2.1.2/02

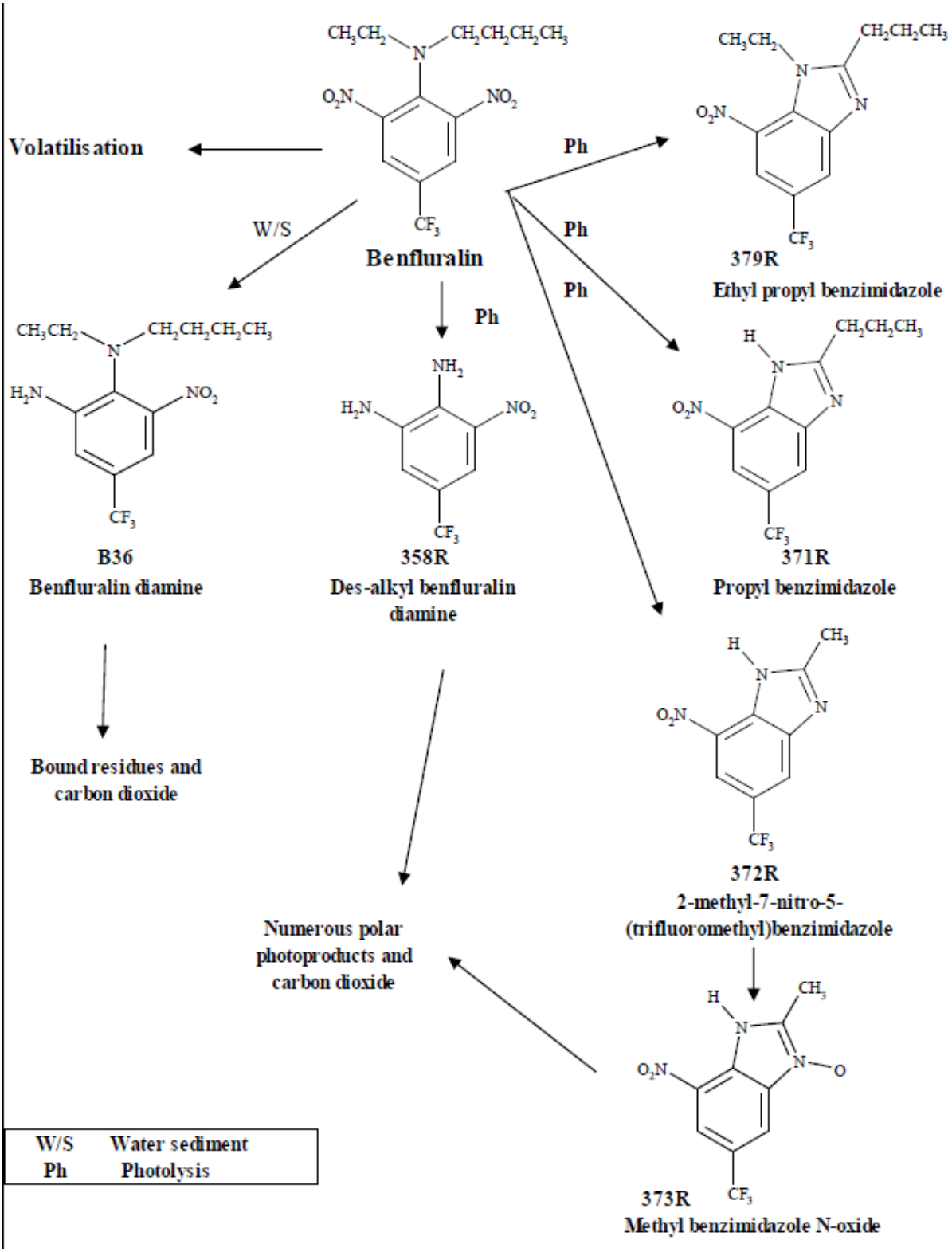


Figure 1. Routes of dissipation and degradation of benfluralin in aquatic systems.

11.1.1 Ready biodegradability

A ready biodegradation study is available following OECD Test Guideline 301D (Closed bottle test) and GLP (Lebertz and Heim, 2002). The control item sodium benzoate was degraded by 82% after 28 days (threshold for ready biodegradability was ≥ 60% after 7 days), therefore confirming the suitability of the inocula used. The degradation in the control + benfluralin samples indicated that the presence of the test item did not hinder

the effectiveness of the inocula used. The study was run at ~1.6 mg/l benfluralin, considered to be above the test item water solubility. A low degradation of 5% of the calculated biological oxygen demand (BOD) was observed over 28 days compared to controls and benfluralin was considered to be 'not readily biodegradable'. Validation criteria were met.

11.1.2 BOD_5/COD

No data.

11.1.3 Hydrolysis

An aqueous hydrolysis study (Knoch and Heim, 2002a) using benfluralin was conducted following GLP and Test guideline EEC method C 7.3, based on OECD Test Guideline 111. The study used [Phenyl-U-^{14}C]-benfluralin (radiochemical purity ≥99%) at 0.03 mg a.s./l in sterile buffer solutions at pH 4, 7 and 9. Samples were incubated at 50 °C in the dark for 5 days.

Analysis by High Performance Liquid Chromatography (HPLC) and Thin Layer Chromatography (TLC) indicated that at 50 °C benfluralin was stable at all tested pHs. No further testing at other temperatures was performed.

A second study (Saunders et al., 1985) was available, showing that benfluralin was stable to hydrolysis at 26 °C. However, the study was non-GLP and the reporting of the study was very brief. The study can be used for supporting information only. No further details of the study are therefore given here.

Overall, benfluralin is considered hydrolytically stable.

11.1.4 Other convincing scientific evidence

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

No relevant data.

11.1.4.2 Inherent and enhanced ready biodegradability tests

No data.

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

OECD Test Guideline 309 Study

A study investigating the aerobic mineralisation in surface water – simulation biodegradation test following OECD Test Guideline 309 and GLP is available (Blüthgen-Schiller, 2016). The study used benfluralin (phenyl-label) at test item concentrations of 2.94 (low dose) and 32.6 µg/L (high dose) in natural water samples from two different locations (pH 8.2, TOC 3.6, 6.5 or 14.9) and the high dose was also tested under sterile conditions. The experimental set-up was adapted due to the critical properties (i.e. low water solubility, high volatility) of the test item, using foam plugs soaked with paraffin oil (2%) to sample the benfluralin that volatilised at the neck, inlet and outlet of the flasks, as well as excluding volatile traps and ventilation with moist air of the test system and additional washing steps of the glassware to minimize the amount of substance adsorbing to it. Samples were incubated in the dark for a period of 17 days (high dose) and 16 days (low dose), respectively, at approx. 20.5 °C.

Radioactivity in the water was quantified directly by Liquid Scintillation Counting (LSC) and analysed by HPLC. In all test systems the overall recovery radioactivity ranged from 74.8% to 88.5% of the applied radioactivity (AR).

Benfluralin did not degrade during the test period of 16-17 days but was mainly volatilized from the test system due to its relatively high Henry's constant and medium vapour pressure (see also chapter 11.3). 7.2 – 17.5% AR remained in the water phase after 16-17 days. The test system according to OECD test guideline 309 is not entirely suitable for the investigation of the biodegradation of benfluralin in surface water due to its volatility and its strong adsorption to particles, which would lead to a rapid partitioning to the sediment (see Knoch and Heim, 2002b).

BBA Part IV, Section 5-1 Study

An aerobic water/sediment study is available following the guideline BBA Part IV, Section 5-1 (December 1999; Knoch and Heim, 2002b). The study used benfluralin (phenyl-label) and two natural water/sediment systems (2 cm sediment covered with 6 cm deep water).

The water/sediment systems were treated with ^{14}C-benfluralin dissolved in acetonitrile. Traps were connected to the systems to recover evolved organic volatiles and CO_2. The ^{14}C-benfluralin was applied to the surface water in each vessel to give a nominal initial concentration of 0.03 mg/L in the water phase. Flasks were incubated at 20 °C in the dark for up to 100 days.

Concentrated extracts from water and sediment were chromatographically analysed by reversed phase HPLC and normal phase TLC. The radioactivity remaining in the non-extractable residue was quantified by combustion analysis applied radioactivity (AR). The recovery radioactivity from the test systems ranged from 95.9% to 110.7% AR.

Results at the end of the incubation (100 days) showed that the mineralisation was low (1.7% to 2.0% AR). Bound residue reached a maximum level of 26% to 31.4% AR after 100 days. Benfluralin was highly volatile in the water/sediment systems (64.9% to 63.2% AR as volatile benfluralin at study end). Hence, the dissipation of benfluralin from the water phase in the water sediment systems was mainly via volatilisation and dissipation to the sediment. At the end of the study (100 days) benfluralin was not detected in the water phase of the two systems (< 0.1% AR). As the major part of radioactivity was evaporated from the water phase, it was considered that no significant degradation occurred in the water phase. The major part of degradation occurred in sediment only. When calculating the degradation rates for the water sediment systems, volatilisation losses were corrected for, according to the latest FOCUS kinetics guidance (FOCUS, 2014). The degradation of benfluralin was biphasic and was best described by the Hockey Stick kinetic model in both water sediment systems. The geometric mean DT50 of the total system was estimated to be 3.1 days. No degradation rate could be calculated for benfluralin in the water phase.

One significant degradation product was observed, benfluralin diamine, which was observed almost exclusively in the sediment layer and reached a maximum of 8.7% AR (day 2). There were not presented any acceptable degradation kinetics for this metabolite and the degradation rate of this metabolite could therefore not be determined.

Benfluralin is a substance that adsorbes strongly to particles and sediment (see chapter 11.3) and it would therefore be expected that benfluralin quickly dissipates to sediments in aquatic systems, as demonstrated in this study. However, even though this study demonstrates that the primary degradation of benfluralin in the test water/sediment systems is rapid, environmental conditions may vary and result in lower dissipation through volatilisation (e.g. due to water stratification) and possibly also less binding to sediment (e.g. due to greater distances to the sediments). This could potentially result in higher levels of benfluralin in the water phase, where it will not be degraded. Although the study is deemed acceptable in the dRAR and as such can be regarded as valid, the results are therefore not considered appropriate to demonstrate the rapid degradability of benfluralin in the aquatic environment. Furthermore, there are data available from studies that are, according to the guidance on the CLP criteria (v.05, July 2017), considered preferred over this type of water/sediment simulation study, i.e. studies on hydrolysis, rapid biodegradability and mineralisation in water.

11.1.4.4 Photochemical degradation

Two aqueous photolysis studies using benfluralin are available following SETAC 1995 and OECD 316 – Phototransformation of Chemicals in Water-Direct Photolysis and GLP (Knoch and Heim, 2003 and Ding, 2016, respectively). Both studies are considered valid, but the benfluralin endpoints calculated from the Knoch and Heim 2003 study are considered less reliable and are therefore not presented here. Since the results from Ding (2016) are given precedence over the results from Knoch and Heim (2003), only the methodology of the Ding (2016) study is described here.

The study used phenyl labelled benfluralin (radiochemical purity 98.7%) at a concentration of approx. 0.03 mg/l (corresponding to half of the test item's water solubility). Test solutions were prepared with either sterile buffer (pH 7) and continuously irradiated at 25 °C for up to 18 days with a xenon arc light filtered to restrict the wavelength range to 295 – 800 nm. The 18 day study duration was considered equivalent to 4.7 days summer sunlight at 40ºN assuming 12 hour days.

Radioactivity was quantified directly by LSC and analysed by UHPLC. Identification of transformation products was done by co-chromatography of reference standards and LC-MS analysis.

Benfluralin is rapidly photolytically degraded in aqueous media. The direct photolytic transformation half-life of benfluralin in sterile buffered water (pH 7) is determined from Ding (2016), and was 7.8 hours under continuous (artificial) irradiation. Recalculated to conditions of natural summer sunlight at 40 °N, the DT50 in sterile water was 1.7 hours. In the study by Knoch and Heim (2003b), metabolites 371R (propyl benzimidazole), 372R (methyl benzimidazole), 379R (ethyl propyl benzimidazole), and 358R (des-alkyl benfluralin diamine) were observed at levels exceeding 10% AR (average of duplicates). In the study by Ding (2016), the metabolites 358R and 372R were observed at levels exceeding 10% AR (average of duplicates) with maximum concentrations of 14.1% (day 1) and 19.8% AR (day 4), respectively, and metabolite 371R was observed to exceed 10% AR in two single samples (10.8% AR at day 4 and 10.2% AR at day 7).

11.1.5 Rapid degradation conclusion

Benfluralin is considered hydrolytically stable at environmentally relevant pH and temperature.

In an OECD Test Guideline 301D study, benfluralin was considered not readily biodegradable on the basis of a degradation equal to 5% of the calculated BOD at the study end (28 days). On the basis of this test, benflurain is considered not to meet the rapid degradability criteria.

Benfluralin did not degrade in a surface water mineralisation study (simulation test) following OECD TG 309 and disappeared from the test system mainly through volatilisation. However, the study is not considered entirely suitable for benfluralin, as benfluralin in the environment would quickly volatilise and partition to the sediment due to its high volatility and strong adsorption to particles, respectively. It is noted that the degree of volatilisation from the aquatic environment is highly dependent on the prevailing environmental conditions. Furthermore, the level of dissipation to the sediment may also be variable in the aquatic environment (e.g. due to proximity of the sediments). Hence, even though the test is not entirely suitable for benfluralin, the study can be used for the purpose of assessing the rapid degradability criteria. Benfluralin is, on the basis of this aquatic simulation test, considered not to meet the rapid degradability criteria.

In a water/sediment simulation study, benfluralin dissipated rapidly from the water phase to sediment. Minimal mineralisation was observed with 1.7 – 2.0% AR mineralisation seen at study termination (day 100). Several aquatic metabolites were formed, with one at levels above 5% AR (benfluralin diamine in sediment). The low rate of mineralisation indicates that only small amounts of benfluralin were degraded in the test system, while the majority of benfluralin had either volatilised or been transported to sediment where it formed bound residues or was rapidly degraded. Total system DT50 values at a study temperature of 20 °C were calculated to 3.1 days, equivalent to 6.1 days at 12 °C. The rate of degradation of benfluralin in the water phase could not be determined. Even though the study does demonstrate that the primary degradation of benfluralin in water/sediment systems may be rapid, environmental conditions may vary and result in lower dissipation through volatilisation and binding to sediment, resulting in higher levels of benfluralin in the water phase,

where it will not be degraded. Furthermore, the studies on hydrolysis, rapid biodegradation and degradation in a water simulation study are according to the guidance on the CLP criteria (v.05, July 2017) considered preferred data over this water/sediment study. The study is therefore not considered appropriate to demonstrate the rapid degradability of benfluralin in the aquatic environment..

Benfluralin is rapidly degraded by photodegradation. However, it is noted that the actual degree of photodegradation in the aquatic environment depends on local conditions and seasons and is difficult to quantify. Given the available data, there is insufficient information to evaluate photodegradation in the European environment in terms of mineralisation or transformation to non-classifiable substances. Therefore the data on aquatic photolysis is not considered to meet the criteria for rapid degradation.

Overall, benfluralin is not considered to be rapidly degradable for the purpose of classification according to the CLP criteria.

11.2 Environmental transformation of metals or inorganic metals compounds

Not relevant.

11.2.1 Summary of data/information on environmental transformation

Not relevant.

11.3 Environmental fate and other relevant information

Adsorption

An adsorption coefficient study is available (Knoch and Batzer, 2003) following GLP and OECD Test Guideline 106. The study used benfluralin and 3 soils that can be considered relevant for the EU. K_{fOC} values were 10736 to 14400 ml/g, indicating that benfluralin will adsorb strongly to particles and is not mobile in soil.

Volatility

The vapour pressure of benfluralin is 1.8 x 10^{-3} Pa at 20 °C and the water solubility at 20 °C is 0.064 mg/L with a calculated Henry's Law constant of 9.1 $Pa \times m^3 \times mol^{-1}$. These figures suggest that benfluralin can volatilize from water surfaces, as indicated by its Henry's law constant. This is confirmed by results from the simulation studies investigating the degradation of benfluralin in the aquatic environment (e.g. OECD TG 309).

11.4 Bioaccumulation

Table 60: Summary of relevant information on bioaccumulation

Method	Results	Remarks	Reference
Partition coefficient *n*-octanol/water OECT TG107, (shake flask method + analysis by GC-FID) Benfluralin (99.9%) (batch 251-198-OD-118 = TSN100015)	Log K_{ow} 5.27 ± 0.11 at 20 °C, pH 6.0-7.0		Dunning, J. (2016d)
Bioaccumulation in fish: aqueous exposure, US EPA FIFRA 165 4, evaluated according to OECD 305 (2012), GLP	Bioconcentration factor (BCF): 1740.8	Study not valid	Author (1987)

11.4.1 Estimated bioaccumulation

No data.

11.4.2 Measured partition coefficient and bioaccumulation test data

Only one bioaccumulation study with the bluegill sunfish, *Lepomis macrochirus*, is available. The bioaccumulation study had a 28 days exposure period and 14 days depuration period in a flow-through system and a maximum whole fish bioconcentration factor (BCF) of 1740.8 was derived. The study is not considered reliable and did not fulfil all of the validity criteria in OECD test guideline 305 (2012) ; i.e. the concentration of the test substance in the chambers was not maintained within ± 20 % of the mean of the measured concentration during the uptake phase. Furthermore, the growth of the juvenile fish and the lipid content were not reported. The study was therefore not considered valid. The complete evaluation of the study is given in the Annex I to the CLH-report (RAR section B.9.2.2.3; Report No. ABC-0362, ABC-0365/ CA 8.2.2.3/01). BCF values from studies of low quality is not recommended used for classification purposes. Given the experimentally determined log Kow of 5.27 for benfluralin, it can be concluded that benfluralin has the potential to bioaccumulate according to the CLP-criteria (v.05, July 2017).

11.5 Acute aquatic hazard

A summary of available valid information on the aquatic toxicity of benfluralin is presented in the table below. All the listed studies are considered reliable and suitable for use in hazard classification. In the pesticide review program, a total of nine and six aquatic studies were considered unacceptable and supplemental, respectively. Ten of these studies did not fulfil the validity criteria of their respective test guideline and four studies fulfilled the validity criteria but were tested with doses far above the water solubility of benfluralin and further showed clear evidence of precipitation and/or discoloration of the test solution. One open literature study confirmed the high toxicity of benfluralin to fish but was considered supplemental as the methodology was not comparable to any standard study guidelines.

Table 61: Summary of relevant information on acute aquatic toxicity

Method	Species	Endpoint	Exposure		Results		Reference
			Design	Duration	Endpoint	Toxicity	
Acute toxicity to fish, ASTM E729 80 (1980), evaluated according to OECD 203 , GLP	*Oncorhynchus mykiss* (Rainbow trout)	Mortality	Semi-static	96 hours	LC_{50}	0.081 mg a.s./L (mm)	Author (1985) Report No. F00185/ CA 8.2.1/01
Acute toxicity to fish, OECD TG 203, GLP	*Oncorhynchus mykiss* (Rainbow trout)	Mortality	Static	96 hours	LC_{50}	1.00 mg TR-6/L[1]	Author (2001a) Report No. 011092/ CA 8.2.1/06
Acute toxicity to fish, OECD TG 203, GLP	*Oncorhynchus mykiss* (Rainbow trout)	Mortality	Static	96 hours	LC_{50}	5.46 mg TR-15/L[2]	Author (2001b) Report No. 011106/ CA 8.2.1/07
Acute toxicity to fish, OCSPP Draft Guideline 850.1075, evaluated according to OECD 203, GLP	*Oncorhynchus mykiss* (Rainbow trout)	Mortality	Flow-through	96 hours	LC_{50}	> 0.048 mg a.s./L (mm)	Author (2014a) Report No. 14050.6160/ CA 8.2.1/08
Acute toxicity to fish, OCSPP Draft Guideline 850.1075, evaluated according to OECD 203 GLP	*Lepomis macrochirus* (Bluegill sunfish)	Mortality	Flow-through	96 hours	LC_{50}	> 0.042 mg a.s./L (mm)	Author (2013a) Report No. 14050.6125/ CA 8.2.1/09
Acute toxicity to fish, OCSPP Draft Guideline 850.1075, evaluated according to OECD 203, GLP	*Cyprinodon variegatus* (Sheepshead Minnow)	Mortality	Flow-through	96 hours	LC_{50}	> 0.027 mg a.s./L (mm)	Author (2013b) Report No. 14050.6161/ CA 8.2.1/10

Acute toxicity to fish, OECD 203, GLP	*Cyprinus carpio* (carp)	Mortality	Flow-through	96 hours	LC_{50}	> 0.029 mg a.s./L (mm)	Author (2004) Report No. 12550.6332/ CA 8.2.1/11
Daphnia sp., Acute Immobilisation Test , OECD 202, GLP	*Daphnia magna*	Immobility	Static	48 hours	EC_{50}	3.52 mg TR-6/L (mm)[1]	Marino et al. (2001c) Report No. 011093/ CA 8.2.4.1/02
Daphnia sp., Acute Immobilisation Test , OECD 202, GLP	*Daphnia magna*	Immobility	Static	48 hours	EC_{50}	9.36 mg TR-15/L (mm)[2]	Marino et al. (2001d) Report No. 011105/ CA 8.2.4.1/03
Daphnia sp., Acute Immobilisation Test , OCSPP Guideline 850.1010, evaluated according to OECD 202, GLP	*Daphnia magna*	Immobility	Flow-through	48 hours	EC_{50}	> 0.034 mg a.s./L (mm)	Urann (2014b) Report No. 14050.6154/ CA 8.2.4.1/04
Mysid Acute Toxicity Test , US EPA FIFRA 72 3, GLP	*Mysidopsis bahia* (Mysid shrimp)	Mortality	Flow-through	96 hours	LC_{50}	0.043 mg a.s./L (mm)	Sousa (1990b) Report No. 90-6-3343/ CA 8.2.4.2/01
Freshwater Alga and Cyanobacteria, Growth Inhibition Test, US EPA FIFRA 123 2 evaluated according to OECD 201, GLP	*Pseudokirchneriella subcapitata* (Green algae)	Growth-rate	Static	72 hours	E_rC_{50} E_yC_{50}	> 5.56 mg TR-6/L (mm) 4.09 mg TR-6/L (mm)[1]	Henry et al. (2002) Report No. 011101/ CA 8.2.6.1/02

Freshwater Alga and Cyanobacteria, Growth Inhibition Test, OECD 201, GLP	*Pseudokirchneriella subcapitata* (Green algae)	Growth-rate	Static	72 hours	E_rC_{50} E_yC_{50}	> 9.15 mg TR-15/L (mm) 3.82 mg TR-15/L (mm)[2]	Marino et al. (2001) Report No. 011102/ CA 8.2.6.1/03
Freshwater Alga and Cyanobacteria, Growth Inhibition Test, OECD 201, GLP	*Pseudokirchneriella subcapitata* (Green algae)	Growth-rate	Static	96 hours	E_rC_{50} E_yC_{50}	> 0.0132 mg a.s./L (mm) > 0.0132 mg a.s./L (mm)	Softcheck (2015a) Report No. 14050.6228/ CA 8.2.6.1/04
Lemna sp. Growth Inhibition Test, OECD 221, GLP	*Lemna gibba* (Duck weed)	Growth-rate	Semi-static	7 days	E_rC_{50} E_yC_{50}	> 0.032 mg a.s./L (mm) 0.017 mg a.s./L (mm)	Hoberg (2007) Report No. 12550.6485/ CA 8.2.7/01

mm = mean measured
[1]Tested with the metabolite Trifluralin Metabolite TR-6
[2]Tested with the metabolite Trifluralin Metabolite TR-15

11.5.1 Acute (short-term) toxicity to fish

The seven valid studies considered suitable for use in hazard classification are presented below. In two of the studies the metabolites TR-6 and TR-15 are used as test substance.

Study 1 – Author (1985) Report No. F00185/ CA 8.2.1/01

The 96-hour semi-static study was conducted with *Oncorhynchus mykiss* (rainbow trout) exposed to benfluralin with a nominal exposure range of 56, 90, 140, 225, 330 and 500 µg a.s./L. Exposure solutions were prepared with the aid of the solvent acetone and a solvent control was included. Study conditions were considered acceptable. Measured concentrations were 22 to 44% of nominal over the study period and results were based on mean measured concentrations of the 24-hour aged solutions. This is considered conservative and acceptable for the purpose of hazard classification. The 96-hour endpoint was above the water solubility of 64 µg/L and estimated to be:

LC_{50} = 81 µg a.s./L

Study 2 – Author (2001a) Report No. 011092/ CA 8.2.1/06

The 96-hour static study was conducted with *Oncorhynchus mykiss* (rainbow trout) exposed to trifluralin metabolite TR-6 with a nominal exposure range of 0.117, 0.194, 0.324, 0.540, 0.900, 1.50 mg trifluralin metabolite TR-6/L. Exposure solutions were prepared with the aid of the solvent dimethylformamide and a solvent control was included. Study conditions were considered acceptable. Measured concentrations were 92 to 103% of nominal over the study period and results were based on nominal concentrations. The 96-hour endpoint was estimated to be:

LC_{50} = 1 mg TR-6/L

Study 3 – Author (2001b) Report No. 0111006/ CA 8.2.1/07

The 96-hour static study was conducted with *Oncorhynchus mykiss* (rainbow trout) exposed to trifluralin metabolite TR-15 with a nominal exposure range of 1.01, 1.68, 2.81, 4.68, 7.80, 13.0 mg trifluralin metabolite TR-15/L. Exposure solutions were prepared with the aid of the solvent dimethylformamide and a solvent control was included. Study conditions were considered acceptable. Measured concentrations were 100 to 103% of nominal over the study period and results were based on nominal. The 96-hour endpoint was estimated to be:

LC_{50} = 5.46 mg TR-15L

Study 4 – Author (2014a) Report No. 14050.6160/ CA 8.2.1/08

The 96-hour flow-through study was conducted with *Oncorhynchus mykiss* (rainbow trout) exposed to benfluralin with a nominal exposure range of 3.1 , 6.3, 12, 25, and 50 µg a.s./L. The test substance was dosed with a saturator column made of glass and Teflon packed with glass wool. The column with wool was covered evenly with a benfluralin in acetone solution and a vacuum pump was used to evaporate all the acetone. The column was designed to provide a constant flow of saturated aqueous solution without the use of a carrier solvent. Study conditions were considered acceptable. Measured concentrations were 42 to 95% of nominal over the study period and the endpoints were given as mean measured concentrations. The 96-hour endpoint was estimated to be:

LC_{50} > 48 µg a.s./L

Study 5 – Author. (2013a) Report No. 14050.6125/ CA 8.2.1/09

The 96-hour flow-through study was conducted with *Lepomis macrochirus* (bluegill sunfish) exposed to benfluralin with a nominal exposure range of 3.1 , 6.3, 12, 25, and 50 µg a.s./L. The test substance was dosed with a saturator column made of glass and Teflon packed with glass wool. The column with wool was covered evenly with a benfluralin in acetone solution and a vacuum pump was used to evaporate all the acetone. The column was designed to provide a constant flow of saturated aqueous solution without the use of a carrier solvent. Study conditions were considered acceptable. Measured concentrations were 72 to 92% of nominal over the study period and the endpoints were given as mean measured concentrations. The 96-hour endpoint was estimated to be:

LC_{50} > 42 µg a.s./L

Study 6 – Author (2013b) Report No. 14050.6161/ CA 8.2.1/10

The 96-hour flow-through study was conducted with *Cyprinodon variegatus* (sheepshead minnow) exposed to benfluralin with a nominal exposure range of 3.1 , 6.3, 12, 25, and 50 µg a.s./L. The test substance was dosed with a saturator column made of glass and Teflon packed with glass wool. The column with wool was covered evenly with a benfluralin in acetone solution and a vacuum pump was used to evaporate all the acetone. The column was designed to provide a constant flow of saturated aqueous solution without the use of a carrier solvent. Study conditions were considered acceptable. Measured concentrations were 55 to 68% of nominal over the study period and the endpoints were given as mean measured concentrations. The 96-hour endpoint was estimated to be:

LC_{50} > 27 µg a.s./L

Study 7 – Author (2004) Report No. 12550.6332/ CA 8.2.1/11

The 96-hour flow-through study was conducted with *Cyprinus carpio* (carp) exposed to benfluralin with a nominal exposure range of 6.3, 13, 25, 50 and 100 µg a.s./L. Exposure solutions were prepared with the aid of the solvent acetone and a solvent control was included. Study conditions were considered acceptable. Measured concentrations were 20 to 39% of nominal over the study period and the endpoints were given as mean measured concentrations. The 96-hour endpoint was estimated to be:

LC_{50} > 29 µg a.s./L

11.5.2 Acute (short-term) toxicity to aquatic invertebrates

The four valid studies considered suitable for use in hazard classification are presented below. In two of the studies the metabolites TR-6 and TR-15 are used as test substance.

Study 1 – Marino *et al.* (2001c) Report No. 011093/ CA 8.2.4.1/02

The 48-hour static study was conducted with *Daphnia magna* (water flea) exposed to trifluralin metabolite TR-6 with a nominal exposure range of 0.778, 1.30, 2.16, 3.60, 6.00, 10.0 mg trifluralin metabolite TR-6/L. Study conditions were considered acceptable. Measured concentrations were 81 to 98% of nominal over the study period and the endpoints were given as nominal concentrations. The 48-hour endpoint was estimated to be:

EC_{50} = 3.52 mg TR-6/L

Study 2 – Marino *et al.* (2001d) Report No. 011105/ CA 8.2.4.1/03

The 48-hour static study was conducted with *Daphnia magna* (water flea) exposed to trifluralin metabolite TR-15 with a nominal exposure range of 1.56, 2.59, 4.32, 7.20, 12.0, 20.0 mg trifluralin metabolite TR-15/L. Study conditions were considered acceptable. Measured concentrations were 97 to 106% of nominal over the study period and the endpoints were given as nominal concentrations. The 48-hour endpoint was estimated to be:

EC_{50} = 9.36 mg TR-15/L

Study 3 – Urann (2014b) Report No. 14050.6154/ CA 8.2.4.1/04

The 48-hour flow-through study was conducted with *Daphnia magna* (water flea) exposed to benfluralin with a nominal exposure range of 3.1 , 6.3, 12, 25, and 50 µg a.s./L. The test substance was dosed with a saturator column made of glass and Teflon packed with glass wool. The column with wool was covered evenly with a benfluralin in acetone solution and a vacuum pump was used to evaporate all the acetone. The column was designed to provide a constant flow of saturated aqueous solution without the use of a carrier solvent. Study conditions were considered acceptable. Measured concentrations were 45 to 80% of nominal over the study period and the endpoints were given as mean measured concentrations. The 48-hour endpoint was estimated to be:

EC_{50} > 34 µg a.s./L

Study 4 – Sousa (1990b) Report No. 90-6-3343/ CA 8.2.4.2/01

The 96-hour flow-through study was conducted with *Mysidopsis bahia* (mysid shrimp) exposed to benfluralin with a nominal exposure range of 19, 32, 54, 90 and 150 µg a.s./L. Exposure solutions were prepared with the aid of the solvent acetone and a solvent control was included. Study conditions were considered acceptable. In one of two replicates in the solvent control, all of the mysids exhibited darkened pigmentation at the first 48 hours and were lethargic at the 72 hours sampling time point, thus not fulfilling the validity criterion in OCSPP 850.1035. These effects were absent after 96 hours exposure. The reason behind these effects is unclear as no irregular conditions was observed in this replicate, no effects were observed in the other solvent control replicate or in the two lowest exposure groups, and the solvent concentration was below the maximum recommended concentration of 0.1 mL/L. The effects are thus unlikely to be a toxic effect of the solvent, and the deviation does not undermine the observed mortal effects. Measured concentrations were 44 to 53% of nominal over the study period and the endpoints were given as mean measured concentrations. The 96-hour endpoint was estimated to be:

LC_{50} = 43 µg a.s./L

11.5.3 Acute (short-term) toxicity to algae or other aquatic plants

Three valid toxicity studies with algae and one with aquatic plants are available and presented below. In two of the studies on algae the metabolites TR-6 and TR-15 are used as test substance.

Study 1 - Henry et al. (2002) Report No. 011101/ CA 8.2.6.1/02

A static algal growth inhibition test following GLP and OECD Test Guideline 201 with *Pseudokirchneriella subcapitata* exposed to trifluralin metabolite TR-6/L is available. The nominal exposure range was 0.078, 0.156, 0.313, 0.625, 1.25, 2.50, 5.00, 10.0 mg trifluralin metabolite TR-6/L. Exposure solutions were prepared with the aid of the solvent acetone and a solvent control was included. The mean measured concentrations were 55.6 to 100% of nominal concentrations. Endpoint and validity criteria was investigated for both the 72h and the 96h timeperiod. The validity criteria were not fulfilled for the 96h, breaching the trigger for CV% for the section-by-section specific growth rates. The validity criteria were neither fulfilled for the 72h time period, by slightly exceeding the trigger for the CV% for the average specific growth rate (8.39% in study against a trigger of 7% in study guideline). However, deviation of the 0–72 hour specific growth rate are considered to be too close to the accepted value to discard the study. The 72h is also the recommended study duration in OECD TG 201. The endpoints for *Pseudokirchneriella subcapitata* were re-calculated to the geometric mean measured concentrations. As measurements were only conducted at test initiation and test termination (96 hours), concentrations are based on the geometric mean of these two timepoints, which is considered conservative.

The 72-hour acute endpoints based on mean measured concentrations were estimated to be:

$E_rC50 > 5.56$ mg TR-6L

$E_yC50 = 4.09$ mg TR-6/L

Study 2 - Marino et al. (2001) Report No. 011102/ CA 8.2.6.1/03

A static algal growth inhibition test following GLP and OECD Test Guideline 201 with *Pseudokirchneriella subcapitata* exposed to trifluralin metabolite TR-6/L is available. The nominal exposure range was 0.78, 0.156, 0.313, 0.625, 1.25, 2.50, 5.00, 10.0 mg trifluralin metabolite TR-15/L. Exposure solutions were prepared with the aid of the solvent acetone and a solvent control was included. The mean measured concentrations were 64.7 to 106% of nominal concentrations. Endpoint and validity criteria were investigated for both the 72h and the 96h time period. The validity criteria were not fulfilled for the 96h timeperiod, breaching the trigger for CV% for the section-by-section specific growth rates. The validity criteria were neither fulfilled for the 72h time period, by slightly exceeding the trigger for the CV% for the average specific growth rate (7.02% in study against a trigger of 7% in study guideline). However, deviation of the 0–72 hour specific growth rate are considered to be too close to the accepted value to discard the study. The 72h is also the recommended study duration in OECD TG 201. The endpoints for *Pseudokirchneriella subcapitata* were re-calculauted to the geometric mean measured concentrations. As measurements were only conducted at test initiation and test termination (96 hours), concentrations are based on the geometric mean of these two timepoints, which is considered conservative.

The 72-hour acute endpoints based on mean measured concentrations were estimated to be:

$E_rC50 > 9.15$ mg TR-15/L

$E_yC50 = 3.82$ mg TR-15/L

Study 3 - Softcheck (2015a) Report No. 14050.6228/ CA 8.2.6.1/04

A static algal growth inhibition test following GLP and OECD Test Guideline 201 with *Pseudokirchneriella subcapitata* exposed to benfluralin is available. The nominal exposure range was 4.1, 8.1, 16, 33, and 65 µg a.s./L. Exposure solutions were prepared with the aid of the solvent dimethylformamide (DMF) and a solvent control was included. The initial measured concentrations were 76 to 87% of nominal. The measured 96h concentrations were ≈ 0 to 9.2% of nominal . The endpoints for *Pseudokirchneriella subcapitata* were re-calculated to the geometric mean of the initial measured concentrations (0h) and the 96h measured concentrations. The concentrations of benfluralin dropped to levels below LOQ in the two lowest treatment levels at the end of the study (96h) and these two values were set to LOQ/2 when calculating the geometric mean. No inhibitory effects on yield or growth rate were observed up to the highest concentration tested of13.2 µg a.s./L(mm). The concentrations falling below the LOQ at the two lowest exposure groups does not

invalidate the establishment of the endpoint, which was calculated from measured concentration in the upper exposure group.

The 96-hour acute endpoints based on mean measured concentrations were estimated to be:

E_rC50 > 13.2 µg a.s./L

E_yC50 > 13.2 µg a.s./L

Study 4 - Hoberg (2007) Report No. 12550.6485/ CA 8.2.7/01

A semi-static 7-day study following GLP and OECD Test Guideline 221 with *Lemna gibba* exposed to benfluralin is available. The nominal exposure range was 4.2, 8.3, 17, 34 and 66 µg a.s./l. Validity criteria were met and the test is considered reliable.

The concentrations of the test item ranged from 41 to 49% of the nominal values and the endpoints were reported as geometic mean mesasured concentrations. The 7-day acute endpoints were estimated to be:

E_rC50 > 32 µg a.s./L

E_yC50 = 17 µg a.s./L (based on frond density)

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

No relevant data.

11.6 Long-term aquatic hazard

A summary of available valid information on the chronic toxicity of benfluralin is presented in the table below. All the listed studies are considered reliable and suitable for use in hazard classification.

Table 62: Summary of relevant information on chronic aquatic toxicity

Method	Species	Endpoint	Exposure		Results		Reference
			Design	Duration	Endpoint	Toxicity	
Fish Early Life-Stage (ELS) toxicity test, US EPA FIFRA 72-4, evaluated according to OECD 210, GLP	*Oncorhynchus mykiss* (Rainbow trout)	Growth	Flow-through	49 days	NOEC	0.0019 mg a.s./L (mm)	Author (1990) Report No. F00690/ CA 8.2.2.1/01
Daphnia magna Reproduction test, OCSPP Draft Guideline 850.1300, evaluated according to OECD 211, GLP	*Daphnia magna*	Reproduction, survival and growth, NOEC	Flow-through	21 days	NOEC	0.046 mg a.s./L (mm)	Urann (2013c) Report No. 14050.6156/ CA 8.2.5.1/02

Freshwater Alga and Cyanobacteria, Growth Inhibition Test, OECD 201, GLP	*Pseudokirchneriella subcapitata* (Green algae)	Growth-rate	Static	96 hours	NOE_rC NOEyC	0.0132 mg a.s./L (mm)	Softcheck (2015a) Report No. 14050.6228/ CA 8.2.6.1/04
Lemna sp. Growth Inhibition Test, OECD 221, GLP	*Lemna gibba* (Duck weed)	Growth-rate	Semi-static	7 days	NOE_rC	0.0069 mg a.s./L (mm)	Hoberg (2007) Report No. 12550.6485/ CA 8.2.7/01

mm = mean measured

11.6.1 Chronic toxicity to fish

One valid chronic toxicity study with fish is considered suitable for use in hazard classification and presented below.

Study 1 – Author (1990) Report No. F00690/ CA 8.2.2.1/01

A 49-day flow-through chronic toxicity study following GLP and OECD Test Guideline 210 with *Oncorhynchus mykiss* (rainbow trout) exposed to benfluralin is available. The nominal exposure range was 1.2, 3.7, 11, 33 and 100 µg/l. Mean measured concentrations ranged from 45–67% of the nominal concentrations. The following endpoints were recorded: larval survival at complete hatch, larval survival at test termination, hatchability, time to hatch and growth (length and weight). General observations were also recorded. The reported maximum temperature span of 1.6 °C between some of the test chambers deviated slightly from one of the validity criteria. This is not considered to impact the test results as the only registered measurement outside the 1.5 °C temperature range was in the highest treatment at only one time point (day 2). At this treatment level, a 100% cumulative mortality was observed among the fish larvae. Exposure solutions were prepared with the aid of the solvent acetone and a solvent control was included. The endpoints based on mean measured concentrations are summarised in the table below:

Table 63: Summary of analytical measurements and effect data

Nominal concentration (µg a.s./l)	**Mean measured concentration (µg a.s./l) ± SD**	**Larval survival at complete hatch (%)**	**Larval survival at test termination (%) ± SD**	**Mean length (mm) ± SD**	**Mean weight (g) ± SD**
Control	Control	100	92.5 ± 9.6	41.0 ± 1.6	0.70 ± 0.08
Solvent control	Solvent control	100			
1.2	0.8 ± 0.2	100	95.0 ± 5.8	41.1 ± 0.5	0.73 ± 0.03
3.7	1.9 ± 0.2	100	90.0 ± 8.2	39.7 ± 1.6	0.66 ± 0.11
11	5.0 ± 0.3	100	92.5 ± 2.9	38.1* ± 1.0	0.65 ± 0.09
33	14.8 ± 1	98.8	39.1* ± 6.9	26.5* ± 1.9	0.21* ± 0.06
100	45.5 ± 2.2	100	0.0*	-	-

*Statistically significantly different from the pooled controls ($p \leq 0.05$)

No effects were seen on time to hatch and hatchability. The different endpoints were determined to be:

Table 64: Summary of EC_{10}, EC_{20} and NOEC w/confidence intervals.

Endpoint	**EC_x/LC_x (µg a.s./l)**	**95% confidence limit (µg a.s./l; LL-UL)**
Survival		
LC_{10}	5.2	1.3 - 20.0
LC_{20}	6.9	2.3 - 21.1
LC_{50}	12.2	4.9 - 30.3
NOEC	5.0	
Weight		
EC_{10}	5.6	3.4 - 9.1
EC_{20}	7.1	4.5 - 11.1
EC_{50}	0.0111	6.5 - 19.2
NOEC	0.005	
Length		
EC_{10}	6.0	4.5 - 8.0
EC_{20}	9.4	7.0 - 12.5
EC_{50}	21.8	14.1 - 33.0
NOEC	**1.9**	

LL – lower limit, UL – upper limit

The chronic endpoint relevant for the hazard classification of benfluralin is considered to be:

NOEC = 1.9 µg a.s./L (based on length)

11.6.2 Chronic toxicity to aquatic invertebrates

One valid chronic toxicity study with aquatic invertebrates is considered suitable for use in hazard classification and presented below.

Study 1 – Urann (2013c) Report No. 14050.6156/ CA 8.2.5.1/02

The flow-through study was conducted with *Daphnia magna* (water flea) exposed to benfluralin with a nominal exposure range of 3.1 , 6.3, 12, 25, and 50 µg a.s./l. The test substance was dosed with a saturator column made of glass and Teflon packed with glass wool. The column with wool was covered evenly with a benfluralin in acetone solution and a vacuum pump was used to evaporate all the acetone. The column was designed to provide a constant flow of saturated aqueous solution without the use of a carrier solvent. Study conditions were considered acceptable. Measured concentrations were 58 to 92% of nominal over the study period. The following endpoints were recorded: survival, cumulative offspring per female and growth (length and dry weight). No effects were observed in the study. Consequently, the mean measured long-term endpoint for survival, offspring per female, body length and dry weight was determined to be:

NOEC = 0.046 mg a.s./L

11.6.3 Chronic toxicity to algae or other aquatic plants

The chronic toxicity endpoints with algae and aquatic plants are presented below.

Study 1 - Softcheck (2015a) Report No. 14050.6228/ CA 8.2.6.1/04

See section 11.5.3 for further study details. No inhibitory effects on yield or growth rate were observed up to the highest concentration tested (13.2 µg a.s./L(mm)), and an EC_{10} value can thus not be determined. The chronic endpoints for *Pseudokirchneriella subcapitata* exposed to benfluralin were estimated to be:

NOE_rC = 13.2 µg a.s./L

NOE_yC = 13.2 µg a.s./L

Study 2 - Hoberg (2007) Report No. 12550.6485/ CA 8.2.7/01

See section 11.5.3 for further study details. The chronic endpoints for *Lemna gibba* exposed to benfluralin were estimated to be:

ErC_{10} = 0.012 mg a.s./L (based on frond density)

EyC_{10} = 0.0085 mg a.s./L (based on frond density)

NOE_rC = 0.0069 mg a.s./L (based on frond density)

NOE_yC = 0.0069 mg a.s./L (based on frond density)

11.6.4 Chronic toxicity to other aquatic organisms

No relevant data.

11.7 Comparison with the CLP criteria

The data for the toxicity of benfluralin to aquatic organisms are compared with the criteria for classification according to CLP Regulation (EC) No 1272/2008. Adequate acute and chronic toxicity data are available for all taxonomic levels (fish, crustacea and algae or other aquatic plants).

11.7.1 Acute aquatic hazard

The lowest available acute toxicity value was obtained with crustacea (LC_{50} = 0.043 mg a.s./L).

This endpoint is lower than the classification criterion for Category Acute 1: ≤ 1 mg/L. The appropriate M-factor is 10, since the toxicity is within the range $0.01 < L(E)C_{50} \leq 0.1$.

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

On the basis of results from tests investigating hydrolysis, rapid biodegradability and surface water mineralisation, benfluralin is considered not to meet the rapid degradability criteria. A water/sediment simulation study demonstrated that when sediment was present, benfluralin quickly dissipated to the sediment and was rapidly degraded there. However, since this type of study may not be relevant for all aquatic systems, where dissipation of benfluralin may be less pronounced due to for instance water stratification or greater distance to sediments, the results were not considered appropriate to demonstrate rapid degradability of benfluralin in the aquatic environment. Furthermore, the studies on hydrolysis, rapid biodegradation and surface water mineralisation are considered preferred data over the water/sediment simulation study. Overall, benfluralin is not considered to be rapidly degradable for the purpose of classification according to the CLP criteria.

One bioaccumulation study with the bluegill sunfish, *Lepomis macrochirus*, gave a maximum whole fish bioconcentration factor (BCF) of 1740.8. However, the study was not considered reliable and did not fulfil all of the validity criteria in OECD test guideline 305 (2012). Given the experimentally determined log K_{ow} of 5.27 for benfluralin, , it can be concluded that benfluralin has the potential to bioaccumulate according to the CLP-criteria (v.05, July 2017).

The lowest available chronic toxicity value was observed in a fish study (NOEC = 0.0019 mg a.s./L). This endpoint is lower than the classification criterion for Category Chronic 1: ≤ 0.1 mg/L. The appropriate M-factor is 10, since the toxicity is within the range of 0.001 < NOEC ≤ 0.01 and the substance is non-rapidly biodegradable.

11.8 Conclusion on classification and labelling for environmental hazards

The following classifications are considered appropriate according to the CLP criteria:

'Aquatic Acute 1', Acute M-Factor: 10

'Aquatic Chronic 1', Chronic M-Factor: 10

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier Submitter's proposal

DS proposal: Aquatic Acute 1; H400with an M-factor of 10 based on the acute toxicity to Mysid shrimp *Mysidopsis bahia* (96h EC_{50} of 0.043 mg/L), and Aquatic Chronic 1; H410 with an M-factor of 10, based a 49-day NOEC of 0.0019 mg/L to *Oncorhynchus mykiss* (Rainbow trout) and substance not rapidly degradable.

Degradation

Ready biodegradability

The DS considered the substance not readily biodegradable based on a GLP ready biodegradation study performed according to the OECD TG 301D (Closed bottle test). A low degradation of 5% was observed for test item at concentration 1.6 mg/L after 28 days. The validation criteria were met: the control item sodium benzoate degraded by 82% after 28 days (threshold for ready biodegradability was ≥ 60% after 7 days) and the degradation of control and benfluralin samples indicated that the presence of the test item did not hinder the effectiveness of the inoculum used.

Hydrolysis

DS accepted as key a GLP aqueous hydrolysis study based on OECD TG 111. Hydrolysis of 0.03 mg/L radiolabelled [Phenyl-U-^{14}C]-benfluralin (radiochemical purity ≥99%) was examined in sterile buffer solutions at pH 4, 7 and 9. Samples were incubated at 50 ºC in the dark for 5 days and the results indicated that benfluralin was stable at all tested pH values. In a second, non-GLP study, benfluralin was stable to hydrolysis at 26 ºC. However, the reporting of the study was very brief without details therefore this study can be used as supporting information only.

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

The aerobic degradation of benfluralin was investigated according to OECD TG 309 at two concentration levels 0.00294 (low dose) and 0.0326 mg/L (high dose) in surface waters from

two different locations (pH 8.2, TOC 3.6, 6.5 or 14.9 mg/L). Samples were incubated in the dark for a period of 17 days (high dose) and 16 days (low dose), respectively, at approx. 20.5 °C. The experimental set-up was specifically adapted due to the critical properties (i.e. low water solubility and high volatility) of the test item. Benfluralin did not degrade during the test period of 16-17 days but was mainly volatilized from the test system due to its relatively high Henry's constant and medium vapour pressure. AR remained in the water phase after 16-17 days was between 7.2 – 17.5%. The test system according to OECD TG 309 is not entirely suitable for the investigation of the biodegradation of benfluralin in surface water due to its volatility and its strong adsorption to particles, which would lead to a rapid partitioning to the sediment.

Aerobic mineralisation of benfluralin (phenyl-label) was studied also in two natural water/sediment systems (2 cm sediment covered with 6 cm deep water) following the guideline BBA Part IV, Section 5-1. The ^{14}C-benfluralin was applied to the surface water in each vessel to give a nominal initial concentration of 0.03 mg/L in the water phase. Flasks were incubated at 20 °C in the dark for up to 100 days. Results at the end of the incubation (100 days) showed that the mineralisation was low (1.7% to 2.0% AR). The dissipation of benfluralin from the water phase in the water sediment systems was mainly via volatilization (64.9% to 63.2% AR measured as volatile benfluralin at study end) and dissipation to the sediment (26% to 31.4% AR after 100 days as bound residue). At the end of the study (100 days) benfluralin was not detected in the water phase of the two systems (< 0.1% AR). As the major part of radioactivity was evaporated from the water phase, it was considered that no significant degradation occurred in the water phase. The major part of degradation occurred in sediment only. The geometric mean DT_{50} of the total system was estimated to be 3.1 days. No degradation rate could be calculated for benfluralin in the water phase. One significant degradation product was observed, benfluralin diamine, which was observed almost exclusively in the sediment layer and reached a maximum of 8.7% AR (day 2). There were not presented any acceptable degradation kinetics for this metabolite and the degradation rate of this metabolite could therefore not be determined. DS concluded that although the primary degradation of benfluralin in the test water/sediment systems is rapid and benfluralin quickly dissipates through volatilization or adsorption to sediments, higher concentrations in water phase could not be excluded due to variations of environmental conditions. DS considered studies on hydrolysis, rapid biodegradability and mineralisation in water are indicative of classification of benfluralin as not rapidly degradable.

Bioaccumulation

Benfluralin has a log K_{OW} of 5.27 ± 0.11 at 20 °C, pH 6.0-7.0 which is experimentally determined according to the OECT TG 107, (shake flask method and analysis by GC-FID). The potential for aquatic bioaccumulation could not be excluded.

Only one experimental bioaccumulation study was available that was considered by the DS as not valid and the calculated BCF values were deemed of low quality, not recommended to be used for classification purposes. The bioaccumulation study with the bluegill sunfish, *Lepomis macrochirus* had a 28 days exposure period and 14 days depuration period in a flow-through system and a maximum whole fish bioconcentration factor (BCF) of 1740.8 was derived L/kg. The study did not fulfil all of the validity criteria in OECD TG 305 (2012); i.e. the concentration of the test substance in the chambers was not maintained within ± 20% of the mean of the measured concentration during the uptake phase, the growth of the juvenile fish and the lipid content were not reported.

Based on the experimentally determined log Kow of 5.27, DS concluded that benfluralin has the potential to bioaccumulate, according to the CLP-criteria.

Acute toxicity

Valid acute toxicity studies were available for all three trophic levels with benfluralin and with main degradation products TR-15 and TR-6, summarised in table below.

***Table**: Summary of valid studies for acute toxicity of benfluralin and metabolites TR-15 and TR-6*

Method	Species	Endpoint	Exposure		Results		Reference
			Design	Duration	Endpoint	Toxicity	
Acute toxicity to fish, ASTM E729 80 (1980), evaluated according to OECD TG 203 , GLP	*Oncorhynchus mykiss* (Rainbow trout)	Mortality	Semi-static	96 hours	LC_{50}	0.081 mg a.s./L (mm)	Anonymous,1985 Report No. F00185/ CA 8.2.1/01
Acute toxicity to fish, OECD TG 203, GLP	*Oncorhynchus mykiss* (Rainbow trout)	Mortality	Static	96 hours	LC_{50}	1.00 mg TR-6/L (mm)[1]	Anonymous,2001a Report No. 011092/ CA 8.2.1/06
Acute toxicity to fish, OECD TG 203, GLP	*Oncorhynchus mykiss* (Rainbow trout)	Mortality	Static	96 hours	LC_{50}	5.46 mg TR-15/L (mm)[2]	Anonymous,2001b Report No. 011106/ CA 8.2.1/07
Acute toxicity to fish, OCSPP Draft Guideline 850.1075, evaluated according to OECD TG 203, GLP	*Oncorhynchus mykiss* (Rainbow trout)	Mortality	Flow-through	96 hours	LC_{50}	> 0.048 mg a.s./L (mm)	Anonymous,2014a Report No. 14050.6160/ CA 8.2.1/08
Acute toxicity to fish, OCSPP Draft Guideline 850.1075, evaluated according to OECD TG 203 GLP	*Lepomis macrochirus* (Bluegill sunfish)	Mortality	Flow-through	96 hours	LC_{50}	> 0.042 mg a.s./L (mm)	Anonymous,2013a Report No. 14050.6125/ CA 8.2.1/09
Acute toxicity to fish, OCSPP Draft Guideline 850.1075, evaluated according to OECD TG 203, GLP	*Cyprinodon variegatus* (Sheepshead Minnow)	Mortality	Flow-through	96 hours	LC_{50}	> 0.027 mg a.s./L (mm)	Anonymous,2013b Report No. 14050.6161/ CA 8.2.1/10

Acute toxicity to fish, OECD TG 203, GLP	*Cyprinus carpio* (carp)	Mortality	Flow-through	96 hours	LC_{50}	> 0.029 mg a.s./L (mm)	Anonymous, 2004 Report No. 12550.6332/ CA 8.2.1/11
Daphnia sp., Acute Immobilisation Test, OECD TG 202, GLP	*Daphnia magna*	Immobility	Static	48 hours	EC_{50}	3.52 mg TR-6/L (mm)[1]	Marino *et al*., 2001c Report No. 011093/ CA 8.2.4.1/02
Daphnia sp., Acute Immobilisation Test, OECD TG 202, GLP	*Daphnia magna*	Immobility	Static	48 hours	EC_{50}	9.36 mg TR-15/L (mm)[2]	Marino *et al*., 2001d Report No. 011105/ CA 8.2.4.1/03
Daphnia sp., Acute Immobilisation Test, OCSPP Guideline 850.1010, evaluated according to OECD 202, GLP	*Daphnia magna*	Immobility	Flow-through	48 hours	EC_{50}	> 0.034 mg a.s./L (mm)	Urann, 2014b Report No. 14050.6154/ CA 8.2.4.1/04
Mysid Acute Toxicity Test, US EPA FIFRA 72 3, GLP	*Mysidopsis bahia* (Mysid shrimp)	Mortality	Flow-through	96 hours	LC_{50}	**0.043 mg a.s./L (mm)**	Sousa, 1990b Report No. 90-6-3343/ CA 8.2.4.2/01
Freshwater Alga and Cyanobacteria, Growth Inhibition Test, US EPA FIFRA 123 2 evaluated according to OECD TG 201, GLP	*Pseudokirchneriella subcapitata* (Green algae)	Growth-rate	Static	72 hours	E_rC_{50} E_yC_{50}	> 5.56 mg TR-6/L (mm) 4.09 mg TR-6/L (mm)[1]	Henry et al., 2002 Report No. 011101/ CA 8.2.6.1/02

Freshwater Alga and Cyanobacteria, Growth Inhibition Test, OECD TG 201, GLP	*Pseudokirchneriella subcapitata* (Green algae)	Growth-rate	Static	72 hours	E_rC_{50} E_yC_{50}	> 9.15 mg TR-15/L (mm) 3.82 mg TR-15/L (mm)[2]	Marino *et al.*, 2001 Report No. 011102/ CA 8.2.6.1/03
Freshwater Alga and Cyanobacteria, Growth Inhibition Test, OECD TG 201, GLP	*Pseudokirchneriella subcapitata* (Green algae)	Growth-rate	Static	96 hours	E_rC_{50} E_yC_{50}	> 0.0132 mg a.s./L (mm) > 0.0132 mg a.s./L (mm)	Softcheck, 2015a Report No. 14050.6228/ CA 8.2.6.1/04
Lemna sp. Growth Inhibition Test, OECD TG 221, GLP	*Lemna gibba* (Duck weed)	Growth-rate	Semi-static	7 days	E_rC_{50} E_yC_{50}	> 0.032 mg a.s./L (mm) 0.017 mg a.s./L (mm)	Hoberg, 2007 Report No. 12550.6485/ CA 8.2.7/01

[1] Tested with the metabolite Trifluralin Metabolite TR-6
[2] Tested with the metabolite Trifluralin Metabolite TR-15
mm: mean measured

As can be seen both metabolites TR-15 and TR-6 are with much lower toxicity than parent compound benfluralin for all three trophic levels and will not be discussed any longer.

Short term toxicity to fish

Acute toxicity of benfluralin to fish was investigated in 5 studies, which were considered valid by the DS and equivalent to OECD TG 203.

96-hour fish toxicity was estimated to be LC_{50} = 0.081 mg/L in one study with *Oncorhynchus mykiss* (Rainbow trout) exposed to benfluralin under semi-static conditions was above the water solubility of 0.064 mg/L.

Four different fish species *Oncorhynchus mykiss* (Rainbow trout) *Lepomis macrochirus* (Bluegill sunfish) *Cyprinodon variegatus* (Sheepshead Minnow) *and Cyprinus carpio* (carp) were exposed to benfluralin under flow-through conditions for 96 h. The lowest endpoint value expressed as mean measured concentration was the 96-hour LC_{50} > 0.027 mg/L found for *Cyprinodon variegatus*. The fish species were exposed to benfluralin (nominal exposure range of 0.031, 0.063, 0.012, 0.025, and 0.050 mg/L.) under flow-through conditions for 96 h. Test design included a saturator column made of glass and Teflon packed with glass wool to dose the substance. The column was designed to provide a constant flow of saturated aqueous solution without the use of a carrier solvent. A slightly different design was used in the 96-hour flow-through study conducted with *Cyprinus carpio* (carp) exposed to benfluralin, The nominal exposure range is 0.0063, 0.013, 0.025, 0.050 and 0.100 mg/L and exposure

solutions were prepared with the aid of the solvent acetone, a solvent control was included. The 96-hour endpoint was estimated to be: LC_{50} > 0.029 mg a.s./L.

Short term toxicity to invertebrates

There were two valid acute toxicity studies available for invertebrates, both conducted in a flow-through system, under analytical control of benfluralin concentrations.

The acute toxicity study to *Daphnia magna* was conducted following the procedures described and the recommendations provided in the OECD TG 202 with a nominal exposure range of 0.0031, 0.0063, 0.012, 0.025, and 0.050 mg/L benfluralin. The test substance was dosed with a saturator column made of glass and Teflon packed with glass wool. The column was designed to provide a constant flow of saturated aqueous solution without the use of a carrier solvent. The 48-hour endpoint was estimated to be: EC_{50} > 0.034 mg benfluralin/L.

The acute toxicity of benfluralin to the Mysid shrimp *Mysidopsis bahia* was tested in 96-hour flow-through study at a nominal exposure range of 0.019, 0.032, 0.054, 0.090 and 0.150 mg/L. Exposure solutions were prepared with the aid of the solvent acetone and a solvent control was included. Although some unclear effects (lethargic species with darkened pigmentation at the first 48 hours and 72 hours sampling time point) observed in two replicates of solvent control, the study was accepted as valid because these effects were absent after 96 hours exposure, no effects were observed in the other solvent control replicate or in the two lowest exposure groups, in addition the solvent concentration was below the maximum recommended concentration of 0.1 mL/L. The 96-hour endpoint was estimated to be: LC_{50} = 0.043 mg/L benfluralin.

Short-term toxicity to algae

Two algae tests are given for benfluralin with *Pseudokirchneriella subcapitata* and *Lemna gibba.*

Toxicity of benfluralin to *Pseudokirchneriella subcapitata* was studied under static conditions as an algal growth inhibition test following GLP and OECD TG 201. The nominal exposure range was 0.0041, 0.0081, 0.016, 0.033, and 0.065 mg/L with exposure solutions prepared in solvent dimethylformamide (DMF) (solvent control was included). The measured 96h concentrations were ≈ 0 to 9.2% of nominal and the endpoints for *Pseudokirchneriella subcapitata* were re-calculated to the geometric mean of the initial measured concentrations (0h) and the 96h measured concentrations. No inhibitory effects on yield or growth rate were observed up to the highest concentration tested of 0.0132 mg/L. The concentrations falling below the LOQ at the two lowest exposure groups does not invalidate the establishment of the endpoint, which was calculated from measured concentration in the upper exposure group.
The 96-hour acute endpoints based on mean measured concentrations were presented as:
E_rC_{50} > 0.0132 mg/L and E_yC_{50} > 0.0132 mg/L

A valid and reliable toxicity test to *Lemna gibba* was conducted for 7 days under semi-static conditions. The nominal exposure range was 0.0042, 0.0083, 0.017, 0.034 and 0.066 mg a.s./L and the endpoints were reported as geometric mean measured concentrations. The 7-day acute endpoints were: E_rC_{50} > 0.032 mg/L and E_yC_{50} = 0.017 mg/L (based on frond density).

Based on these, the DS proposed a classification of Category Acute 1: ≤ 1 mg/L, based on the lowest available acute toxicity value for crustacea (LC_{50} = 0.043 mg a.s./L) and an appropriate M-factor of 10, since the toxicity is within the range 0.01 < L(E)C_{50} ≤ 0.1.

Chronic toxicity

Valid and reliable long-term toxicity studies are summarised in table below.

***Table**: Summary of valid studies for chronic toxicity of benfluralin*

Method	Species	Endpoint	Exposure		Results		Reference
			Design	Duration	Endpoint	Toxicity	
Fish Early Life-Stage (ELS) toxicity test, US EPA FIFRA 72-4, evaluated according to OECD TG 210, GLP	*Oncorhynchus mykiss* (Rainbow trout)	Growth	Flow-through	49 days	NOEC	**0.0019 mg a.s./L (mm)**	Anonymous, 1990 Report No. F00690/ CA 8.2.2.1/01
Daphnia magna Reproduction test, OCSPP Draft Guideline 850.1300, evaluated according to OECD TG 211, GLP	*Daphnia magna*	Reproduction, survival and growth, NOEC	Flow-through	21 days	NOEC	0.046 mg a.s./L (mm)	Urann, 2013c Report No. 14050.6156/ CA 8.2.5.1/02
Freshwater Alga and Cyanobacteria, Growth Inhibition Test, OECD TG 201, GLP	*Pseudokirchneriella subcapitata* (Green algae)	Growth-rate	Static	96 hours	NOE_rC NOE_yC	0.0132 mg a.s./L (mm)	Softcheck, 2015a Report No. 14050.6228/ CA 8.2.6.1/04
Lemna sp. Growth Inhibition Test, OECD TG 221, GLP	*Lemna gibba* (Duck weed)	Growth-rate	Semi-static	7 days	NOE_rC	0.0069 mg a.s./L (mm)	Hoberg, 2007 Report No. 12550.6485/ CA 8.2.7/01

mm: mean measured

Long-term toxicity to fish

A 49-day flow-through chronic toxicity study following GLP and OECD TG 210 with *Oncorhynchus mykiss* (rainbow trout) exposed to benfluralin (prepared in solvent acetone and a solvent control was included) in the nominal exposure range 0.0012, 0.0037, 0.011, 0.033 and 0.100 mg/L was conducted. The following endpoints based on mean measured concentrations were recorded: larval survival at complete hatch, larval survival at test termination, hatchability, time to hatch and growth (length and weight). The chronic endpoint relevant for the hazard classification of benfluralin is considered to be: NOEC = 0.0019 mg/L (based on length).

Long-term toxicity to invertebrates

One GLP chronic toxicity study was available for aquatic invertebrate *Daphnia magna* conducted under flow-through conditions, for 21 days in accordance with OECD testing guideline 211. The test substance with a nominal exposure range of 0.0031, 0.0063, 0.012, 0.02525, and 0.050 mg a.s./L. was dosed with a saturator column made of glass and Teflon packed with glass wool ensuring constant flow of saturated aqueous solution of tested substance without the use of a carrier solvent. No effects were observed for endpoints considered: survival, cumulative offspring per female and growth (length and dry weight). Consequently, the mean measured long-term endpoint for survival, offspring per female, body length and dry weight was determined as NOEC = 0.046 mg/L.

Long-term toxicity to algae

Valid toxicity studies available for two algae species *Pseudokirchneriella subcapitata* and *Lemna gibba* have been already described in a previous section of this ODD. For *Pseudokirchneriella subcapitata* no inhibitory effects on yield or growth rate were observed up to the highest concentration tested – 0.0132 mg/L (mean measured). The chronic endpoints for *Pseudokirchneriella subcapitata* exposed to benfluralin as mean measured concentrations were NOE_rC = 0.0132 mg a.s./L and NOE_yC = 0.0132 mg a.s./L.

The calculated chronic endpoints for *Lemna gibba* exposed to benfluralin were: E_rC_{10} - 0.012 mg/L (based on frond density) and E_yC_{10} - 0.0085 mg/L (based on frond density);
NOE_rC = 0.0069 mg/L (based on frond density) and NOE_yC = 0.0069 mg/L (based on frond density).

Based on these, the DS proposed a classification of Aquatic Chronic 1 based on a lowest available toxicity value of 0.0019 mg a.s./L, with an M-factor of 10 since the toxicity is within the range of 0.001 < NOEC ≤ 0.01 and the substance is non-rapidly degradable.

Comments received during consultation

One comment was received during the consultation, supporting the proposed environmental classification Aquatic Acute 1; H400 (M=10) and Aquatic Chronic 1; H410 (M=10).

Assessment and comparison with the classification criteria

Degradation

RAC agrees with the DS to consider benfluralin as 'not rapidly degradable' due to a 5% degradation in 28 days in a ready biodegradability test (OECD TG 301D, Closed bottle test) and the substance being hydrolytically stable in sterile buffer solutions at pH 4, 7 and 9.

Bioaccumulation

Based on the value of log Kow of 5.27 ± 0.11 at 20 °C bioaccumulation could not be excluded. An experimentally determined BCF of 1740.8 L/kg was available, however the study did not meet the relevant validity criteria. RAC agrees with the DS that given the experimentally determined log Kow of 5.27, it can be concluded that benfluralin has the potential to bioaccumulate according to the CLP-criteria.

Acute aquatic toxicity

There were acute toxicity data available for three trophic levels.

The lowest available acute toxicity value was obtained with Mysid shrimp *Mysidopsis bahia* (LC_{50} = 0.043 mg/L). This endpoint is lower than the classification criterion for Category Acute 1 ≤ 1 mg/L. The appropriate M-factor is 10, since the toxicity is within the range 0.01 < $L(E)C_{50}$ ≤ 0.1.

Chronic aquatic toxicity

There were chronic toxicity data available for all three trophic levels.

The lowest available chronic toxicity value was observed in a fish study *Oncorhynchus mykiss* (Rainbow trout) (NOEC = 0.0019 mg/L). This endpoint is lower than the classification criterion for Category Chronic 1 ≤ 0.1 mg/L. The appropriate M-factor is 10, since the toxicity is within the range of 0.001 < NOEC ≤ 0.01 and the substance is non-rapidly degradable.

In conclusion, RAC agrees with the DS that Benfluralin warrants classification as:

- **Aquatic Acute 1; H400, M = 10 and**
- **Aquatic Chronic 1; H410, M = 10.**

12 EVALUATION OF ADDITIONAL HAZARDS

12.1 Hazardous to the ozone layer

An Ozone Depleting Potential (ODP) is not reported for benfluralin and it is not listed in Annex I to Regulation (EC) No. 1005/2009. This hazard is therefore not considered further in this report.

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

Data conclusive but not sufficient for classification

RAC evaluation of hazards to the ozone layer

Summary of the Dossier Submitter's proposal

An Ozone Depleting Potential is not reported for benfluralin and it is not listed in Annex I to Regulation (EC) No. 1005/2009. This hazard was therefore not considered further in this report. The overall DS conclusion was that no classification for hazards to the ozone layer is warranted in the presence of conclusive but not sufficient for classification data.

Comments received during consultation

No comments have been received.

Assessment and comparison with the classification criteria

RAC agrees with the DS conclusion for no classification for hazards to the ozone layer due to conclusive but not sufficient for classification data.

13 ADDITIONAL LABELLING

None

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15 ANNEXES

Annex I Annex I to CLH Report

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON BENFLURALIN (ISO); *N*-BUTYL-*N*-ETHYL-α,α,α-TRIFLUORO-2,6-DINITRO-*p*-TOLUIDINE

Annex I to the CLH Report is provided separately.