Committee for Risk Assessment

RAC

Annex 1

Background document

to the Opinion proposing harmonised classification and labelling at Community level of

Benzovindiflupyr (ISO);
***N*-[9-(dichloromethylene)-1,2,3,4-tetrahydro-1,4-methanonaphthalen-5-yl]-3-(difluoromethyl)-1-methyl-1*H*-pyrazole-4-carboxamide**

EC number: -
CAS number: 1072957-71-1

CLH-O-0000001412-86-28/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

04 December 2014

CLH report

Proposal for Harmonised Classification and Labelling

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

Substance Name: Benzovindiflupyr

EC Number: -

CAS Number: 1072957-71-1

Index Number: -

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Version number: 002 **Date:** March 2014

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Part A.

Part A.

1 PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

1.1 Substance

Table 1: Substance identity

Substance name:	benzovindiflupyr
EC number:	none
CAS number:	1072957-71-1
Annex VI Index number:	none
Degree of purity:	Minimum purity 96% w/w The substance consists of 2 enantiomers: N-[(1R,4S)-9-(dichloromethylidene)-1,2,3,4-tetrahydro-1,4-methanonaphthalen-5-yl]-3-(difluoromethyl)-1-methyl-1H-pyrazole-4-carboxamide and N-[(1S,4R)-9-(dichloromethylidene)-1,2,3,4-tetrahydro-1,4-methanonaphthalen-5-yl]-3-(difluoromethyl)-1-methyl-1H-pyrazole-4-carboxamide. The two main constituents are enantiomers. The substance contains both enantiomers at a 1:1 ratio, thus the substance is a racemate.
Impurities:	No impurities of toxicological or environmental significance

1.2 Harmonised classification and labelling proposal

Table 2: The current Annex VI entry and the proposed harmonised classification

	CLP Regulation	**Directive 67/548/EEC (Dangerous Substances Directive; DSD)**
Current entry in Annex VI, CLP Regulation	Not included	Not included
Current proposal for consideration by RAC	Acute Tox. 3 H301: Toxic if swallowed. H331: Toxic if inhaled. Aquatic Acute 1: H400: Very toxic to aquatic life. Aquatic Chronic 1: H410: Very toxic to aquatic life with long lasting effects. M-Factor acute: 100 M-Factor chronic: 100	Not necessary anymore

Resulting harmonised classification (future entry in Annex VI, CLP Regulation)	Acute Tox. 3 H301: Toxic if swallowed. H331: Toxic if inhaled. Aquatic Acute 1: H400: Very toxic to aquatic life. Aquatic Chronic 1: H410: Very toxic to aquatic life with long lasting effects. M-Factor acute: 100 M-Factor chronic: 100	

2.1 Proposed harmonised classification and labelling based on CLP Regulation criteria

Table 3: Proposed classification according to the CLP Regulation

CLP Annex I ref	Hazard class	Proposed classification	Proposed SCLs and/or M-factors	Current classification [1)2)]	Reason for no classification [3)]
2.1.	Explosives	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
2.2.	Flammable gases	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
2.3.	Flammable aerosols	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
2.4.	Oxidising gases	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
2.5.	Gases under pressure	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
2.6.	Flammable liquids	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
2.7.	Flammable solids	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
2.8.	Self-reactive substances and mixtures	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
2.9.	Pyrophoric liquids	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
2.10.	Pyrophoric solids	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
2.11.	Self-heating substances and mixtures	Not classified	Not applicable	Not classified	conclusive but not sufficient for

CLP Annex I ref	Hazard class	Proposed classification	Proposed SCLs and/or M-factors	Current classification [1)2)]	Reason for no classification [3)]
					classification
2.12.	Substances and mixtures which in contact with water emit flammable gases	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
2.13.	Oxidising liquids	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
2.14.	Oxidising solids	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
2.15.	Organic peroxides	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
2.16.	Substance and mixtures corrosive to metals	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
3.1.	Acute toxicity - oral	**Acute Tox. 3: H301**	Not applicable	Not classified	Not applicable
	Acute toxicity - dermal	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
	Acute toxicity - inhalation	**Acute Tox. 3: H331**	Not applicable	Not classified	Not applicable
3.2.	Skin corrosion / irritation	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
3.3.	Serious eye damage / eye irritation	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
3.4.	Respiratory sensitisation	Not classified	Not applicable	Not classified	No data available
3.4.	Skin sensitisation	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
3.5.	Germ cell mutagenicity	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
3.6.	Carcinogenicity	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
3.7.	Reproductive toxicity	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
3.8.	Specific target organ toxicity – single exposure	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
3.9.	Specific target organ toxicity – repeated exposure	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
3.10.	Aspiration hazard	Not classified	Not applicable	Not classified	Data lacking
4.1.	Hazardous to the aquatic	**Aquatic Acute**	**M-Factor acute:**	Not classified	Not applicable

CLP Annex I ref	Hazard class	Proposed classification	Proposed SCLs and/or M-factors	Current classification [1)2)]	Reason for no classification [3)]
	environment	**1; H400** **Aquatic Chronic 1; H410**	**100** **M-Factor chronic: 100**		
5.1.	Hazardous to the ozone layer	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification

[1)] Including specific concentration limits (SCLs) and M-factors
[2)] Data lacking, inconclusive, or conclusive but not sufficient for classification

Labelling: Signal word: Danger
Hazard statements: H301, H331, H410
Precautionary statements: P273, P301+P310, P304+P340, P391

Proposed notes assigned to an entry:

Note C : The supplier must state on the label that the substance is a mixture os isomers.

3 BACKGROUND TO THE CLH PROPOSAL

3.1 History of the previous classification and labeling

No previous classification and labeling agreed.

3.2 Short summary of the scientific justification for the CLH proposal

No classification is warranted for physical-chemical hazards.

With an oral Median Lethal Dose (MLD) of >55 and < 175 mg/kg bw and acute inhalation Median Lethal Concentration (MLC) of 0.56 mg/L, benzovindiflupyr warrants classification Acute Tox. 3: H301, H331 according to CLP. It has low dermal toxicity and is not a skin, eye or respiratory tract irritant, corrosive or sensitiser and, therefore, no classification is warranted under CLP for these hazard classes.

In repeated dose toxicity studies the main effect noted in all species was an initial body weight loss or a reduction in body weight gain. No evidence of immunotoxicity or neurotoxicity was seen in specific studies to address these endpoints. Specific target organ effects were minor. Increased liver weight and centrilobular hypertrophy in rats were concluded to be adaptive changes resulting from UDPGT induction and are not relevant to human health. Effects on kidney were observed in preliminary studies in rats and mice at high dose levels but not in the 90-day studies at similar dose levels. All effects were minor, occurred at dose levels associated with body weight effects and, as such, provide no evidence of specific target organ toxicity. Some evidence of gastrointestinal disturbance was seen in mice and dogs (soft faeces and mucosal hyperplasia in the rectum and colon in mice; vomiting, mucoid faeces in dogs). These effects were accompanied by effects on body weight, were of minimal severity and there was no evidence of organ dysfunction. Consequently, none of the effects reported are considered to warrant classification STOT RE under CLP.

Genotoxicity of benzovindiflupyr was tested in three *in vitro* and one *in vivo* test. The results of all studies were negative whilst positive and negative controls demonstrated the validity of the tests. Benzovindiflupyr can be considered as not genotoxic and no classification is warranted.

Carcinogenicity studies in rats and mice showed an increased incidence of thyroid follicular cell adenoma in rats but no evidence of carcinogenicity in the mouse. Additional studies have concluded that the thyroid tumours in male rats are attributable to induction of hepatic UDPGT, which results in a series of downsteam events, ultimately leading to tumourigenesis. The available data also demonstrates that this mode of action is not relevant for human hazard/risk assessment purposes. Indeed, there is known qualitative and quantitative differences between rats and human in response to UDPGT induction and increased T_3/T_4 clearance.

The reproductive toxicity of benzovindiflupyr has been investigated in a two generation reproduction toxicity study in the rat and developmental toxicity studies in rats and rabbits. No evidence of treatment-related effects on fertility, sexual function or other parameters of reproductive performance were seen and there was no indication of developmental toxicity.

In aquatic toxicity studies the relevant (lowest) acute LC_{50} value for technical benzovindiflupyr (pure active substance + impurities) was observed for *C. carpio* with an acute 96 hr LC50 of 0.0035 mg a.s./L. In long-term toxicity studies, the lowest NOEC (32 d) of 0.00095 mg/L was for the fish, *P. promelas*. As benzovindiflupyr is not rapidly biodegradable, it fullfils the criteria for environmental classification.

3.3 Current harmonised classification and labelling

3.3.1 Current classification and labelling in Annex VI, Table 3.1 in the CLP Regulation

Not included in Annex VI.

3.3.2 Current classification and labelling in Annex VI, Table 3.2 in the CLP Regulation

Not included in Annex VI.

3.4 Current self-classification and labelling

3.4.1 Current self-classification and labelling based on the CLP Regulation criteria

Classification and labelling notified to CLP inventory

Classification:	Acute Tox 3; H301
	Acute Tox 3; H331
	Aquatic Acute 1; H400 (M-Factor: 100)
	Aquatic Chronic 1; H410 (M-Factor: 100)
Labelling:	Hazard Statement Code: H301+H331; H410
	Precautionary Statement Code: P273, P301+P310, P304+P340, P391
	Signal word: Danger
	Hazard Pictogram: GSH06, GSH09
	Supplemental hazard statement code: None

4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

Application has been made for the approval of benzovindiflupyr for use as a pesticidal active substance under Regulation (EC) No 1107/2009, with the France as the Rapporteur Member State. Within this context, classification and labeling according to Regulation (EC) No 1272/2008 should be agreed at Community level.

Part B.

SCIENTIFIC EVALUATION OF THE DATA

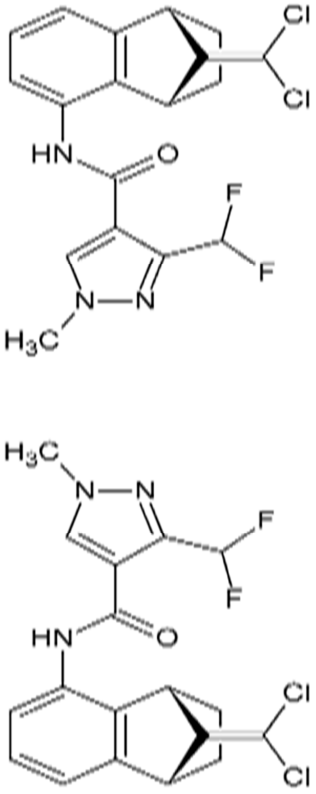
1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 5: Substance identity

EC number:	
EC name:	benzovindiflupyr
CAS number (EC inventory):	1072957-71-1
CAS number:	1072957-71-1
CAS name:	1H-pyrazole-4-carboxamide, N-[9-(dichloromethylene)-1,2,3,4-tetrahydro-1,4-methanonaphthalen-5-yl]-3-(difluoromethyl)-1-methyl-
IUPAC name:	N-[(1RS,4SR)-9-(dichloromethylene)-1,2,3,4-tetrahydro-1,4-methanonaphthalen-5-yl]-3-(difluoromethyl)-1-methylpyrazole-4-carboxamide
CLP Annex VI Index number:	-
Molecular formula:	$C_{18}H_{15}Cl_2F_2N_3O$
Molecular weight range:	398.2

Structural formula:



1.2 Composition of the substance

Table 6: Constituents (non-confidential information)

Constituent	Typical concentration	Concentration range	Remarks
Benzovindiflupyr	≥96% w/w	-	-
N-[(1R,4S)-9-(dichloromethylidene)-1,2,3,4-tetrahydro-1,4-methanonaphthalen-5-yl]-3-(difluoromethyl)-1-methyl-1H-pyrazole-4-carboxamide	≥48 %	-	-
N-[(1S,4R)-9-(dichloromethylidene)-1,2,3,4-tetrahydro-1,4-methanonaphthalen-5-yl]-3-(difluoromethyl)-1-methyl-1H-pyrazole-4-carboxamide	≥48%	-	-

Table 7: Impurities (confidential information)

Impurity	Typical concentration	Concentration range	Remarks
Confidential	-	-	-

Impurities in benzovindiflupyr present at quantities ≥ 1 g/kg. The impurities have been taken into consideration in the classification of this substance. Details on the impurities are considered to be confidential and further information is provided in the technical (IUCLID) CLH dossier.

Table 8: Additives (confidential information)

Additive	Function	Typical concentration	Concentration range	Remarks
-	-	-	-	Not relevant

1.2.1 Composition of test material

The purity of Benzovindiflupyr tested in the studies ranged from 96.8-99.4% w/w. Information on the actual composition used is provided in the relevant tables of this report and also in associated IUCLID summaries (where provided). The tested material in all cases is considered to be equivalent to and representative of that specified above.

1.3 Physico-chemical properties

Table 9: Summary of physico-chemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
State of the substance at 20°C and 101,3 kPa	Pure grade substance: colour: white, physical state: solid (powder), odour: odourless technical grade substance (pure active substance + impurities): colour: off-white, physical state: solid (powder), odour: odourless	Das, 2010a & b	Visual assessment Technical grade purity: 97.7% Pure grade purity: 99.4%
Melting/freezing point	148.4°C at 101.3 kPa	Kühne 2010a	OECD 102 Test material purity: 99.4%
Boiling point	boiling point: decomposition of the substance started at about 285°C before boiling occurred (at 100.7 kPa)	Kühne 2010b	OECD 103 Test material purity: 99.4%
Relative density	density at 20°C: 1.466 g/mL	Kühne 2010c	OECD 109 Test material purity: 99.4%
Vapour pressure	vapour pressure at 25 °C: 3.2×10^{-9} Pa	Weissenfeld, 2010	OECD 104 Test material purity: 99.4%
Surface tension	63.0 mN/m at 20°C (technical grade)	Kühne, 2010d	OECD 115 Test material purity: 97.7%
Water solubility	water solubility at 25°C: 0.98 mg/L	Vijayakumar, 2011	OECD 105 Test material purity: 99.4%
Partition coefficient n-octanol/water	Pow at 25°C: 21000 and log Pow at 25°C: 4.3	Vijayakumar, 2010	OECD 107 Test material purity: 99.4%
Flash point	not applicable	The substance is a solid with a melting point of 148.4°C and is not handled in the molten state. Knowledge of the flash point is not considered necessary	

Property	Value	Reference	Comment (e.g. measured or estimated)
		for safe handling.	
Flammability	No ignition detected below the melting point	Jackson, 2010	EC Method A.16 Test material purity: 97.7%
Explosive properties	not explosive (sensitivity to shock, friction or heating under confinement)	Jackson, 2010	EC Method A.14 Test material purity: 97.7%
Self-ignition temperature	No ignition detected below the melting point	Jackson, 2010	EC Method A.16 Test material purity: 97.7%
Oxidising properties	not oxidising	Jackson, 2010	EC Method A.17 Test material purity: 97.7%
Granulometry	median (particle size distribution): 6.05 µm (dispersion in a liquid)	Das, 2010c	CIPAC method 187 Technical grade purity: 98.2% & 97.7%
Stability in organic solvents and identity of relevant degradation products	Not applicable Inspection of the chemical structure and experience in the handling and use of the substance have not indicated that stability in organic solvents is of concern.		
Dissociation constant	no pKa value of the substance within the range 2.0 to 12.0	Kühne 2010e	OECD 112 Test material purity: 99.4%
Viscosity	not applicable (the substance is a solid and hence testing for viscosity is not possible)		

2 MANUFACTURE AND USES

2.1 Manufacture

The active substance is manufactured inside and outside of the EU.

2.2 Identified uses

Benzovindiflupyr is proposed for use as a fungicide in the EU (not yet approved under EC Reg.1107/2009).

3 CLASSIFICATION FOR PHYSICO-CHEMICAL PROPERTIES

Table 10: Summary table for relevant physico-chemical studies

Method	Results	Remarks	Reference
Not applicable – no relevant properties for classification (see Table 9)			

3.1 Physical-chemical properties

3.1.1 Summary and discussion of physical-chemical properties

The physico-chemical properties of benzovindiflupyr are summarised in Table 9. There is no property which warrants classification under CLP.

3.1.2 Comparison with criteria

As detailed in Table 9, benzovindiflupyr does not meet the criteria for classification for physico-chemical properties under either CLP.

3.1.3 Conclusions on classification and labelling

CLP: No classification

RAC evaluation of physical hazards
Summary of the Dossier submitter's proposal The results of the tests on physico-chemical properties indicate that benzovindiflupyr is neither explosive, flammable nor self-reactive. The dossier submitter (DS) proposed no classification for physical hazards. **Comments received during public consultation** No comment were received during the public consultation. **Assessment and comparison with the classification criteria** RAC agrees with the DS that no classification for physical hazards is warranted.

4 HUMAN HEALTH HAZARD ASSESSMENT

4.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

4.1.1 Non-human information

The mammalian metabolism of benzovindiflupyr has been assessed in studies investigating the absorption, distribution, metabolism and excretion of benzovindiflupyr in rats (Green and MacDonald, 2011; Shaw, 2011a, 2011b, 2011c, 2011 d, 2011e, 2011f). In a biotransformation study, the nature of the metabolites formed (both qualitative and quantitative) was determined. The fate of benzovindiflupyr following both single and multiple doses was also investigated. Preliminary investigations of biliary elimination, pharmacokinetics and biotransformation using [pyrazole-5-^{14}C]- or [phenyl-U-^{14}C]- radiolabelled benzovindiflupyr indicated that very little cleavage (<3%) of benzovindiflupyr occurred between the pyrazole and phenyl moieties. Similarly, in a Quantitative whole-body autoradiography (QWBA) study there were no clear differences in tissue distribution profiles between the [pyrazole-5-^{14}C]- or [phenyl-U-^{14}C]-benzovindiflupyr. Therefore, based on the results of these preliminary studies, subsequent ADME studies used only [pyrazole-5-^{14}C]- radiolabeled material.

Absorption

The oral absorption of total radioactivity was estimated following a single low or high oral gavage dose of benzovindiflupyr (1 or 40 mg/kg bw) to bile duct cannulated male and female rats (Shaw, 2011c). Oral absorption was estimated as the radioactivity present in urine, bile, cage wash and carcass up to 48 hours post dose by which time the majority of the administered radioactivity had been excreted (97 and 89% following 1 mg/kg bw and 90 and 86% following 40 mg/kg bw in males and females, respectively). Absorption was similar in male and female rats following both doses. Absorption following 1 mg/kg bw (low dose) was estimated to be 81% in males and 79% in females. Following the 40 mg/kg bw (high dose), absorption was estimated to be 61 and 62% in males and females, respectively.

A study was conducted to examine the kinetics of total radioactivity in the blood following oral and intravenous administration of [^{14}C]-benzovindiflupyr to rats. The oral bioavailability was determined by comparing the dose normalised exposure following intravenous and oral administration of [^{14}C]-benzovindiflupyr. The systemic oral bioavailability of total radioactivity, after oral administration of 1 mg/kg bw [^{14}C] benzovindiflupyr, in males of 129% and 99% in females indicates that absorption of total radioactivity was essentially complete.

Excretion

Irrespective of dose or sex, following a single oral dose of 1 or 40 mg/kg bw of benzovindiflupyr in rat, radioactivity was distributed throughout the body and was rapidly eliminated with the majority being excreted within the first 72 hours post dose (95 and 91% following the low dose and 97 and 91% following the high dose in males and females, respectively) (Shaw, 2011f). Following both doses the predominant route of elimination was *via* the faeces. Following a single oral dose of 1 mg/kg bw, elimination *via* the faeces accounted for 84 and 90% of the administered dose and urinary excretion accounted for 12 and 6 % of the administered dose in males and females, respectively. Following a single oral dose of 40 mg/kg bw, the faeces accounted for 93 and 90% of the administered dose in males and females, respectively, with urinary excretion accounting for 7% in both sexes.

With the high recovery of radioactivity in faeces, biliary elimination was shown to be an important route of excretion following both doses in both sexes (Shaw, 2011c). In the 48 hours following dosing 76 and 69% of the administered dose was excreted via bile following 1 mg/kg bw and 47 and 57% of the administered dose was excreted via bile following 40 mg/kg bw in males and females, respectively.

In a preliminary study (QWBA), using both radiolabels, and in the excretion and distribution study, using pyrazole labelled benzovindiflupyr, radioactivity was measured in expired air over the first 24 hours post dose and shown to be negligible (<0.1% of administered dose) in both sexes. This was consistent with the generally high recoveries of dose and the anticipated metabolically stable location of the radiolabels in the molecule.

Distribution

In the preliminary QWBA study (Shaw, 2011f) using both radiolabels of benzovindiflupyr total radioactivity was extensively distributed throughout the body by the first sampling time of 5 hours in males and 1 hour in females and had declined markedly in both sexes by 72 hours post dose. There were no clear differences in tissue distribution profiles between the labels nor were any pronounced sex or dose differences apparent. The highest tissue concentrations were present in the Harderian gland and liver with lower concentrations in the adrenal gland, brown fat and kidney.

The excretion and distribution study demonstrated that following both doses in both sexes the residues of radioactivity at 7 days post dose were very low in the tissues and carcass with only 1.8 and 1.0% of the dose remaining following the 1 mg/kg bw dose of benzovindiflupyr and 1.4 and 0.8% remaining following the 40 mg/kg bw dose in males and females, respectively.

Seven days following administration of the low dose to male rats, radioactivity was detected in the blood and plasma at a concentration of 0.033 and 0.040 µg equiv/g, respectively. Mean tissue concentrations in kidney and liver were 0.055 µg equiv/g and 0.046 µg equiv/g, respectively. However, concentrations of radioactivity in all other tissues were below that of the blood concentration. Radioactivity in the blood and plasma of female rats was 0.004 and 0.003 µg equiv/g, respectively. The highest mean tissue concentrations were also present in the kidney and liver, with a common mean of 0.016 µg equiv/g. Lower concentrations of radioactivity were also found in the thyroid, adrenals and renal fat ranging between 0.013-0.015 µg equiv/g. All other tissues, except bone mineral and brain were also above that of the blood concentration.

Seven days following administration of the high dose to male rats, the concentration of radioactivity in the blood and plasma was 0.53 and 0.63 µg equiv/g, respectively. The highest mean tissue concentrations were present in the kidney and liver at 1.48 and 1.30 µg equiv/g, respectively. Progressively lower concentrations, but above those in blood, were present in the thyroid, heart, adrenal glands, pancreas, lungs and spleen. Concentrations of radioactivity in the remaining tissues were below that of the blood concentration or were not reliably detected. The concentration of radioactivity in the blood and plasma of female rats was 0.23 and 0.15 µg equiv/g, respectively. The highest mean tissue concentrations were present in the kidney and the liver at 0.82 and 0.76 µg equiv/g, respectively. Progressively lower concentrations, but above those in blood, were present in the renal fat, heart, pancreas, lungs, spleen and ovaries. Concentrations of radioactivity in the remaining tissues were below that of the blood concentration or were not reliably detected.

Pharmacokinetics

Following a single oral dose of benzovindiflupyr to male and female rats, peak plasma concentrations of radioactivity were reached after approximately 2 - 4 hours and 6 - 24 hours following doses of 1 and 40 mg/kg bw, respectively (Shaw, 2011b). Following the low dose the terminal phase half-life was 55 hours in males and 28 hours in females and following the high dose was 30 hours and 33 hours in males and females, respectively. Systemic exposure to total radioactivity was comparable between plasma and whole blood but there was evidence to suggest a greater systemic exposure to total radioactivity in males compared to females. Increases in exposure with respect to Cmax and AUC were generally less than proportional with the increase in dose but there appeared to be a trend towards broad dose proportionality in AUC estimates in females.

Tissue depletion on radioactivity following a single oral dose of radiolabelled benzovindiflupyr

A single oral dose of 1 mg or 40 mg/kg bw of benzovindiflupyr was administered to male and female rats to investigate the tissue distribution of radioactivity (Shaw 2011d). At intervals over a period of 6 days after dosing, the rats were killed in groups of 3 per sex and residual radioactivity was measured in selected

tissues/organs and the remaining carcasses. Tissue distribution was extensive throughout and generally similar between the sexes following both doses. Following an initial rapid decline terminal phase half-life estimates for tissue depletion appeared slightly longer in male animals than in female animals following the low dose. However, this trend was not apparent following the high dose and, therefore, may not reflect real physiological differences between the sexes. Collectively tissue concentrations of radioactivity were highest at the first sampling time point and progressively declined thereafter with terminal phase half-lives ranging between 1.4 and 8.8 days. The calculation of half-lives for some tissues was made difficult because of low and variable tissue concentrations measured over the course of the study.

The highest concentration of radioactivity following both doses was present in the liver of both sexes with liver, kidney and adrenal concentrations remaining the highest throughout the course of the study. The total residues in tissues and carcass at the end of the study accounted for 2.4% of the dose in males and 2.0% in females following the low dose and for just 1.1% and 3.8% following the high dose in males and females, respectively.

Tissue depletion on radioactivity following repeated oral dosing of radiolabelled benzovindiflupyr

Daily oral doses of 1 mg/kg bw benzovindiflupyr was administered to male rats for 14 days to determine the extent of accumulation of radioactivity in tissues and the remaining carcasses and its subsequent elimination (Shaw, 2011d). Radioactivity was well distributed into the tissues and the concentration generally increased during the period of dosing and appeared to be approaching steady state concentrations by the end of the 14 day dosing period. Following the cessation of dosing, all tissue concentrations steadily declined.

Tissue concentrations of radioactivity were highest in the liver and kidney consistent with both biliary and urinary elimination of [^{14}C]-benzovindiflupyr and its metabolites. Following the liver and kidney, the adrenals and thyroid had the greatest concentrations of radioactivity with residues in all other tissues being generally below plasma concentrations until around 10 days post dose 14. Thereafter, concentrations were generally above those in plasma and by the final sampling time (63 days post dose 14) concentrations were still measurable in most tissues but, were approaching the limit of reliable measurement. The total tissue and carcass residues at the final sampling time accounted for less than 0.2% of the total radiolabelled dose administered.

As was observed following a single dose the terminal phase half-life for tissue depletion was variable reflecting the low concentrations at or around the limit of reliable measurement for several tissues. As a result the shortest estimate was obtained in the plasma and the longest in the testes being 2.5 and 69 days, respectively.

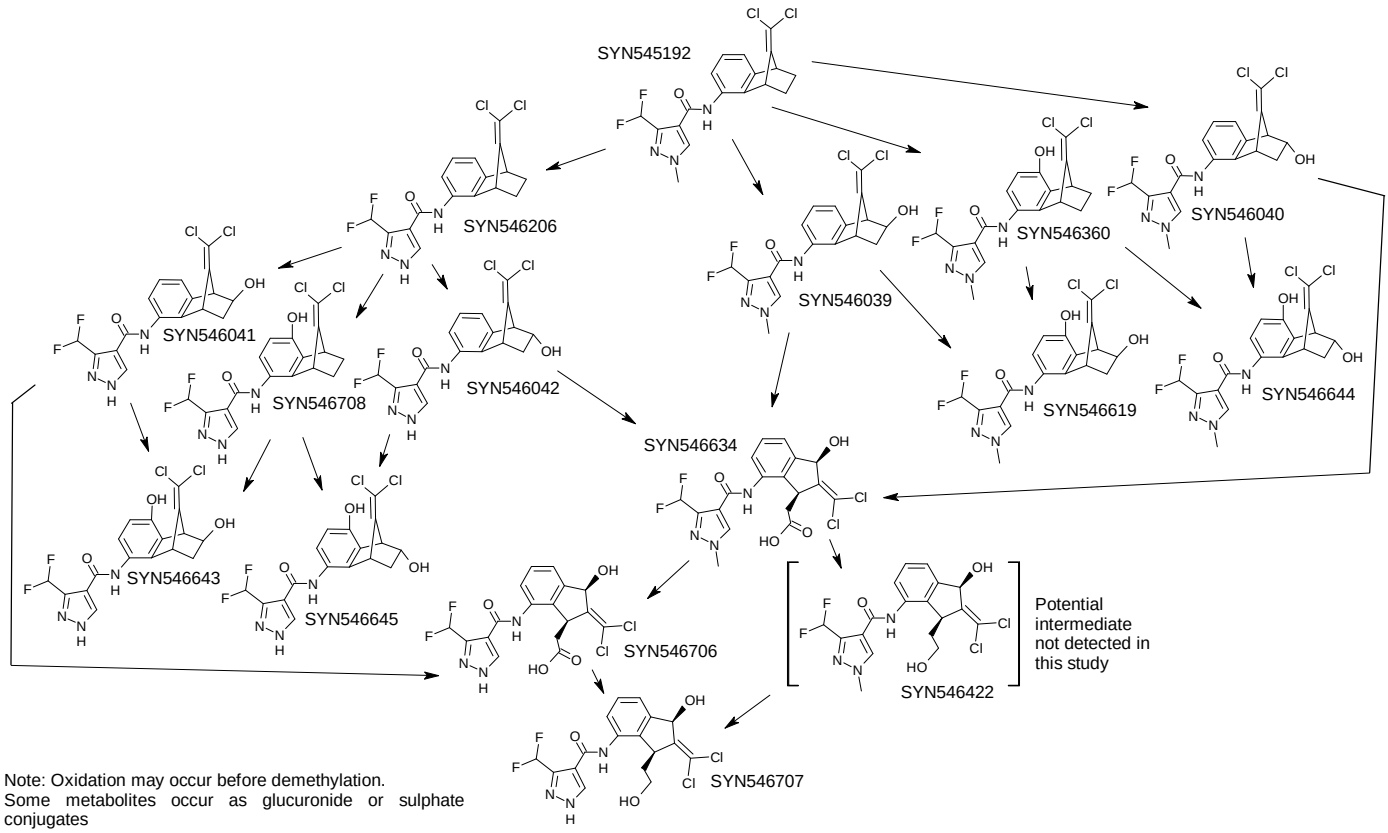
Biotransformation

Benzovindiflupyr was extensively metabolised in rat giving rise to at least 8 types of metabolite (*e.g.* desmethyl, hydroxy, dihydroxy, desmethyl hydroxy, desmethyl dihydroxy, ring-open, glucuronide conjugate, sulphate conjugate) (Green and MacDonald, 2011). The majority of the administered radioactivity (70-85% dose) was identified following a single oral dose of benzovindiflupyr. The major metabolites were identified as SYN546041, SYN546360, SYN546643, SYN546645 and SYN546619. Other identified components included SYN546039, SYN546042, SYN546708 and SYN546644. Glucuronide and, in some cases, sulphate conjugates of these metabolites were also present. While some quantitative differences were observed between males and females, SYN546041 and SYN546360 together accounted for a major proportion of the dose (35-60% dose). No significant differences were observed following a single dose at 40 mg/kg bw or 1 mg/kg bw or repeat daily dosing at 1 mg/kg bw/day. Minor differences were observed between males and females as indicated by the presence of ring-open metabolites, SYN546634, SYN546706 and SYN546707 primarily in males and the presence of a sulphate conjugate of SYN546042 in females only. There was little evidence to indicate cleavage of benzovindiflupyr between the pyrazole and phenyl moieties with possible metabolites present at <2% of the dose in urine only being consistent with the results of the preliminary study.

The biotransformation proceeded by:

- Formation of SYN546206 by N-demethylation of benzovindiflupyr

- Hydroxylation and demethylation to give the major metabolite SYN546041
- Hydroxylation of benzovindiflupyr to give the major phenolic metabolite SYN546360
- Hydroxylation of both benzovindiflupyr and SYN546206 to give the metabolites SYN546039, SYN546360, SYN546040, SYN546042 and SYN546708
- Further hydroxylation to give dihydroxylated metabolites of both benzovindiflupyr and SYN546206 (*e.g.* SYN546619, SYN546644, SYN546645 and SYN546643)
- Opening of the bicyclo moiety of both benzovindiflupyr and SYN546206 to give metabolites SYN546634, SYN546706 and SYN546707
- Glucuronic acid conjugation and some sulphate conjugation



4.1.2 Human information

No information.

4.1.3 Summary and discussion on toxicokinetics

See 4.1.1 above.

4.2 Acute toxicity

4.2.1 Non-human information

Table 11: Summary table of relevant acute toxicity studies

Method	Results	Remarks	Reference
rat (CRL:(WI)BR) female oral: gavage (vehicle 1% carboxymethylcellulose) Test material purity: 97% Doses: 17.5, 55, 175 mg/kg bw. OECD Guideline 425 (Acute Oral Toxicity: Up-and-Down Procedure) GLP	LD_{50}: 55 mg/kg bw (female) based on: test mat.	**175 mg/kg bw** 3 females dosed, one died 6 hours after dosing, the remaining 2 died on day 1. Clinical signs included decreased activity (3/3), prone position (3/3), incoordination (3/3), piloerection (3/3), dyspnoea (3/3), decreased respiratory rate (1/3), clonic convulsion (1/3), decreased body temperature (3/3). **55 mg/kg bw** 4 females dosed, 3/4 survived, 1/4 died 2 hours after dosing. Clinical observations decreased activity (4/4), dyspnoea (4/4), incoordination (4/4) and hunched back (1/4). **17.5 mg/kg bw** 1 female dosed, survived, no clinical observations.	Tavaszi J (2010)
rat (Wistar CRL:(WI)BR) male/female inhalation: aerosol of dust (nose only) Test material purity: 97% Single dose: 0.5 mg/L (nominal); 0.56 mg/L (analytical concentration). OECD Guideline 403 (Acute Inhalation Toxicity) GLP	LC_{50} (4 h): > 0.56 mg/L air (male/female) based on: test mat. (nose only)	One female was found dead on day 1. Significant clinical signs noted during exposure: increased respiratory rate (2/5 males, 2/5 females) and laboured respiration (5/5 males, 5/5 females). Significant observations on removal from restraint and/or after 1 hour: laboured respiration (5/5 males, 4/5 females), ataxia (1/5 males, 4/5 females), lethargy (1/5 males, 4/5 females) and clonic convulsions (1/5 females). Three of the four surviving females were emaciated for a couple of days after exposure. The majority of animals recovered from Day 3 with exception of one female where clinical signs persisted until Day 10.	Nagy K (2010)
rat (CRL:(WI)BR) male/female Single dose: 2000 mg/kg bw Test material purity: 97% Coverage: semiocclusive OECD Guideline 402 (Acute Dermal Toxicity) GLP	LD_{50}: > 2000 mg/kg bw (male/female) based on: test mat.	No mortality, no adverse clinical or dermal signs were observed in any of the animals. There were no effects on bodyweight and no macroscopic findings at necropsy.	Zelenák V (2010a)

4.2.1.1 Acute toxicity: oral

The estimated acute median lethal dose (MLD) for benzovindiflupyr via the oral route was 55 mg/kg bw based on an acute up-and-down procedure (Tavazi, 2010a). However, in a single dose neurotoxicity study (Sommer, 2011a; Table 21) there was no mortality at 80 mg/kg bw. Based on the mortality pattern (3/3 at 175 mg/kg bw; 0/20 at 80 mg/kg bw; 1/4 at 55 mg/kg bw) it can be concluded that the MLD lies within the range >55 < 175 mg/kg bw.

4.2.1.2 Acute toxicity: inhalation

The median lethal concentration (MLC) following acute inhalation exposure to aerosol of the solid substance was estimated to be 0.56 mg/L (Nagy, 2010).

4.2.1.3 Acute toxicity: dermal

The acute dermal MLD was shown to be >2000 mg/kg bw (Zelenák, 2010a).

4.2.1.4 Acute toxicity: other routes

No information.

4.2.2 Human information

No information.

4.2.3 Summary and discussion of acute toxicity

The estimated acute oral median lethal dose (MLD) for benzovindiflupyr was >55 and < 175 mg/kg bw and the median lethal concentration (MLC) following acute inhalation exposure was estimated to be 0.56 mg/L. The acute dermal MLD was shown to be >2000 mg/kg bw.

4.2.4 Comparison with criteria

With an oral MLD of >55 < 175 mg/kg bw and acute inhalation MLC of 0.56 mg/L, benzovindiflupyr warrants classification as Acute Tox. 3: H301, H331 according to CLP.

It has low dermal toxicity (MLD > 2000 mg/kg bw) and, therefore, no classification is warranted under CLP.

4.2.5 Conclusions on classification and labelling

CLP: Acute Tox 3; H301 + H331

RAC evaluation of acute toxicity
Summary of the Dossier submitter's proposal Three studies on acute toxicity, one for each route of exposure, were included in the CLH report. In an acute oral toxicity study (conducted in accordance with OECD test guideline (TG) 425) with female rats, mortalities were observed at the middle dose (55 mg/kg bw, 1 animal) and at the high dose (175 mg/kg bw, all animals) (Tavaszi, 2010). No deaths were observed in an oral single dose neurotoxicity study up to the highest dose of 80

mg/kg bw (Sommer, 2011a).
Based on the results of these two studies, the acute oral median lethal dose (MLD) for benzovindiflupyr was estimated to be >55 and <175 mg/kg bw. The DS proposed to classify benzovindiflupyr as Acute Tox. 3; H301.

A dermal limit dose study (conducted in accordance with OECD TG 402) reported no mortalities when male and female rats were administered 2000 mg/kg bw (Zelenák, 2010a). No classification was proposed for acute dermal toxicity.

The acute inhalation toxicity was evaluated in a GLP study (OECD TG 403) in rats. In this study, one female was found dead on day 1, while significant clinical signs were noted during exposure in all animals. The median lethal concentration (MLC) was estimated to be >0.56 mg/L. The DS proposed to classify benzovindiflupyr as Acute Tox. 3; H331 in accordance with the classification criteria for inhalation of dusts and mists.

Comments received during public consultation
No comments were received during public consultation.

Assessment and comparison with the classification criteria
RAC agrees with the assessment of the DS that the MLD for benzovindiflupyr is >55 and <175 mg/kg bw, and therefore based on the comparison of the oral MLD with the criteria, RAC agrees with the conclusion of the DS that benzovindiflupyr should be classified as acute Tox. 3; H301.

For the inhalation route, RAC discussed the feasibility of classification based on the a minimum MLC value , i.e. >0.56 mg/L (proposed by the DS), which may be considered inconclusive.
Based on evident toxicity observed during the fixed dose study the classification as acute Tox. 3; H331 is warranted.

RAC concludes, in agreement with the DS proposal, to classify the substance for inhalation toxicity as acute Tox. 3; H331 in accordance with CLP.

As the dermal LD_{50} was estimated to be >2000 mg/Kg RAC agrees with the conclusion of the DS that benzovindiflupyr should not be classified for dermal toxicity in accordance with CLP.

4.3 Specific target organ toxicity – single exposure (STOT SE)

4.3.1 Summary and discussion of Specific target organ toxicity – single exposure

All clinical signs observed in the acute toxicity studies via the oral, dermal and inhalation routes (see Tables 11 and 21) were considered to be non-specific signs of general acute toxicity. The acute neurotoxicity study demonstrated no evidence of neurotoxicity at 80 mg/kg bw.

4.3.2 Comparison with criteria

Substances that have produced significant non-lethal toxicity in humans or that, on the basis of evidence from studies in experimental animals, can be presumed to have the potential to produce significant non-lethal toxicity in humans following single exposure, are classified as STOT SE 1 or 2. Classification is supported by evidence associating single exposure to the substance with a constant and identifiable effect.

Classification in STOT SE 3 is reserved for transient target organ effects and is limited to substances that have narcotic effects or cause respiratory tract infection.

The signs that were apparent after single oral and inhalation exposure (no adverse effects were observed after dermal exposure) to benzovindiflupyr were indicative of non-specific, general acute toxicity. As there was no clear evidence of specific effects on a target organ or tissue that were independent of mortalities, no definitive signs of respiratory tract irritation or narcotic effects, no classification for specific target organ toxicity (single exposure) under CLP is required.

4.3.3 Conclusions on classification and labelling

CLP: No classification

RAC evaluation of specific target organ toxicity – single exposure (STOT SE)
Summary of the Dossier submitter's proposal All clinical signs observed in the acute toxicity studies via the oral, dermal and inhalation routes were considered to be non-specific signs of general acute toxicity. The acute neurotoxicity study demonstrated no evidence of neurotoxicity at 80 mg/kg bw. Therefore, the dossier submitter concluded that no classification is warranted for STOT SE. **Comments received during public consultation** No comments were received during public consultation. **Assessment and comparison with the classification criteria** The signs that were apparent after single oral and inhalation exposure (no adverse effects were observed after dermal exposure) to benzovindiflupyr were indicative of non-specific, general acute toxicity. There was no clear evidence of specific effects on a target organ or tissue that were independent of mortalities, no definitive signs of respiratory tract irritation or narcotic effects. In the absence of constant and identifiable effects, RAC agrees with the DS conclusion that benzovindiflupyr need not be classified for STOT SE in accordance with CLP.

4.4 Irritation

4.4.1 Skin irritation

4.4.1.1 Non-human information

Table 12: Summary table of relevant skin irritation studies

Method	Results	Reference
rabbit (New Zealand White) Purity: 97% 0.5 g of benzovindiflupyr technical Vehicle: none Coverage: semiocclusive OECD Guideline 404 (Acute	Mean scores at 24, 48 and 72 hours for each of three rabbits: Erythema: 0.3, 0, 0 (mean: 0.1); Oedema: 0, 0, 0 (mean: 0)	Zelenák V (2010b)

Dermal Irritation / Corrosion) GLP		

4.4.1.2 Human information

No information.

4.4.1.3 Summary and discussion of skin irritation

No signs of oedema were observed in any animal. Very slight erythema was seen in all rabbits at 1 hour which persisted to 24 hours in one animal.

4.4.1.4 Comparison with criteria

The application of benzovindiflupyr did not result in any significant signs of skin irritation. Therefore, benzovindiflupyr does not meet the criteria for classification according to CLP regulation.

4.4.1.5 Conclusions on classification and labelling

CLP: No classification

RAC evaluation of skin corrosion/irritation

Summary of the Dossier submitter's proposal

One rabbit skin irritation study, conducted according to OECD TG 404, was summarised in the CLH report (Zelenák, 2010b). No signs of oedema were observed in any animal; the mean scores for each of the three rabbits at 24, 48 and 72h were all equal to zero. Very slight erythema was seen in all rabbits at 1 hour which persisted to 24 hours in one animal; the mean scores at 24, 48 and 72h were 0.3, 0 and 0 respectively. The DS proposed no classification for skin corrosion/irritation.

Comments received during public consultation

No comments were received during public consultation.

Assessment and comparison with the classification criteria

Dermal exposure to benzovindiflupyr did not result in any significant signs of skin corrosion/irritation.

Therefore, RAC agrees with the DS that benzovindiflupyr should not be classified for skin corrosion/ irritation in accordance with CLP.

4.4.2 Eye irritation

4.4.2.1 Non-human information

Table 13: Summary table of relevant eye irritation studies

Method	Results	Reference

rabbit (New Zealand White Rabbit, SPF) Purity: 97% 0.1g benzovindiflupyr Vehicle: none OECD Guideline 405 (Acute Eye Irritation / Corrosion) (adopted April 24, 2002) GLP	Mean scores at 24, 48 and 72 hours each of 3 rabbits: Cornea: 0, 0, 0 (mean: 0) Iris: 0, 0, 0 (mean: 0) Conjunctiva (redness): 1, 1, 1 (mean: 1) Conjunctiva (chemosis): 0, 0, 0 (mean: 0)	Mallaun M (2011)

4.4.2.2 Human information

No information.

4.4.2.3 Summary and discussion of eye irritation

A study has been conducted (Mallaun, 2011). One hour after application, conjunctival redness was noted in all animals which persisted in two animals until 72 hours after treatemtn and in one animal until 7 days after treatment. There were no corneal or iridial effects in any animal and all animals showed full recovery at 10 days after treatment.

4.4.2.4 Comparison with criteria

Benzovindiflupyr was mildly irritating to the eyes with transient signs of irritation that reversed after 10 days post-treatment. All mean irritation scores were <2, therefore, no classification is required in accordance with CLP.

4.4.2.5 Conclusions on classification and labelling

CLP: No classification

RAC evaluation of eye corrosion/irritation

Summary of the Dossier submitter's proposal

One rabbit eye irritation study, conducted according to OECD TG 405, was summarised in the CLH report (Mallaun, 2011). In this study, conjunctival redness was noted in all animals one hour after application, which persisted in two animals until 72 hours after treatment and in one animal until 7 days after treatment. There were no corneal or iridial effects in any animal and all animals showed full recovery at 10 days after treatment. Dossier Submitter proposes no classification for this endpoint.

Comments received during public consultation

No comments were received during public consultation.

Assessment and comparison with the classification criteria

Benzovindiflupyr was mildly irritating to the eyes with transient signs of irritation (conjunctival redness) that reversed after 10 days post-treatment.

All mean irritation scores were <2, therefore, RAC agrees with the DS conclusion that no classification is required in accordance with CLP.

4.4.3 Respiratory tract irritation

4.4.3.1 Non-human information

See 4.3 above (STOT SE).

No repeated dose toxicity studies via the inhalation route have been conducted.

4.4.3.2 Human information

No information.

4.4.3.3 Summary and discussion of respiratory tract irritation

Benzovindiflupyr is not a respiratory irritant.

4.4.3.4 Comparison with criteria

There is no data to discuss the effect of Benzovindiflupyr as a respiratory irritant, therefore, no classification is discussed.

4.4.3.5 Conclusions on classification and labelling

CLP: No classification

4.5 Corrosivity

4.5.1 Non-human information

Table 14: Summary table of relevant corrosivity studies

Method	Results	Remarks	Reference
Not relevant			

No signs of corrosion were observed in the available irritation studies with benzovindiflupyr (see section 4.4 above).

4.5.2 Human information

No information.

4.5.3 Summary and discussion of corrosivity

No evidence of corrosion was observed in the available irritation studies with benzovindiflupyr.

4.5.4 Comparison with criteria

Benzovindiflupyr was not corrosive in the available irritation studies, does not have a pH of ≤2 or ≥11.5, and, therefore, does not meet the criteria for classification according to CLP.

4.5.5 Conclusions on classification and labelling

CLP: No classification

RAC evaluation of skin corrosion/irritation

Summary of the Dossier submitter's proposal

One rabbit skin irritation study, conducted according to OECD TG 404, was summarised in the CLH report (Zelenák, 2010b). No signs of oedema were observed in any animal; the mean scores for each of the three rabbits at 24, 48 and 72h were all equal to zero. Very slight erythema was seen in all rabbits at 1 hour which persisted to 24 hours in one animal; the mean scores at 24, 48 and 72h were 0.3, 0 and 0 respectively. The DS proposed no classification for skin corrosion/irritation.

Comments received during public consultation

No comments were received during public consultation.

Assessment and comparison with the classification criteria

Dermal exposure to benzovindiflupyr did not result in any significant signs of skin corrosion/irritation.

Therefore, RAC agrees with the DS that benzovindiflupyr should not be classified for skin corrosion/ irritation in accordance with CLP.

4.6 Sensitisation

4.6.1 Skin sensitisation

Table 15: Summary table of relevant skin sensitisation studies

Method	Results	Reference
mouse (CBA/J Rj) female Local lymph node assay 5 or 6 females/group Test material purity: 97% 25 µL to each ear Concentrations: 0, 0.01, 0.1, 1.0, 5, 10, 25% w/v Vehicle: acetone: olive oil (4:1) OECD Guideline 429 (Skin Sensitisation: Local Lymph Node Assay) GLP	Not sensitising Stimulation index: 0.8, 0.7, 0.8, 0.4, 0.9 and 0.6 at concentrations of 0.01, 0.1, 0.1, 5, 10 and 25% w/v, respectively.	Török-Bathó M (2010)

4.6.1.1 Non-human information

No stimulation index is above 3, whatever the concentration tested. Benzovindiflupyr was negative for skin sensitisation potential in the mouse local lymph node assay (Török-Bathó, 2010).

4.6.1.2 Human information

No information.

4.6.1.3 Summary and discussion of skin sensitisation

Stimulation index values of the test item were all <3 (0.6, 0.9 and 0.4 at concentrations of 25, 10 and 5% (w/v), respectively and 0.8, 0.7 and 0.8 at concentrations of 1.0, 0.1 and 0.01% (w/v), respectively). The positive control substance had a stimulation index > 3.0.

4.6.1.4 Comparison with criteria

Benzovindiflupyr was negative for skin sensitisation potential in the mouse local lymph node assay and does not meet the criteria for classification according to CLP.

4.6.1.5 Conclusions on classification and labelling

CLP: No classification

RAC evaluation of skin sensitisation
Summary of the Dossier submitter's proposal A local lymph node assay (LLNA) in female mice was conducted according to OECD TG 429 (Török-Bathó, 2010). Stimulation index values for the test item were all <3 (0.6, 0.9 and 0.4 at concentrations of 25, 10 and 5% (w/v), respectively and 0.8, 0.7 and 0.8 at concentrations of 1.0, 0.1 and 0.01% (w/v), respectively). The positive control substance had a stimulation index >3.0. Benzovindiflupyr was negative for skin sensitisation potential in the mouse LLNA and according to the DS does not meet the criteria for classification according to CLP. **Comments received during public consultation** No comments were received during public consultation. **Assessment and comparison with the classification criteria** Benzovindiflupyr was negative for skin sensitisation potential in the mouse local lymph node assay. The stimulation index values for the test item were all <3. The Guidance on the Application of the CLP Criteria, version 4.0 – November 2013 (CLP Guidance) indicates that a substace may be classified as a skin sensitiser on the basis of positive results in an LLNA (criteria for a positive result include a stimulation index >3). Therefore RAC concludes that the substance does not meet the criteria for classification according to CLP.

4.6.2 Respiratory sensitisation

4.6.2.1 Non-human information

There is no data that benzovindiflupyr is a respiratory sensitiser.

4.6.2.2 Human information

No information.

4.6.2.3 Summary and discussion of respiratory sensitisation

There is no data on that benzovindiflupyr is a respiratory sensitiser.

4.6.2.4 Comparison with criteria

There is no evidence that benzovindiflupyr is a respiratory sensitiser and, therefore, no classification is warranted.

4.6.2.5 Conclusions on classification and labelling

CLP: No classification

4.7 Repeated dose toxicity

4.7.1 Non-human information

Table 16: Summary table of relevant repeated dose toxicity studies

Method	Results	Reference
Studies in Rats		
rat (Wistar HanTM: HsdRccHanTM: WIST) male/female 28 day study 5/sex/dose Test material purity: 97% 0, 100, 400, 1200 ppm (nominal in diet) Exposure: 28 days (Continuous in the diet) equivalent or similar to OECD Guideline 407 (Repeated Dose 28-Day Oral Toxicity in Rodents) GLP	**<u>1200 ppm (99 mg/kg bw/day)</u>** *Body weight:* ↓ 11.2% (males), ↓ 11.9% (females) *Body weight gain*: ↓ 26.8% (males), ↓ 53.5% (females) *Food consumption*: ↓ during week 1 (42.7% males, 69% females), extending throughout the treatment period in females (8.8 – 38.1%). *Food efficiency*: Generally ↓ throughout the treatment period in both sexes. *Functional observations*: 50% ↑ (females only) landing foot splay measurements. *Clinical chemistry*: ↓ total protein (7.1%), ↓ albumin (7.9%), ↑ (50.5%) AST activity in females, ↓ (15.4%) glucose in males. *Organ weights*: ↑ absolute (2.9%) and relative (16.5%) liver weight; ↑ absolute (2.8%) and relative (16.3%) heart weights in males. *Histopathology:* Minimal tubular basophilia of the kidneys (3/5 females). Minimal centrilobular hepatocyte hypertrophy of the liver (5/5 males). **<u>400 ppm (36 mg/kg bw/day)</u>** *Histopathology:* Minimal tubular basophilia in the kidneys (2/5 females). Minimal centrilobular hepatocyte hypertrophy of the liver (2/5 males). **<u>100 ppm (9 mg/kg bw/day)</u>** No treatment-related effects. NOAEL was 100 ppm (9 mg/kg bw/day) in both males and females based on minimal tubular basophilia in the kidneys of females and minimal centrilobular hepatocyte hypertrophy in males at 400 ppm.	Marr A (2010)
rat (Han Wistar (CRL: WI(Han))) male/female 90-day study 10/sex/dose Test material purity: 98.3% 0, 100, 750, 1500 ppm (nominal in diet) Exposure: 90 days (Continuous in the diet) OECD Guideline 408 (Repeated Dose 90-Day Oral Toxicity in Rodents) GLP	**<u>1500 ppm (108.7/108.8 mg/kg bw/day)</u>** *Body weight*: ↓ from Day 31 (9.4%) in males and Day 3 in females (9.0%); ↓ overall body weight gain (males 29.8%, females 54.8%). *Food consumption*: ↓ throughout the treatment period (up to 37.8% in males, 42.2% in females). *Food utilisation*: ↓ in both sexes (overall 30.6% males, 42.3% females). *Functional observation*: ↓ in forelimb grip strength in females, ↓ core body temperature (males 36.4°C/2.2%, females 36.8°C/2.6%). *Clinical chemistry*: ↓ alkaline phosphatase (males 25%, females 26.7%), ↓ glucose (males 16.6%, females 18.8%) and ↑ urea (20.7% males only). *Organ weights*: Adjusted liver weights↑ 26.5% in males.	Robertson B (2010a)

Method	Results	Reference
	Histopathology: Centrilobular hepatocyte hypertrophy of the liver of all males and 4/10 females. **<u>750 ppm (53.8/58.8 mg/kg bw/day)</u>** *Body weight*: ↓ from Day 38 (8.9%) in males and Day 14 in females (7.2%); ↓ overall body weight (males 20.1%, females 33.3%). *Food consumption*: ↓ throughout (up to 22.3% males, 36.1% females). *Food utilisation*: ↓ overall 16.3% males, 25% females. *Functional observation*: ↓ core body temperature (37.0°C/2.1%) in females. *Clinical chemistry:* ↓ alkaline phosphatase (35.6%), ↓ glucose (15.2%) in females; ↑ urea (19%) in males. *Organ weights*: Adjusted liver weights ↑ 7.1% in females. *Histopathology*: Centrilobular hepatocyte hypertrophy in 4/10 males. **<u>100 ppm (7.6/8.2 mg/kg bw/day)</u>** No treatment-related effects. NOAEL was 100 ppm (7.6/8.2 mg/kg bw/day) for both males and females on the basis of lower body weights, food consumption and food utilisation and clinical chemistry changes in both sexes, and increased liver weights in females and centrilobular hepatocyte hypertrophy in males at 750 ppm.	
rat (Wistar) male/female 28-day dermal 10/sex/dose Test material purity: 97% 100, 300, 1000 mg/kg bw/day(nominal) Exposure: 28 days (5 d/wk, 6h/d) OECD Guideline 410 (Repeated Dose Dermal Toxicity: 21/28-Day Study) GLP.	There were no treatment-related effects at any dose level. NOAEL was 1000 mg/kg bw/day.	Sommer EW (2010)
Studies in mice		
mouse (CD-1 (Crl:CD-1(ICR))) male/female Preliminary dose setting study 5/sex/dose (main study) + 7, 15, 15 and 15 /sex/group for toxicokinetic sampling Test material purity: 98.3% 0, 100, 300 or 500 ppm (nominal in diet) Exposure: 28 days (Continuous in the diet) OECD Guideline 407 (Repeated Dose 28-Day Oral Toxicity in	**<u>500 ppm (81.8/91.5 mg/kg bw/day)</u>** *Body weight*: ↓ (21.0% males, 10.7% females). *Body weight gain*: ↓ (215.2% males, 80.0 % females) overall. *Clinical chemistry*: no effect *Histopathology:* ↑ in tubulointerstitial nephritis in the kidneys (2/5 males (minimal); 1/5 females (slight)). **<u>300 ppm (47.4/57.9 mg/kg bw/day)</u>** *Body weight gain*: Body weight loss in both sexes days 0-3. Male bodyweight ↓ 8.2% day 5. **<u>100 ppm (15.6/19.0 mg/kg bw/day)</u>** No treatment-related effects.	Shearer J (2010)

Method	Results	Reference
Rodents) GLP.	NOAEL was 100 ppm (15.6/19.0 mg/kg bw/day in males/females), based on a clear reduction in body weight and increased incidence of tubulointerstitial nephritis in the kidneys of both sexes at 500 ppm and an initial body weight loss/statistically significantly lower body weight at 300 ppm.	
mouse (CD-1 (Crl:CD-1(ICR))) male/female 90-day study 10/sex/dose + 4/sex/group for toxicokinetic sampling Test material purity: 97.0% 0, 100, 300 or 500 ppm (nominal in diet) Exposure: At least 91 days (Continuous in the diet) OECD Guideline 408 (Repeated Dose 90-Day Oral Toxicity in Rodents) GLP.	**500 ppm (97.9/102.8 mg/kg bw/day)** *Mortality*: 2/14 males removed from the study (on days 9 and 15) due to treatment related effects. *Clinical observations*: Soft faeces (6/10 males; 1/10 females). *Body weight*: Males ↓ 17.8% by 7 days, females ↓ 12.9% after 2 days. *Body weight gain*: ↓ 71.4% males, 46% females overall. *Food consumption*: Slight variances in both sexes. Food utilisation ↓ 72.7% males, 40% females. *Clinical chemistry*: 36.9% ↓ plasma triglyceride and 5.6% ↑ creatinine levels in males. 5.1% ↑ in plasma calcium levels in females. *Gross pathology*: Large intestine distended (1/10 males; 1/10 females). *Histopathology*: Minimal to moderate mucosal hyperplasia in the colon (8/10 males, 9/10 females) and/or rectum (4/10 males, 7/10 females). No histopathology finding on kidney related to treatment. **300 ppm (55.6/59.6 mg/kg bw/day)** *Clinical observations*: Soft faeces (2/10 males; 2/10 females). *Body weight*: Males and females body weight loss during the initial 4 days (12.4% males, 6.3% females). *Body weight gain*: ↓ over the study period 35.3% males, 36% females. *Food utilisation* ↓ 40.9% males, 30% females. *Clinical chemistry*: 31.1% ↓ plasma triglyceride levels in males. 3.8% ↑ in plasma calcium levels in females. *Gross pathology*: Large intestine distended (1/10 males; 1/10 females). *Histopathology*: Minimal or mild mucosal hyperplasia in the colon (6/10 males, 5/10 females) and/or rectum (3/10 males, 5/10 females). **100 ppm (17/20.9 mg/kg bw/day)** No treatment-related effects. NOAEL was 100 ppm for both sexes (17.0/20.9 mg/kg bw/day) based on decreased body weight gain, an increased incidence of soft faeces in males, changes in clinical chemistry parameters and mucosal hyperplasia in the rectum and colon at 300 ppm.	Mackay C (2011)
Studies in dogs		
dog (Beagle) male/female 90-day study	**750 mg/kg bw/day** *Clinical observations*: Salivation (4/4 females), faeces	Pothmann D (2010)

Method	Results	Reference
4/sex/dose Test material purity: 97% 0, 30, 375, 750 mg/kg/day (actual ingested) Exposure: 13 weeks (Daily) oral (capsule) OECD Guideline 409 (Repeated Dose 90-Day Oral Toxicity in Non-Rodents) GLP	containing mucus or white particles or yellow stained faeces (4/4 males; 2/4 females). *Body weight:* Males ↓ 7.2%, females 10.3% during the first week of treatment. There was a statistically significant ↓ in mean body weight in males from day 50 onwards (18.4 % day 50, 19.2% day 92). *Body weight gain*: ↓ in males from day 22 onwards (100% day 50, 81.8% day 92) and sporadically in females between day 8 and the end of the study (92.3% day 50, 66.7% day 92). *Food consumption*: ↓ mean food intake during the first two weeks (males 42.5%, females 48.9% days 1-8); improved in all animals after change of the feeding regimen (food was presented three hours after dosing instead of immediately after). *Clinical chemistry*: ↑ plasma triglyceride values in males and some females during the whole treatment period (week 13 males 114%, females 81%); ↓ plasma calcium values in males during week 8 (4.2%) and 13 (5.1%). *Hystopathology:* no finding on kidney. Some minimal findings on caecum considered substance specific. **375 mg/kg bw/day** *Clinical observations*: Salivation (2/4 females), faeces containing mucus or yellow stained faeces (4/4 males; 1/4 females). *Body weight*: Males ↓ 7.2%, females 10.3% during the first week of treatment. *Body weight gain*: ↓ in males from day 22 onwards (81.3% day 50, 54.5% day 92) *Food consumption*: ↓ mean food intake during the first two weeks (males 46.9%, females 45.8% days 1-8); improved in all animals after change of the feeding regimen (food was presented three hours after dosing instead of immediately after). *Clinical chemistry*: ↓ plasma calcium values were observed in males week 8 (1.9%) and 13 (4.3%). **30 mg/kg bw/day** No treatment-related effects. NOAEL was 30 mg/kg bw/day based on initial bodyweight loss, reduced food consumption, decreased body weight gain and effects on clinical chemistry.	
dog (Beagle) male/female 1-year study 4/sex/dose Test material purity: 97% 0, 25, 250, 500 mg/kg/day (actual ingested) Exposure: 52 weeks (Daily), oral (capsule) OECD Guideline 452 (Chronic Toxicity Studies)	**500 mg/kg bw/day** *Clinical observations*: Salivation (2/4 males; 2/4 females). ↑ incidence of vomiting (of feed, fluid, capsule or mucus) and ↑ incidence of faeces containing mucus. *Body weight*: ↓ body weight gain on day 8 (males 100%, females 150%). Cumulative body weight gain ↓ in both sexes (males 50%, 58.3% and 42.9%, females 57.1%, 45.5% and 63.2% on days 22, 36 and 78). *Clinical chemistry:* no effect.	Braun L (2011)

Method	Results	Reference
GLP	**250 mg/kg bw/day** *Clinical observations*: Salivation (1/4 males; 2/4 females); ↑ incidence of vomiting (of feed, fluid, capsule or mucus) and ↑ incidence of faeces containing mucus. **25 mg/kg bw/day** No treatment-related effects. NOAEL was 250 mg/kg bw/day based on reduced body weight gain at 500 mg/kg bw/day.	

4.7.1.1 Repeated dose toxicity: oral

Short-term toxicity of benzovindiflupyr was studied in rats, mice and dogs and has included specific studies to address neurotoxicity and immunotoxicity (Tables 16, 21 and 22).

Rats

In rats the main effect observed after short-term dietary administration of benzovindiflupyr was a reduction in body weight gain, food consumption and food utilization (Marr, 2010; Robertson 2010a). There were some minor changes in the liver including increased liver weight and centrilobular hypertrophy visible after 90 days only. They were considered indicative of adaptive change. A minor pathological finding of minimal tubular basophilia in the kidney was noted in females at 99 mg/kg bw/day in the 28 day rat study linked with minor modifications of markers of kidney clinical chemistry. However, no renal findings were seen in males or at doses compatible with guidance values for classification (Table 3.9.3 CLP). In both studies the NOAEL was 100 ppm (equivalent to 8-9 mg/kg bw/day)

In a sub-chronic neurotoxicity study the NOAEL for general systemic toxicity was also 100 ppm (6.31/7.48 mg/kg bw/day) and there was no evidence of neurotoxicity at 50.67/37.99 mg/kg bw/day (Sommer, 2011b; Table 21; Section 4.12.2).

Mice

In the 28 day mouse study, benzovindiflupyr caused initial body weight loss at doses of 300 and 500 ppm and tubulointerstitial nephritis was observed in the kidneys in both sexes at 500 ppm (Shearer, 2010). Although coherent with the findings described in the 28d rat study, this effect was considered minor in mice as in female rats. In the 90 day study, initial body weight loss was observed at 500 ppm and two males were terminated *in extremis* (Mackay, 2011). There was no evidence of kidney pathology but hyperplasia in the colon and rectum was observed in both sexes at 300 and 500 ppm. The NOAEL for both the 28 and 90 day mouse studies was 100 ppm (equivalent to 17/20.9 mg/kg bw/day).

There was no indication that benzovindiflupyr was immunotoxic in a 28-day dietary immunotoxicity study and the NOAEL for immunosuppression was 97.1 mg/kg bw/day (Wasil, 2012; Table 22; Section 4.12.2).

Dogs

Toxicity in the dog was assessed in a 90-day toxicity (Pothmann, 2010) and 1-year studies (Braun, 2011).

In the 90 day study, there were signs of general toxicity at the top dose of 750 mg/kg bw/day including salivation, slight initial body weight loss and reduced food consumption and reduced body weight gain. Slight initial body weight loss, reduced body weight gain and initial reduced food consumption were also observed at 375 mg/kg bw/day. Clinical chemistry changes were also seen at 375 and 750 mg/kg bw/day (increased plasma triglycerides in both sexes at 750 mg/kg bw/day, decreased plasma calcium in males at 375 and 750 mg/kg bw/day) that might also indicate intestine disorder. The NAOEL was 30 mg/kg bw/day.

In the 1 year dog study, salivation, vomiting (of feed, fluid, capsule or mucus) and an increased incidence of faeces containing mucus were observed in both sexes at 250 and 500 mg/kg bw/day. This last effect is compatible with intestine disorder. However, the level at which these effects occur are above the classification criteria. Reduced body weight gain was seen in males and females at 500 mg/kg bw/day. The NOAEL for systemic toxicity was 250 mg/kg bw/day.

4.7.1.2 Repeated dose toxicity: inhalation

No information available.

4.7.1.3 Repeated dose toxicity: dermal

In a 28-day dermal toxicity study (Sommer, 2010) there was no evidence of systemic toxicity or local irritation. The NOAEL was 1000 mg/kg bw/day.

4.7.1.4 Repeated dose toxicity: other routes

No information available.

4.7.1.5 Human information

No information available.

4.7.1.6 Other relevant information

No other relevant information available.

4.7.1.7 Summary and discussion of repeated dose toxicity

The repeated dose toxicity of benzovindiflupyr has been evaluated by the oral route of administration in rats, dogs and mice and by the dermal route in a 28-day study in the rat. In all species the main effect observed after short-term oral administration was an initial body weight loss or a reduction in body weight gain. No evidence of toxicity was seen following dermal exposure. Effects on specific target organs were minor and are summarised below:

Bodyweight

Effects on body weight were observed at the lowest observed adverse effect level in all species. Although adverse, this effect is not severe enough to support classification in itself.

Liver

In the rat minor changes in the liver included increased liver weight and centrilobular hypertrophy in both the 28-day and 90-day toxicity studies at doses of 36 mg/kg bw/day and above. There was no evidence of liver toxicity in mice or dogs. Increased liver weight and liver histopathology was seen in rats at the LOAEL (400 ppm, 36 mg/kg bw/day). Investigative studies have demonstrated that benzovindiflupyr induces hepatic UDP glucuronyltransferase (UDPGT) leading to hepatocellular hypertrophy and increased liver weight (Robertson, 2012b). The available data (see Annex) also show that the mode of action in the rat has no relevance in humans due to the well documented qualitative and quantitative differences in response to UDPGT induction between rats and humans (Dellarco *et al*, 2006). Therefore, these adaptive changes do not warrant classification.

Kidney

Minimal tubular basophilia was seen female rats in the 28-day study at 400 ppm (36 mg/kg bw/day) and above but not in the 90-day study at higher doses (1500 and 750 ppm, 108.8 and 58.8 mg/kg bw/day). These effects are observed above the concentration limits described in the CLP (based on 90d studies). In the mouse, tubulointerstitial nephritis was observed in both sexes at 500 ppm (81.8 mg/kg bw/day) in the 28-day study but not in the 90 day study at the same dose level. There was no indication of kidney toxicity in the dog.

Effects on kidney were observed in preliminary studies in rats and mice at high dose levels but not in the 90-day studies at similar dose levels. All effects were minor, occurred at dose levels associated with body weight effects and, as such, provide no evidence of specific target organ toxicity and do not warrant classification

Gastrointestinal tract

In the 90-day mouse study an increased incidence of soft faeces and hyperplasia in the colon and rectum which was observed in both sexes at 300 and 500 ppm (55.6/59.6 mg/kg bw/day and 97.9/102.8 mg/kg bw/day). In the 1 year dog study, vomiting and mucoid faeces were observed in both sexes at 250 and 500 mg/kg/day. Some evidence of gastrointestinal disturbance was seen in mice and dogs (soft faeces and mucosal hyperplasia in the rectum and colon in mice; vomiting, mucoid faeces in dogs). These effects were accompanied by effects on body weight, were of minimal severity and there was no evidence of organ dysfunction. No classification is warranted

4.7.2 Comparison with criteria of repeated dose toxicity findings relevant for classification as STOT RE

There is no evidence that benzovindiflupyr produces specific target organ toxicity (repeated exposure) i.e. produces significant health effects considered to impair function, both reversible and irreversible. Significant effects (increased weight and histopathology) observed in the liver in the rat were considered to be adaptive changes resulting from UDPGT induction (see Annex to this report) and are not relevant to human health. Consequently, no classification is warranted.

4.7.3 Conclusions on classification and labelling of repeated dose toxicity findings relevant for classification as STOT RE

CLP: No classification

RAC evaluation of specific target organ toxicity– repeated exposure (STOT RE)
Summary of the Dossier submitter's proposal The CLH report contained a detailed description and assessment of the benzovindiflupyr data on repeated dose toxicity (RDT). The RDT of benzovindiflupyr has been evaluated by the oral route in rats (28 day study equivalent to OECD TG 407; Marr, 2010 and 90 day study, OECD TG 408; Robertson, 2010a), mice (28 day study, OECD TG 407; Shearer, 2010 and 90 day study, OECD TG 408; Mackay, 2011) and dogs (90 day study OECD TG 409; Pothman, 2010, 1 year study OECD TG 452; Braun, 2011; in both studies capsules were provided in the diet) and by the dermal route in a 28 day study in rat (OECD TG 410; Sommer, 2010). In all species tested, the main effect observed after short-term oral administration was an initial body weight loss or a reduction in body weight gain. No evidence of toxicity was seen following dermal exposure. Toxic effects were minor and are summarised below: *Bodyweight* Effects on body weight were observed in all the species at different doses but this was

associated with a decrease in food consumption in the oral studies. Thus this effect was not considered severe enough to support classification in itself.

Liver
In the rat, minor changes in the liver included increased liver weight and centrilobular hypertrophy in both the 28 day and 90 day toxicity studies at doses of 36 mg/kg bw/day and above. There was no evidence of liver toxicity in mice or dogs. Increased liver weight and liver histopathology was seen in rats at the LOAEL (400 ppm, 36 mg/kg bw/day in the 28 day study and 750 ppm, 53 mg/kg bw/day in the 90 day study). Investigative studies have demonstrated that benzovindiflupyr induces hepatic UDP glucuronyltransferase (UDPGT) leading to hepatocellular hypertrophy and increased liver weight (Robertson, 2012b). The available data also showed that the mode of action in the rat has no relevance in humans due to the well documented qualitative and quantitative differences in response to UDPGT induction between rats and humans (Lake, 2012a; Lake, 2012B; Green, 2012).

Therefore, these adaptive changes do not warrant classification as STOT RE.

Kidney
Minimal tubular basophilia was seen in female rats in the 28 day study at 400 ppm (36 mg/kg bw/day; Marr, 2010) and above but not in the 90 day study at higher doses (1500 and 750 ppm, 108.8 and 58.8 mg/kg bw/day; Robertson, 2010a). These effects are observed above the concentration limits described in the CLP (based on 90 day studies). In the mouse, tubulointerstitial nephritis was observed in both sexes at 500 ppm (81.8 mg/kg bw/day) in the 28 day study but not in the 90 day study at the same dose level. There was no indication of kidney toxicity in the dog.
Effects on kidney were observed in in 28 day studies in rats (Marr, 2010) and mice (preliminary studies; Shearer, 2010) at high dose levels but not in the 90 day studies at similar dose levels.

All effects were minor, occurred at dose levels associated with body weight effects and, as such, provide no evidence of specific target organ toxicity and do not warrant classification.

Gastrointestinal tract
In the 90 day mouse study, an increased incidence of soft faeces and hyperplasia in the colon and rectum was observed in both sexes at 300 and 500 ppm (55.6/59.6 mg/kg bw/day and 97.9/102.8 mg/kg bw/day). In the 1 year dog study, vomiting and mucoid faeces were observed in both sexes at 250 and 500 mg/kg/day. Some evidence of gastrointestinal disturbance was seen in mice and dogs (soft faeces and mucosal hyperplasia in the rectum and colon in mice; vomiting, mucoid faeces in dogs). These effects were accompanied by effects on body weight, were of minimal severity and there was no evidence of organ dysfunction.

Study	**NO(A)EL (mg/kg bw/d)**	**LO(A)EL effects (mg/kg bw/d)**
Rat 28 d (dietary)	9.0 (male and female)	36.0 (male and female) Minimal tubular basophilia in kidneys (female). Centrilobular hepatocyte hypertrophy in liver (male).
Mouse 28 d (dietary)	15.,6 and 19.0 (male and female)	47.5 and 57.9 (male and female) ↓ bw in males
Rat 90 d (dietary)	7.6 and 8.2 (male and female)	53.8 and 58.8 (male and female) ↓ bw, bw gain, food consumption, food utilization in both sexes; ↑ adjusted liver weights in females;

		Centrilobular hepatocyte hypertrophy in males (4/10).
Mouse 90 d (dietary)	17.0 and 20.9 (male and female)	55.6 and 59.6 (male and female) ↑incidence of soft faeces in males; ↓ bw, bw gain, food consumption in males and females; Minor changes in clinical chemistry: ↓ plasma triglycerides in males, ↑ plasma calcium level in females Distended large intestine was observed in one animal of each sex. Mucosal hyperplasia of the colon in males (6/10) and in females (5/10) and/or mucosal hyperplasia of the rectum in males (3/10) and in females (5/10)
Dog 13 w (capsule)	30.0	375.0 (male and female) ↓ bw and bw gain in males ↓ plasma calcium level in males (w8 and w13).
Dog 1y (capsule)	250.0	500.0 (male and female) ↓ body weight gain in males and females

The DS proposed no classification for STOT RE.

Comments received during public consultation

No comments were received during public consultation.

Assessment and comparison with the classification criteria

There is no evidence that benzovindiflupyr produces specific target organ toxicity (repeated exposure) i.e. produces significant health effects considered to impair function, both reversible and irreversible. Significant effects observed in the liver (increased weight and histopathology) in rat were considered to be adaptive changes resulting from UDPGT induction and are not relevant to human health. The effects do not support classification for specific target organ toxicity following repeated exposure and fall within the effects mentioned in Annex I: 3.9.2.8.1 of the CLP Guidance. Based on the data presented, RAC concludes that the substance should not be classified for STOT RE.

In conclusion, RAC agrees with the DS that no classification is warranted for STOT RE in accordance with CLP.

4.8 Germ cell mutagenicity (Mutagenicity)

4.8.1 Non-human information

Table 17: Summary table of relevant *in vitro* and *in vivo* mutagenicity studies

Method	Results	Remarks	Reference
***In vitro* Studies**			
Bacterial reverse mutation assay (e.g. Ames test) (gene mutation) S. typhimurium TA 1535, TA 1537, TA 98 and TA 100, E. coli WP2 uvr A pKM 101 and E. coli, WP2 pKM 101 (met. act.: with and without) Test material purity: 97% Test concentrations: 3, 10, 33, 100, 333, 1000, 2500 and 5000 µg/plate (active ingredient) OECD Guideline 471 (Bacterial Reverse Mutation Assay) GLP	**Negative** with and without metabolic activation	**Negative** for S. typhimurium TA 1535, TA 1537, TA 98 and TA 100; met. act.: with and without **Negative** for E. coli WP2 uvr A pKM 101 and E. coli, WP2 pKM 101; met. act.: with and without ; Cytotoxicity: no but tested up to limit concentration Vehicle (DMSO) controls, negative controls and positive controls were used in each test. Results valid.	Sokolowski A (2009)
Mammalian cell gene mutation assay (gene mutation) mouse lymphoma L5178Y cells (met. act.: with and without) Test material purity: 97% Test concentrations: Pre-experiment: 11.7, 23.4, 46.9, 93.8, 187.5, 375.0, 750.0 and 1500.0 µg/mL Main experiment: 1.3, 2.5, 5.0, 10.0, 15.0, 20.0, 30.0, 40.0, 50.0 and 60 µg/mL OECD Guideline 476 (In vitro Mammalian Cell Gene Mutation Test) GLP	**Negative** with and without metabolic activation	**Negative** for mouse lymphoma L5178Y cells; met. act.: with and without Cytotoxicity: yes ; Vehicle (DMSO) controls and positive controls were used in both assays. Results valid	Wollny H-E (2010)
In vitro mammalian chromosome aberration test (chromosome aberration) lymphocytes: human peripheral blood (met. act.: with and without) Test material purity: 97% Test concentrations: 0.34, 0.60, 1.83, 2.5, 3.1, 3.20, 5.0, 5.5, 7.5, 9.6 and 10.0 µg/mL (chromosomal aberration analysis) 3.1, 5.5, 9.6, 16.9, 29.5, 51.7, 90.5, 158.3, 277.1, 484.9, 848.6 and 1485.0 µg/mL (cytogenetic tests) OECD Guideline 473 (*In vitro* Mammalian Chromosome Aberration Test)	**Negative** with and without metabolic activation	**Negative** for lymphocytes: human peripheral blood; met. act.: with and without Cytotoxicity: yes (precipitation was seen in cultures treated with benzovindiflupyr at concentrations of 51.7 µg/mL (without S9) and 29.5 µg/mL (with S9) and above). Vehicle (DMSO) controls and positive controls were used in both assays. Results valid	Bohnenberger S (2010)

GLP			
***In vivo* Studies**			
Micronucleus assay (chromosome aberration) rat (Wistar (Han)) male/female oral: gavage Test material purity: 97% 0, 43.8, 87.5, 175 mg/kg/day (males); 0, 75 mg/kg/day (females) (nominal conc.) OECD Guideline 474 (Mammalian Erythrocyte Micronucleus Test) GLP	**Negative**	Genotoxicity: **negative** (male/female); toxicity: yes (assessed in preliminary study. MTD 175 mg/kg/day for males and 75 mg/kg/day for females) Vehicle controls (DMSO) and positive controls used. Results valid	Innes D (2010)

4.8.1.1 *In vitro* data

Benzovindiflupyr showed no genotoxic activity in a reverse gene mutation assay in bacteria using six tester strains (Sokolowski, 2009), in a mammalian cell gene mutation assay (Wollny, 2010) and in an *in vitro* mammalian chromosome aberration test (Bohnenberger, 2010). All studies were tested both in the presence and absence of exogenous metabolic activation and used positive and negative controls to confirm the validity of the tests.

4.8.1.2 *In vivo* data

Benzovindiflupyr was negative in a rat micronucleus assay (Innes, 2010).

4.8.2 Human information

No information.

4.8.3 Other relevant information

No other relevant information.

4.8.4 Summary and discussion of mutagenicity

Benzovindiflupyr has been demonstrated to be negative for genotoxicity in a comprehensive package of *in vitro* and *in vivo* assays for genotoxicity.

4.8.5 Comparison with criteria

Genotoxicity of benzovindiflupyr was tested in three *in vitro* and one *in vivo* test (Table 17). The results of all studies were negative whilst positive and negative controls demonstrated the validity of the tests. Benzovindiflupyr can be considered not to be genotoxic and no classification is proposed.

4.8.6 Conclusions on classification and labelling

CLP: No classification

RAC evaluation of germ cell mutagenicity

Summary of the Dossier submitter's proposal

Benzovindiflupyr showed no genotoxic activity in a reverse gene mutation assay in bacteria using six tester strains (Sokolowski, 2009), in a mammalian cell gene mutation assay (Wollny, 2010) and in an *in vitro* mammalian chromosome aberration test (Bohnenberger, 2010). All studies were tested both in the presence and absence of exogenous metabolic activation and used positive and negative controls to confirm the validity of the tests.
Negative results were obtained also in a rat micronucleus assay after treatment with Benzovindiflupyr (Innes, 2010).
The negative results in both *in vitro* and *in vivo* assays, lead the DS to propose no classification for mutagenicity.

Comments received during public consultation

No comments were received during public consultation.

Assessment and comparison with the classification criteria

Genotoxicity of benzovindiflupyr was tested in three *in vitro* and one *in vivo* test. The results of all studies were negative whilst positive and negative controls demonstrated the validity of the tests.

RAC concludes that benzovindiflupyr can be considered not to be genotoxic and no classification is warranted in accordance with CLP.

4.9 Carcinogenicity

4.9.1 Non-human information

Table 18: Summary table of relevant carcinogenicity studies

Method	Results	Reference
2-year study rat (Han Wistar (CrL:WI(Han))) male/female 52/sex/dose (main study) plus 12/sex/dose (toxicity study - to provide data at 1 year interim kill) oral: feed Test material purity: 97% 0, 25, 100 ppm (males and females), 600	***Non-neoplastic findings*** **600/400 ppm (30.17/ 27.44 mg/kg bw/day males/females)** *Bodyweight:* diverged from controls throughout the study; ↓ 10.9% males, 24.9% females week 104. *Bodyweight gain:* ↓ 13.8% males, 38.2% females week 104. *Food consumption:* ↓ (4-10%) in males, ~6-20 in females. Food utilisation: ↓ weeks 1-4 (15.2%) and 1-13 (6.4%) in males; weeks 1-4 (17.7%), 9-13 (18.5%) and 1-13 (14.5%) in females. *Clinical chemistry:* ↓ ALP (week 53, 30.4% males, 22.2% females), ALT (week 53, 28.6% males, 30.9% females) and AST (week 53, 21.4% males, 37.0% females). *Organ weights*: ↑ 7.5% absolute liver weight in males week 104. *Pathology*: Non-neoplastic: ↑ centrilobular hypertrophy in the livers of males	Mackay C (2012a)

<table>
<tr><th>Method</th><th>Results</th><th>Reference</th></tr>
<tr><td>(males), 400 (females) ppm (nominal in diet)
0, 1.21, 4.88 and 30.17 mg/kg/day (males); 0, 1.65, 6.66 and 27.44 mg/kg/day (females) (actual ingested)
Exposure: 104 consecutive weeks (Continuously in diet)
OECD Guideline 453 (Combined Chronic Toxicity / Carcinogenicity Studies)
GLP</td><td>(9/12 week 52, 13/52 week 104) and females (10/12 week 52, 36/52 week 104), accompanied by pigmented hepatocytes in females and hepatocyte vacuolation and hepatocellular eosinophilic foci in males.
<u>100 ppm (4.88/6.66 mg/kg bw/day males/females)</u>
Pathology: Non-neoplastic: ↑ hepatic centrilobular hypertrophy in males after 104 weeks of treatment (8/52).
<u>25 ppm (1.21/1.65 mg/kg bw/day males/females</u>)
No treatment-related effects.

Neoplastic findings
Pathology: Neoplastic: increased incidence of thyroid follicular cell adenoma in males at 600 ppm.
Overall tumour incidence (%) males
<table><tr><td></td><td colspan="4">Dietary concentration benzovindiflupyr (ppm)</td></tr><tr><td></td><td>0</td><td>25</td><td>100</td><td>600</td></tr><tr><td>Thyroid follicular cell adenoma</td><td>1/53 (2%)</td><td>4/52 (7.6%)</td><td>5/52 (9.6%)</td><td>9/52* (17.3%)</td></tr><tr><td>Thyroid follicular cell carcinoma</td><td>1/52</td><td>0/52</td><td>1/52</td><td>0/52</td></tr></table>
The toxicity and carcinogenicity NOAEL was 100 ppm (equating to 4.88 mg/kg bw/day in males and 6.66 mg/kg bw/day in females) based on lower bodyweight gain, food consumption/utilisation, clinical pathology changes, increased liver weight, increased incidence of centrilobular hypertrophy in the liver (accompanied by pigmented hepatocytes in females and hepatocyte vacuolation and hepatocellular eosinophilic foci in males) in both males and females, and increased incidence of thyroid follicular cell adenoma in males at 600 ppm.</td><td></td></tr>
<tr><td>80-week study
mouse (CD-1 (Crl:CD-1(ICR))) male/female
50/sex/dose
Test material purity: 97%
oral: feed
0, 20, 60 and 200 ppm (nominal in diet)
0, 2.62, 7.55 and 26.18 mg/kg/day (males); 0, 2.89, 8.67 and 29.26 mg/kg/day (females) (actual ingested)
Exposure: 80 consecutive weeks (Continuously in diet)
OECD Guideline 451 (Carcinogenicity Studies)
GLP</td><td>Non neoplastic findings
<u>200 ppm (26.18/29.26 mg/kg bw/day males/females)</u>
Bodyweight: ↓ 3.5 - 5.2% males first 4 weeks of the study.
Bodyweight gain: ↓ 20.5% males weeks 1-4
Pathology: Non-neoplastic: ↑ simple mucosal hyperplasia in the colon of males (13/49) and females (10/48); ↑ incidence of simple mucosal hyperplasia in the caecum (4/50 males and 2/48 females).
<u>60 ppm (7.55/8.67 mg/kg bw/day males/females)</u>
No treatment-related findings.
<u>20 ppm (2.62/2.89 mg/kg bw/day males/females)</u>
No treatment-related findings.

Neoplastic findings
There were no treatment-related neoplastic findings.
There was a numerically higher (not statistically significant) incidence of Harderian gland adenomas in treated mice at all dose levels compared with controls.
Tumour incidence in the Harderian gland
<table><tr><td></td><td></td><td colspan="4">Dietary concentration of benzovindiflupyr (ppm)</td></tr><tr><td></td><td>Sex</td><td>0</td><td>20</td><td>60</td><td>200</td></tr><tr><td>Adenoma</td><td>M</td><td>2/50</td><td>7/50</td><td>4/50</td><td>8/50</td></tr></table></td><td>Mackay C (2012b)</td></tr>
</table>

Method	Results	Reference

	F	0/50	4/50	3/50	5/50
Adenocarcinoma	M	0/50	1/50	0/50	0/50
	F	0/50	0/50	0/50	0/50

The NOAEL for toxicity was 60 ppm (7.55 mg/kg bw/day in males and 8.67 mg/kg bw/day in females) based on lower body weight and body weight gain and increased incidence of simple mucosal hyperplasia of the colon and caecum at 200 ppm.

4.9.1.1 Carcinogenicity: oral

The chronic toxicity and carcinogenicity of benzovindiflupyr was studied in rats and mice (Table 18).

Rats

In a 2 year combined chronic toxicity/carcinogenicity study significantly lower body weight gain, food consumption and food utilisation were observed in both sexes at 600/400 ppm. ALP, ALT and AST values were consistently lower than control values at the top dose.

Liver

In males at 600 ppm liver weight (covariate analysis with body weight) was statistically significantly increased. There were no other effects on organ weight. The incidence of centrilobular hypertrophy in the liver was statistically significantly higher in top dose males and females at 52 and 104 weeks. In males at 600 ppm, the incidence of eosinophilic cell foci in the liver was statistically significantly higher after 104 weeks and a higher incidence of hepatocyte vacuolation was seen after 104 weeks. A higher incidence of pigmented hepatocytes was observed in females at 400 ppm after 52 and 104 weeks. There was no increased incidence of liver tumours.

Thyroid

In males at 600 ppm there was a treatment-related increase in the incidence of thyroid follicular cell adenomas. Additional studies have been conducted to elucidate the mode of action for the thyroid follicular cell adenomas observed in top dose males (Robertson 2010b, 2012a, 2012b; Lake, 2012a, 2012b; Green, 2012; see Section 4.12.1.3). An in-depth evaluation of the investigative mode of action studies along with detailed summaries of the individual studies is presented in the Annex to this report. The overall database was evaluated according to the mode of action frameworks established by the IPCS and ILSI/HESI (Sonich-Mullin *et al*, 2001; Meek *et al*, 2003) which address both the question of the mode of action in the experimental animal model and the relevance of this mode of action to humans. Based on an evaluation of the mode of action studies and the regulatory toxicology database the following has been demonstrated:

- Benzovindiflupyr has no direct effect on thyroid peroxidase
- Induction of UDPGT leading to increase in conjugation and excretion of (triiodothyronine) T_3 and thyroxine (T_4)
- A decrease in serum T_3 and T_4 levels
- A compensatory increase in thyroid stimulating hormone (TSH) levels secreted via the hypothalamus-pituitary-thyroid (HPT) axis
- Under the chronic proliferative stimulus of TSH, thyroid follicular cells eventually progress to form follicular cell adenomas

The available data support the conclusion that the mode of action that has been shown in the rat has no relevance to human due to the well documented qualitative and quantitative differences in response to UDPGT induction and increased T_3/T_4 clearance between rats and humans (Dellarco *et al*, 2006).

Mice

In a carcinogenicity study in the mouse statistically significantly lower group mean body weight was observed in males treated with 200 ppm during the first 7 weeks of the study. This was associated with a statistically lower group mean body weight gain compared to the control animals over the first 4 weeks of the study. There was a higher incidence of simple mucosal hyperplasia in the large intestine (colon and caecum) at 200 ppm that was considered to be treatment related. The effect was more pronounced in males based on incidence and severity. There was no effect on the large intestine at either 20 or 60 ppm. There were no treatment-related neoplastic findings. Although there was a higher incidence of Harderian gland adenoma in the treated groups when compared with controls, this difference was considered to be incidental to treatment because:

- There was no dose-response relationship
- The incidence did not achieve statistical significance
- There were no indications of any pre-neoplastic micropathology findings
- There were no increases in the incidence of adenocarcinoma and there was no indication of any increase in tumour incidence in the Harderian gland in the rat

An increased incidence of Harderian gland adenomas is of no relevance to humans as the Harderian gland is a rodent specific structure with no anatomical equivalent in humans (Aldert *et al*, 1986).

4.9.1.2 Carcinogenicity: inhalation

No information (oral studies available).

4.9.1.3 Carcinogenicity: dermal

No information (oral studies available).

4.9.2 Human information

No information.

4.9.3 Other relevant information

No other relevant information.

4.9.4 Summary and discussion of carcinogenicity

Carcinogenicity studies in rats and mice showed an increased incidence of thyroid follicular cell adenoma in rats but no evidence of carcinogenicity in the mouse.

Thyroid

A treatment related increase in thyroid follicular cell adenoma was observed in male rats. Additional studies have concluded that the thyroid tumours in male rats are attributable to induction of hepatic UDPGT, which results in a series of down steam events, ultimately leading to tumourigenesis. The available data also demonstrates that this mode of action is not relevant for human hazard/risk assessment purposes due to qualitative and quantitative differences in response to UDPGT induction and increased T_3/T_4 clearance between rats and human.

4.9.5 Comparison with criteria

Thyroid tumours in male rats are induced via a mode of action that is not relevant for human risk assessment (UDPGT induction and increased T_3/T_4 clearance). No classification for carcinogenicity is necessary.

4.9.6 Conclusions on classification and labeling

CLP: No classification

RAC evaluation of carcinogenicity

Summary of the Dossier submitter's proposal

The carcinogenicity potential of benzovindiflupyr was evaluated in a 2 year study in rat and in an 80 weeks study in mice conducted according to OECD TG 453 and 451 respectively.
The carcinogenicity study for benzovindiflupyr in rats showed the induction of thyroid tumors in male rats as reported in the table below:

	Dietary concentration benzovindiflupyr (ppm)			
	0	25	100	600
Thyroid follicular cell adenoma	1/53 (2%)	4/52 (7.6%)	5/52 (9.6%)	9/52* (17.3%)
Thyroid follicular cell carcinoma	1/52	0/52	1/52	0/52

The available data, as reported in the CLH report, also demonstrated that the mode of action of this kind of tumor is species-specific and not relevant for humans.
Based on the evaluation of the mode of action studies and on the regulatory toxicology database the following has been demonstrated:

- Benzovindiflupyr has no direct effect on thyroid peroxidase
- Induction of UDPGT leading to an increase in conjugation and excretion of (triiodothyronine) T3 and thyroxine (T4)
- A decrease in serum T3 and T4 levels
- A compensatory increase in thyroid stimulating hormone (TSH) levels secreted via the hypothalamus-pituitary-thyroid (HPT) axis
- Under the chronic proliferative stimulus of TSH, thyroid follicular cells eventually progress to form follicular cell adenomas

All these findings supported the conclusion of the DS that the mode of action that has been shown in the rat has no relevance to humans due to the well documented qualitative and quantitative differences in response to UDPGT induction and increased T3/T4 clearance between rats and humans.

The carcinogenicity study in the mouse showed an increased incidence of Harderian gland adenomas at all doses compared with control. These Harderian gland tumours were considered not treatment related for the following reasons:

- There was no dose-response relationship. The Harderian gland tumour incidence at the low dose was higher than the historical control range whereas the incidence in the mid-dose was within the historical range.
- There was no statistical significance using pairwise tests and there was no statistically significant trend using the Peto trend test.
- There were no indications of any pre-neoplastic micropathology findings in the Harderian gland.
- There were no increases in the incidence of adenocarcinoma in the Harderian gland in the mouse.
- There were no increases in the incidence of adenocarcinoma in the Harderian gland and there was no indication of any increase in tumour incidence in the Harderian gland in the rat.

Moreover this kind of tumor is not relevant for classification; in fact, according to CLP guidance paragraph 3.6.2.3.2:

" *Tumours in the Harderian glands. Harderian glands are found in all vertebrates that possess a nictitating membrane, or third eyelid. They are located behind the eyeball in the orbit nictitating membrane, encircling the optic nerve. Humans have a rudimentary one* "
"*…the assumed mode of action is required to decide if these findings would support a classification. However, tumours observed only in these tissues, with no other observed tumours are unlikely to lead to classification. However, such determinations must be evaluated carefully in justifying the carcinogenic potential for humans; any occurrence of*

other tumours at distant sites must also be considered..."

Taken together, the DS concluded that benzovindiflupyr does not pose a carcinogenic hazard to humans.

Comments received during public consultation

No comments were received during public consultation.

Assessment and comparison with the classification criteria

Carcinogenicity studies in rats and mice showed an increased incidence of thyroid follicular cell adenoma in rats but no evidence of carcinogenicity in the mouse.
Certain tumour types observed in animal carcinogenicity studies are of questionable or no relevance to humans. This is the case of thyroid tumor observed in rats. In fact, benzovindiflupyr induced thyroid tumours in male rats are attributable to induction of hepatic UDP-glucuronsyltransferase (UDPGT), which results in a series of downstream events, ultimately leading to tumourigenesis (as reported in the annex of the CLH report).

This situation is also clearly stated in paragraph 3.9.2.5.3 of the CLP Guidance: …. "such a mechanism/effect cannot be directly extrapolated to humans, i.e. these thyroid effects observed in rodents caused by an increase in hepatic UDPG-transferase are therefore considered of insufficient concern for classification."

In conclusion, RAC agrees that benzovindiflupyr acts by a mode of action that is not relevant to humans and concluded that no classification for carcinogenicity is needed in accordance with CLP.

4.10 Toxicity for reproduction

4.10.1 Effects on fertility

Table 19: Summary table of relevant reproductive toxicity studies

Method	Results	Reference
rat (Wistar) male/female two-generation study 25/sex/dose Test material purity: 97% 0, 25, 100 and 600 ppm (nominal in diet (males)) 0, 25, 100 and 250 ppm (nominal in diet (females)) Exposure: continuously in diet (70-day pre-pairing period and during the pairing and after pairing periods in males and during the pairing, gestation and lactation periods in females for breeding of the F1 litters. F1 generation received benzovindiflupyr following weaning of the F1 litters on day 21 post partum to adulthood (at least a 70-day pre-pairing period) and also during the pairing, gestation and lactation periods for breeding of the F2 litters.)) OECD Guideline 416 (Two-Generation Reproduction Toxicity Study) GLP	***Parental toxicity*** **<u>600 ppm males (40.5 mg/kg bw/day)</u>** F0: ↓ body weight (e.g. 5.1% at end of pre-pairing period); ↓ food consumption during pre-pairing (e.g. 13.9% days 1-3; 6-8% days 68-70); ↑ 13.2% liver weight adjusted for body weight, ↑ centrilobular hepatocellular hypertrophy (5/25 control, 15/25 treated). F1: ↓ body weight (e.g. 7.1% at the end of the pre-pairing period); ↓ food consumption (e.g. 4.5% days 68-70 of pre-pairing period), ↑ 10.2% liver weight adjusted for body weight, ↑ centrilobular hepatocellular hypertrophy (0/25 control, 15/25 treated); ↑cell hypertrophy in pars distalis of pituitary (9/25 control, 16/25 treated); ↑ patchy fatty change in the liver (1/25 control, 10/25 treated). **<u>250 ppm females (19.4 mg/kg bw/day)</u>** F0: ↓ body weight (e.g. 7.8% at end of pre-pairing period, 13.1% gestation, 11.1% lactation); ↓ food consumption pre-pairing (e.g. 14.1% days 1-3, 5.4% days 68-70) and gestation (e.g. 11.9% days 0-2, 21.0% days 18-21), ↑ incidence of "lactational diestrus" (1/25 control, 10/25 treated), ↓ hepatocellular glycogen deposits (control 19/25, treated 7/25). F1: ↓ body weight gain during pre-pairing (10% at the end), gestation (14.6%) and lactation (10.4%); ↓ food consumption during pre-pairing (e.g. 9.6% days 68-70); gestation (e.g. 10.9% days 0-2, 26.9% days 18-21); ↑ 8.4% liver weight adjusted for body weight; ↑ incidence of "lactational diestrus" (0/25 control, 8/25 treated), ↑ incidence hypertrophy of adrenal cortical zona glomerulosa (4/25 control, 15/25 treated), ↓ hepatocellular glycogen deposits (control 21/25, treated 8/25). **<u>100 ppm (6.8/8.2 mg/kg bw/day males/females)</u>** F0: ↓ body weight in females (e.g. 4.3% at end of pre-pairing period, 13.1% gestation, 11% lactation), ↓ hepatocellular glycogen deposits in females (19/25 in control, 5/25 treated). F1: ↓ hepatocellular glycogen deposits in females (19/25 in control, 14/25 treated), ↑ patchy fatty change in the liver in males (5/25 control, 8/25 treated) **<u>25 ppm (1.7/2.0 mg/kg bw/day males/females)</u>** No treatment related effects. NOAEL 100 ppm (6.8/8.2 mg/kg bw/day) ***Reproductive parameters*** No effects at any dose level.	Whitlow S (2011a)

Method	Results	Reference
	NOAEL 600 ppm (40.5 mg/kg bw/day) in males and 250ppm (19.4 mg/kg bw/day) in females. ***Offspring toxicity*** **<u>250 ppm</u>** *Body weight*: ↓ F1 10.3 and 11.8% in males and females respectively, F2 13.0 and 14.7% in males and females respectively. *Sexual development*: ↑ time to preputial separation (F1, by 1.9 days) *Organ weights*: ↑ liver weight adjusted for body weight F1 7.5% males, 5.2% females, F2 5.6% females only, ↓ spleen weight adjusted for body weight F1 males 16.0% **<u>100 ppm and 25 ppm</u>** No treatment related effects. NOAEL 100 ppm	

4.10.1.1Non-human information

Benzovindiflupyr was evaluated in a two-generation reproduction study in the rat (Whitlow 2011b) preceded by a preliminary dose-range-finding study (Whitlow, 2011a). In the preliminary study, groups of 8 male and female rats were given benzovindiflupyr continuously in diet at 0, 75, 400 or 600 ppm (males) or 0, 75, 200 or 400 ppm (females). The rats were allowed a 10 week pre-mating period, up to 14 day mating period and then allowed to rear their offspring to weaning (day 21 post partum). For mating, the males were exposed to a lower dietary concentration of benzovindiflupyr i.e. that fed to their female partner. Lower food consumption was seen in both males and females at all doses level. Increased liver weight was also seen in males at 600 and 400 ppm. There was no evidence of any effects on reproduction at any dose level. Pup body weight was reduced at 400 ppm.

For the two-generation study, groups of 25 male and 25 female rats received benzovindiflupyr in the diet for 10 weeks and were then paired for mating. The females were allowed to litter and rear their offspring to weaning on day 21 *post partum*. The F1 generation animals were selected from the weaned F1 litters and maintained on test diets for at least 90 days prior to pairing for mating. The F2 offspring were terminated at weaning. Benzovindiflupyr was administered continuously at dose levels of 0 ppm, 25 ppm, 100 ppm and 600/250 ppm (males/females). For mating, the males were exposed to a lower dietary concentration of benzovindiflupyr i.e. that fed to their female partner.

At 600 ppm (males) body weight and/or body weight gain in both the P and F1 generations was lower than control during the pre-pairing and post-pairing periods. Food consumption was also generally lower than in controls. At 250 ppm (females), body weight and/or body weight gain and food consumption in both the P and F1 generations were lower in controls during the pre-pairing, gestation and lactation periods.

The follicle and corpora lutea count made during micropathological examination of ovarian tissues from females of the F1 generation, showed a lower number of growing follicles and corpora lutea in the 250 ppm group compared to the control group. Also, there was an increased incidence of lactational diestrous in parental females at this dose. These observations are consistent with a reduction in ovarian follicle and corpora lutea count occurring as a consequence of prolonged lactational diestrous in dams that nursed their pups for longer due to the significant effect on pup body weight. The prolonged nursing of the pups delayed the dams returning to normal estrous cycling hence the higher incidence of lactational diestrous (Woodside and Jans, 1995). These observations are, therefore, considered to be an indirect consequence of the effects of treatment on pup and maternal body weights and not an effect on reproduction. Furthermore, there were no effects on the length or pattern of oestrus cycles prior to mating, pre-coital interval, gestation length or the proportion of successful matings in P or F1 parents at any dose tested

For males at 600 ppm, the weight of the liver adjusted for body weight was statistically significantly increased in the P and F1 generations and microscopic examination revealed centrilobular hepatocellular hypertrophy. There was also an increased incidence of cell hypertrophy in the pars distalis of the pituitary in the F1 males at 600 ppm, increased patchy fatty change in the liver of males in the F1 generation at all dose levels. The weight of the liver adjusted for body weight was statistically significantly higher than control in the F1 females at 250 ppm and there was an increased incidence of hypertrophy of the adrenal zona glomerulosa. A decreased incidence of hepatocellular glycogen deposits in females at 250 and 100 ppm probably reflected the nutritional condition of the animals associated with lower mean body weight and is considered not to be toxicologically significant.

The body weight of F1 and F2 pups in the 250 ppm dose group was lower than control at weaning. The time until preputial separation was statistically significantly longer in the F1 males in the 600 ppm dose group. However, the body weight at the time of sexual maturation was similar to that of control and other treated groups indicating that this observation is a secondary consequence of the lower body weight of these animals. The time until vaginal patency in the F1 female pups was not affected by treatment and there were no effects on anogenital distance. In the 250 ppm F1 males at weaning, spleen weight was lower than controls and liver weight was higher in both sexes.

In conclusion, the parental NOAEL was 100 ppm (equivalent to 6.8 mg/kg bw/day for males and 8.2 mg/kg bw/day for females). The NOAEL for reproduction was 600 ppm in males (equivalent to 40.5 mg/kg bw/day) and 250 ppm in females (equivalent to 19.4 mg/kg bw/day). The offspring NOAEL for general toxic effects was 100 ppm (equivalent to 7.8/5.2 mg/kg bw/day for F1 generation males/females during pre-pairing).

4.10.1.2 Human information

No information available.

4.10.2 Developmental toxicity

Table 20: Summary table of relevant developmental toxicity studies

Method	Results	Reference
rat (Wistar) Developmental toxicity study 24 females/group Test material purity: 97% 0, 7.5, 15 and 30 mg/kg bw/day (nominal conc.) Exposure: oral gavage days 6-20 post coitum (Daily) OECD Guideline 414 (Prenatal Developmental Toxicity Study) GLP	***Maternal toxicity*** **30 mg/kg bw/day** Decreased activity (14/24), hunched posture (6/24), ataxia (14/24), prostrate (2/24) and ruffled fur (2/24) Body weight gain not adjusted for uterus weight: ↓ 27.2% days 6-21 Body weight gain adjusted for uterus weight: ↓ 75% days 6-21 Food consumption: ↓ 23.0% days 6-21 **15 and 7.5 mg/kg bw/day** No effects. NOAEL 15 mg/kg bw/day ***Developmental toxicity*** **30 mg/kg bw/day** Foetal weight: ↓ 10.4% (sexes combined) Visceral variation: ↑ foetal/litter incidence of thymus long cranial (7%/29% control, 24/74% treated). Skeletal variation: slight delay in ossification related to	Whitlow S (2011b)

Method	Results	Reference
	lower foetal weights, ↑ foetal/litter incidence of: non-ossified cervical vertebral body 1 (18%/50% control, 35%/78% treated); non-ossified cervical vertebral body 2 (9%/21% control, 23%/52% treated); incompletely ossified sternebra 5 (4%/17% control, 18%/52% treated); non-ossified proximal phalanges of digit 5 left (24%/50% control, 37%/74% treated), right (21%/42% control, 34%/70% treated); and non-ossified calcaneus left (69%/96% control, 99%/100% treated), right (66%/92% control, 98%/100% treated). **15 mg/kg bw/day** Visceral variation: ↑ foetal/litter incidence of thymus long cranial (7%/29% control, 18%/52% treated). **7.5 mg/kg bw/day** No effects. NOAEL 15 mg/kg bw/day based on effects on foetal weight and ossification at 30 mg/kg bw/day	
rabbit (New Zealand White) Dose range finding study 10 females/group Test material purity: 97% 0, 25, 50 and 100 mg/kg bw/day (nominal conc.) Exposure: oral gavage days 7-28 of gestation (Once daily) equivalent or similar to OECD Guideline 414 (Prenatal Developmental Toxicity Study) GLP	***Maternal toxicity*** **100 mg/kg bw/day** Mortality: premature termination of the group on day 14 due to severe effects on body weight and food consumption Body weight: ↓ 12.6% below control day 14 Food consumption: ↓ 58.4% of control days 7-10 ↓18.6% of control days 10-13 **50 mg/kg bw/day** Mortality: 2/10 females were euthanized in extremis on gestation days 18 and 20 due to body weight losses and reduced food consumption; 1/10 females aborted on gestation day 27; this female had severely reduced food consumption from day 18. Body weight: losses in individual females, no overall difference in group mean values. Food consumption: ↓ in individual females, no overall difference in group mean values. **25 mg/kg bw/day** No effects ***Developmental toxicity*** **100 mg/kg bw/day** No foetal evaluation. **50 and 25 mg/kg bw/day** No foetal effects (no skeletal evaluation)	Sawhney Coder P (2011a)
rabbit (New Zealand White) Developmental toxicity study 25 females/group Test material purity: 97% 0, 10, 20 and 35 mg/kg bw/day (nominal conc.)	***Maternal toxicity*** No treatment-related effects at any dose level NOAEL 35 mg/kg bw/day.	Sawhney Coder P (2011b)

Method	Results	Reference
Exposure: oral gavage days 7-28 of gestation (Once daily) equivalent or similar to OECD Guideline 414 (Prenatal Developmental Toxicity Study) GLP	***Developmental toxicity*** No treatment-related effects at any dose level NOAEL 35 mg/kg bw/day.	

4.10.2.1Non-human information

Developmental toxicity of benzovindiflupyr has been assessed in preliminary and full developmental toxicity studies in rats and rabbits (Whitlow, 2011c, 2011d; Sawhney Coder, 2011a, 2011b).

The preliminary study in rats dosed groups of 10 pregnant females with 0, 10, 20 or 30 mg/kg bw/day from gestation day 6 to day 20 and terminated on day 21 for evaluation of maternal and developmental effects. The dose level of 30 mg/kg bw/day produced signs of decreased activity, hunched posture, uncoordinated/circling movements during the first week of treatment and food consumption and body weight gain were reduced during the entire treatment period. At 20 mg/kg/day, food consumption and body weight gains were reduced on occasion. The mean foetal body weights were statistically significantly lower at 30 mg/kg bw/day, compared with the control. There were no toxicologically significant effects of treatment on the incidence of external, visceral or skeletal abnormalities.

In the full developmental study in the rat (Whitlow, 2011d), benzovindiflupyr was administered at dose levels of 0, 7.5, 15 and 30 mg/kg bw/day. All female animals survived until the scheduled necropsy. Marked clinical signs of toxicity (ataxia, hunched posture, prostrate, decreased activity and ruffled fur) were observed at 30 mg/kg bw/day. Mean food consumption, body weight and body weight gain were lower at 30 mg/kg bw/day. Mean foetal body weight was lower at 30 mg/kg bw/day and there was a slight delay in ossification which was considered to be related to the treatment with the test item and secondary to maternal toxicity. At visceral examination, the incidence of thymus long cranial attained statistical significance at 30 mg/kg bw/day; the values were outside the historical control range on a foetal (24% vs 14% laboratory historical control range) and litter (74% vs 60% laboratory historical control range) basis. The incidence of this lesion was also increased on a foetal basis at 15 mg/kg bw/day. However, this is a very common spontaneous finding in rats, is known to be a phenomenon that occurs in humans and represents part of a spectrum of physiological change during normal development of migratory tissues and displaced thymic tissue is not associated with compromised function. In the absence of increased incidences of findings in other related structures, the higher incidence of an isolated minor variation was considered to be of no biological significance in terms of foetal development. It was, therefore, concluded that the NOAEL for maternal and developmental toxicity was 15 mg/kg bw/day.

In a dose-range finding study in pregnant rabbit (Sawhney Coder, 2011a), a dose level of 100 mg/kg bw/day produced severe body weight loss and reduced food consumption, resulting in the early termination of all females at this dose level. Excessive body weight losses and reduced food consumption also led to abortion and moribundity in individual females at 50 mg/kg bw/day indicating that this dose level was unsuitably high for a full developmental toxicity study. On this basis, dose levels of 0, 10, 20, and 35 mg/kg bw/day were selected for the definitive prenatal development toxicity study the rabbit (Sawhney Coder, 2011b). In the developmental toxicity study groups of 25 mated female rabbits were administered benzovindiflupyr on gestation days 7 through to 28 and terminated on day 29 for evaluation of maternal and developmental effects. There were no significant treatment-related effects on the dams at any dose level. Marginal effects on maternal body weight gain were seen at 35 mg/kg bw/day during gestation days 13-20. There were no effects on foetal development. The NOAEL for maternal and developmental toxicity was 35 mg/kg bw/day.

The potential for benzovindiflupyr to induce developmental effects has been assessed using two species, rodent and non-rodent. There was no evidence of teratogenicity in either species and no evidence of developmental effects in the absence of maternal toxicity.

4.10.2.2Human information

No information available.

4.10.3 Other relevant information

No other relevant information.

4.10.4 Summary and discussion of reproductive toxicity

The reproductive toxicity of benzovindiflupyr has been investigated in a two generation reproduction toxicity study in the rat and developmental toxicity studies in rats and rabbits. No evidence of treatment-related effects on fertility, sexual function or other parameters of reproductive performance were seen and there was no indication of developmental toxicity.

4.10.5 Comparison with criteria

There is no evidence that benzovindiflupyr produces reproduction or developmental toxicity and, therefore, no classification is warranted.

4.10.6 Conclusions on classification and labelling

CLP: No classification

RAC evaluation of reproductive toxicity

Summary of the Dossier submitter's proposal

The reproductive toxicity of benzovindiflupyr was investigated in a two generation reproduction toxicity study in the rat (Whitlow, 2011a) and the developmental toxicity of benzovindiflupyr was assessed in preliminary and full developmental toxicity studies in rats and rabbits (Whitlow, 2011c, 2011d; Sawhney Coder, 2011a, 2011b).

As part of the alignment with the approval of new PPP active substances, to which benzovindiflupyr is subject to, and to better analyse the reproductive toxicity, additional information about spermatological parameters was received from the DS after the public consultation. The analysis showed that in the P and F1 generations the sperm motility, morphology and sperm head count values were not affected by treatment with the test item. Statistical differences in motility were noted in the 100 ppm dose group but not at 600 ppm, therefore they were considered unrelated to treatment (see tables below, in the DAR, B.6.6.1-8a and B.6.6.1-8b).

Table B.6.6.1-8a: P generation sperm analysis

Observation	Dose Group (mg/kg/day)				HCD
	0	25	100	600	2002-2007
Progressive motile (%)	71	71	63*	70	53-63
Stationary motile (%)	13	13	17*	13	22-35
Not motile (%)	17	17	20	17	9-17
Normal, complete sperm	93.6	-	-	91.7*	89.7-94
Complete sperm, misshapen hook	0.7	-	-	1.5**	0.4-1.2
Complete sperm, abnormally curved hook	2.2	-	-	3.2**	1.1-3.8
Complete sperm, reversed head	0.1	-	-	0.0*	0.0-0.2

* Statistically significant difference from control group mean, p<0.05 (Dunnett test)
** Statistically significant difference from control group mean, p<0.01 (Dunnett test)

Table B.6.6.1-8b: F1 generation sperm analysis

Observation	Dose Group (mg/kg/day)				HCD
	0	25	100	600	2002-2007
Progressive motile (%)	60	58	57	58	44-64
Stationary motile (%)	25	26	27	25	24-33
Not motile (%)	15	16	16	16	10-27
Normal, complete sperm	90.9	-	-	90.6	90.6-94.5
Complete sperm, misshapen hook	1.6	-	-	1.9	0.4-1.1
Complete sperm, abnormally curved hook	3.2	-	-	2.7	2.4-3.2
Complete sperm, reversed head	0.0	-	-	0.1	0.0-0.1

Mating performance and fertility were not affected by treatment with benzovindiflupyr. The follicle and corpora lutea count made during micropathological examination of ovarian tissues from females of the F1 generation showed a statistically significantly lower number of growing follicles and corpora lutea in the high dose group compared to the control group.

No evidence of treatment-related effects on fertility, sexual function or other parameters of reproductive performance were seen.

The developmental toxicity studies were reported as follows (see Table 20 of the CLH report):

Study	Dose Levels	NO(A)EL (mg/kg/day)	LOAEL (mg/kg/day)	Effects
Developmental toxicity in the rat (gavage) Whitlow S, (2011b)	0, 7.5, 15 & 30 mg/kg/day	Maternal: 15 mg/kg/day	At 30 mg/kg/d: Clinical signs: ↓ activity, hunched posture, ataxia, ruffled fur; ↓ body weight gain, food consumption,	At 30 mg/kg/d: Clinical signs: ↓ activity, hunched posture, ataxia, ruffled fur; ↓ body weight gain, food consumption,
		Foetal: 15 mg/kg/day	Foetal: 30 mg/kg/day	At 30 mg/kg/d: ↓ foetal weight, slight delay in ossification in ossification at 30 mg/kg/day.
Range-finding Developmental toxicity in the rabbit (gavage) Coder P, (2011a)	25, 50 & 100 mg/kg/day	Not applicable – range-finding study	Not applicable – range-finding study	At 100 mg/kg/day: ↓ body weight (+++) and food consumption resulting in early termination of all animals. ↓ body weight (++) and food consumption also led to abortion and moribundity in some animals at 50 mg/kg/day.
Developmental toxicity in the rabbit (gavage) Sawhney Coder P, (2010b)	0, 10, 20 & 35 mg/kg/day	Maternal: 35 mg/kg/day	Maternal: None	Maternal: none
		Foetal: 35 mg/kg/day	Foetal: None	Foetal: None

In the rat study (Whitlow, 2011b) foetal weights were statistically significantly reduced and at skeletal examination a slight delay in ossification was observed at 30 mg/kg/day. At this dose clear signs of maternal toxicity were observed. Therefore no biologically significant morphological alterations, including teratogenicity, were observed in this study.

The potential for benzovindiflupyr to induce developmental effects has been assessed using two species, rodent and non-rodent. The experimental results showed no evidence of teratogenicity in both species and no evidence of developmental effects in the absence of maternal toxicity.

In conclusion the reprotoxicity data showed no evidence of treatment-related effects on fertility, sexual function or other parameters of reproductive performance and there was no indication of developmental toxicity.

Comments received during public consultation

No comments were received during public consultation.

Assessment and comparison with the classification criteria

Overall, RAC considers that the data do not provide evidence that benzovindiflupyr induces adverse effects on the reproductive organs or fertility and no classification is supported for fertility.

There is no evidence that benzovindiflupyr produces developmental toxicity effects. In the rat developmental toxicity study the reduction in the body weight and the retardation of ossification were seen in association with maternal toxicity. As reported in the CLP guidance 3.7.2.4.3 "…..*Classification is not necessarily the outcome in the case of minor developmental changes, when there is only a small reduction in foetal/pup body weight or retardation of ossification when seen in association with maternal toxicity."*

RAC therefore agrees with the DS that classification is not justified for reproductive toxicity

in accordance with CLP.

4.11 Other effects

4.11.1 Non-human information

4.11.1.1 Neurotoxicity

Table 21: Studies on neurotoxicity

Method	Results	Reference
rat (RccHan™:WIST(SPF)) male/female Neurotoxicity study single dose 10/sex/dose Test material purity: 97% 0, 10, 30 and 80 mg/kg (nominal conc.) Exposure: Single gavage dose OECD Guideline 424 (Neurotoxicity Study in Rodents) GLP	**80 mg/kg bw** *Clinical signs:* Day 1 (approx. 1 hour post-dose), decreased activity in 3/10 males and 10/10 females. All females also exhibited swaying gait, abnormal gait, collapse, and ruffled fur - severity generally moderate except for ruffled fur and collapse. Day 2 swaying gait in 4/10 females, decreased activity in 3/10 females, abnormal gait in 3/10 females, and ruffled fur in 5/10 females. *Food consumption*: ↓ 24.8% males, 68.7% females, days 1-2. FOB Day 1: Body temperature ↓ 37.7°C males (cf. 38°C controls), 34.4°C females (cf. 37.9°C controls). Forelimb grip strength ↓ 43.3% females. Hind limb grip strength ↓ 25.0% females. Locomotor activity females - total distance ↓ 70.2%, rearing activity ↓ 85.5% and total centre time ↑ 318.5%. **30 mg/kg bw** Day 1 (approx. 1 hour post-dose), decreased activity in 5/10 females at 30 mg/kg. *Food consumption:* ↓ 36.9% females, days 1-2. FOB Day 1: Body temperature ↓ 35.1°C females (cf. 37.9°C controls). Forelimb grip strength ↓ 26.7% females. Locomotor activity females - total distance ↓ 68.1% and rearing activity ↓ 74.5%. **10 mg/kg bw** No effects NOAEL for generalized systemic toxicity was 10 mg/kg for females and 30 mg/kg for males. The NOAEL for neurotoxicity was 80 mg/kg for both males and female	Sommer E (2011a)
rat (RccHan™:WIST(SPF)) male/female Neurotoxicity study subchronic (oral: feed) 12/sex/dose Test material purity: 97% 0, 100, 400 and 800 ppm (males), 0, 100, 250 and 500 ppm (females) (nominal in diet) 0, 6.31, 25.95 and 50.67 mg/kg/day (males), 0, 7.48, 19.17 and 37.99 mg/kg/day (females) (actual ingested)	**800 ppm males (50.67 mg/kg bw/day)** Body weight gain: ↓ 14.8% day 92 Food consumption: ↓ 4.1% overall **500 ppm females (37.99 mg/kg bw/day)** Body weight gain: ↓ 23.2% day 92 Food consumption: ↓ 10.3% overall **400 ppm males (25.95 mg/kg bw/day)** Body weight gain: ↓ 12.7% day 92 **250 ppm females (19.17 mg/kg bw/day)** No effects **100 ppm (6.31/7.48 mg/kg bw/day males/females)**	Sommer E (2011b)

Method	Results	Reference
Exposure: 13 weeks (Continuously in diet) OECD Guideline 424 (Neurotoxicity Study in Rodents) GLP	No effects NOAEL for generalised systemic toxicity is 100 ppm for males and 250 ppm for females, corresponding to 6.31 mg/kg bw/day in males and 19.17 mg/kg bw/day in females. The NOAEL for neurotoxicity is 800 ppm for males and 500 ppm for females, corresponding to 50.67 mg/kg body weight/day in males and 37.99 mg/kg body weight/day in females based on the absence of treatment-related clinical observations, ophthalmoscopic findings, FOB findings, changes in locomotor activity or neuropathological findings (i.e., brain weights, macroscopic findings, microscopic findings).	

4.11.1.2 Immunotoxicity

Table 22: Studies on immunotoxicity

Method	Results	Reference
mouse (Crl:CD-1(ICR)) female 28-day immunotoxicity study 10 females/dose Test material purity: 97.7% 0, 100, 200 and 400 ppm (nominal in diet) 0, 26.4, 47.1 and 97.1 mg/kg (actual ingested) Exposure: 28 consecutive days (Continuously in diet) EPA OPPTS 870.7800 GLP	No suppression of the humoral component of the immune system at any dose level tested. NOAEL for immune suppression was 400 ppm (equivalent to 97.1 mg/kg/day), the highest dose level evaluated.	Wasil J (2012)

4.11.1.3 Specific investigations: other studies

Table 23: Specific investigations

Method	Results	Reference
rat (Wistar) male 28-day study 25 males/group Test material purity: 98.3% 0, 100, 750, 1500 ppm (nominal in diet) Exposure: 28 days (continuous in diet) Animals were terminated after 3, 4, 8, 15 or 29 days of treatment (i.e. after 2, 3, 7, 14 or 28 full days of dietary exposure). Thyroid tissues from all animals terminated on Days 4, 8, 15 and 29 were subjected to a histological examination. GLP, non-guideline.	**1500 ppm** No significant treatment related effect on the incidence of thyroid follicular cell hypertrophy at any time point. Incidence (/5) of minimal diffuse follicular cell hypertrophy Dietary concentration of benzovindiflupyr (ppm) 0 / 100 / 750 / 1500 Day 4: 0 / 0 / 0 / 0 Day 8: 0 / 0 / 0 / 1 Day 15: 0 / 0 / 0 / 2 Day 29: 0 / 0 / 2/4 / 1	Robertson B (2010b), Robertson B (2012a)
rat (Wistar) male Thyroid mode of action *in vivo* 15 males/dose and time point Test material purity: 97% 0, 100, 600, 1200 ppm benzovindiflupyr (nominal in diet) or 1200 ppm phenobarbital sodium salt (positive control). Exposure: Continuous in the diet for 1, 3, 7 or 14 days of treatment or 14 days of treatment plus 63 days recovery. For all animals. Blood samples were collected at termination (days 2, 4, 8, 15 or 78) for thyroid function testing. Liver and	**1200 ppm (112.8 mg/kg bw/day)** ↑ liver weight, ↑ centrilobular hepatocyte hypertrophy, ↑ hepatic UDPGT activity, ↓ serum T_3, ↓ serum T_4, ↑ TSH, ↑ thyroid weight, ↑ thyroid follicular cell proliferation by BrdU incorporation. All effects fully reversed after a 63 day recovery period following 14 days of treatment. **600 ppm (57.7 mg/kg bw/day)** ↑ liver weight, ↑ centrilobular hepatocyte hypertrophy, ↑ hepatic UDPGT activity, ↓ serum T_3 **100 ppm (9.8 mg/kg bw/day)** No treatment-related effects.	Robertson B (2012b)

Method	Results	Reference
thyroid glands were weighed and examined histologically. 5-Bromo-2'-deoxyuridine (BrdU) labelling index was measured (all animals injected 2 hours prior to termination). Frozen samples of the liver from each animal were analysed for hepatic microsomal protein content and UDPGT activity with thyroxine (T_4) as substrate. GLP, non-guideline.		
rat (Wistar) thyroid gland Effects on rat thyroid peroxidise activity *in vitro*. A pooled thyroid gland microsomal preparation from 5 male rats was used. Test material purity: 97% 0, 0.01, 0.1, 1 and 10 µM Thyroid peroxidase activity was assayed by determining the monoiodination of L-tyrosine. As a positive control, the effect of 6-propyl-2-thiouracil (PTU; 10 µM) on rat thyroid peroxidase activity was also determined. Non-GLP, non-guideline.	No inhibition of rat thyroid peroxidase activity *in vitro* at any concentration tested. Treatment with PTU resulted in a 100% inhibition of thyroid peroxidase activity. Benzovindiflupyr is not an inhibitor of rat thyroid peroxidase activity *in vitro*.	Lake BG (2012a)
rat male Effect on hepatic UDPGT activity towards thyroxine as substrate 5 males/dose 0, 100, 750 or 1500 ppm (nominal in diet). Additional groups received 0 or 1200 ppm Phenobarbital for 7 days. Liver microsomes were assayed for protein content and UDPGT activity towards thyroxine as substrate. Enzyme activity was expressed as specific activity (i.e. per unit of microsomal protein), per gram of liver, per total liver and per relative liver weight. GLP, non-guideline.	**1500 ppm** ↑ hepatic microsomal protein (7 days) ↑ hepatic microsomal UDPGT activity (3, 7 and 14 days) ↑ microsomal UDPGT activity 28 days) **750 ppm** ↑ hepatic microsomal protein (7 days) ↑ hepatic microsomal UDPGT (3, 7 and 14 days) **100 ppm** No significant effects (3, 7, 14 or 28 days) Benzovindiflupyr at doses of ≥750 ppm is an inducer of hepatic microsomal UDPGT towards thyroxine as substrate in male rats.	Lake BG (2012b)
Type of effects studied: mechanistic studies (in vivo) oral: feed Exposure: 14 day - 2 years Mode of action hypothesis using the framework developed by the IPCS and ILSI/HESI (Boobis et al, 2006). Non-GLP, non-guideline.	The paper presents the weight of evidence arguments that benzovindiflupyr-induced thyroid tumours in male rats is attributable to induction of hepatic UDPGT, which results in a series of downstream events, ultimately leading to tumour genesis. This mode of action is not relevant for human hazard/risk assessment purposes due to qualitative and quantitative differences in response to UDPGT induction and increased clearance of thyroid hormones (T_3/T_4) between rats and humans.	Green RM (2012)

4.11.1.4Human information

No information.

4.11.2 Summary and discussion

Special studies have been performed to investigate the potential for neurotoxicity (acute and sub-chronic studies), immunotoxicity and to generate data on the proposed mode of action with regard to the thyroid tumours in rats (Robertson 2010b, 2012a, 2012b; Lake, 2012a, 2012b; Green, 2012). As the results of these studies impact on the interpretation of specific target organ toxicity following single (STOT SE) or repeat exposure (STOT RE) and/or carcinogenicity the results of these studies were also considered previously (see sections: 4.3 Specific target organ toxicity – single exposure (STOT SE); 4.7 Repeated dose toxicity; 4.8 Specific target organ toxicity (CLP Regulation) – repeated exposure (STOT RE); and 4.10 Carcinogenicity).

In the acute neurotoxicity study, single gavage doses of 0, 10, 30 or 80 mg/kg did not produce any functional or pathological indications of neurotoxicity, although signs of general toxicity were observed at 30 mg/kg and above (Sommer, 2011a). In the sub-chronic neurotoxicity study, 90 days dietary administration at 0, 100, 250 or 800 ppm (males)/500 ppm (females) did not result in any indications of functional or pathological neurotoxicity. A 28-day dietary immunotoxicity study was conducted in CD-1 female mice (Wasil, 2012). Benzovindiflupyr was administered in the diet for a minimum of 28 consecutive days at dietary concentrations of 0, 100, 200 or 400 ppm. A positive control group was dosed with cyclophosphamide via intraperitoneal injection (50 mg/kg bw/day) for 4 consecutive days. Additionally, all mice were immunised with an intravenous injection of sheep red blood cells (sRBC) on study day 24, approximately 96 hours prior to the scheduled necropsy on study day 28. The liver, spleen, and thymus were weighed. Spleen cell suspensions were prepared, spleen cell counts were performed and the number of specific IgM antibody forming cells directed towards the sRBC was determined. There were no indications that benzovindiflupyr was immunotoxic.

Investigative studies included a 28-day study (Robertson, 2010b; 2012a), a 14 day dietary thyroid mode of action study (Robertson 2012b), an *in vitro* study for effects on thyroid peroxidise activity (Lake, 2012a) and an investigation of hepatic UDPGT activity towards thyroxine as substrate (Lake, 2012b). In addition, a position paper considering all the available data supporting the weight of evidence arguments for the postulated mode of action was prepared (Green, 2012). The information from these studies is presented in detail in the Annex.

4.11.3 Comparison with criteria

There was no indication that benzovindiflupyr has neurotoxic or immunotoxic potential and investigative studies demonstrated that benzovindiflupyr does not pose a carcinogenic hazard to man.

4.11.4 Conclusions on classification and labelling

CLP: No classification

RAC evaluation of neurotoxicity, immunotoxicity and specific investigation
Summary of the Dossier submitter's proposal Specific studies were performed to investigate the potential for neurotoxicity (acute and sub-chronic studies), immunotoxicity and to generate data on the proposed mode of action with regard to the thyroid tumours in rats (Robertson, 2010b, 2012a, 2012b; Lake, 2012a, 2012b; Green, 2012).

In the acute neurotoxicity study, single gavage doses of 0, 10, 30 or 80 mg/kg did not produce any functional or pathological indications of neurotoxicity, although signs of general toxicity were observed at 30 mg/kg and above (Sommer, 2011a). In the sub-chronic neurotoxicity study, 90 days dietary administration at 0, 100, 250 (male and female) or 800 ppm (males)/500 ppm (females) did not result in any indications of functional or pathological neurotoxicity (Sommer, 2011b).

A 28 day dietary immunotoxicity study was conducted in CD-1 female mice (Wasil, 2012). Benzovindiflupyr was administered in the diet for 28 consecutive days at dietary concentrations of 0, 100, 200 or 400 ppm. A positive control group was dosed with cyclophosphamide via intraperitoneal injection (50 mg/kg bw/day) for 4 consecutive days. Additionally, all mice were immunised with an intravenous injection of sheep red blood cells (sRBC) on study day 24, approximately 96 hours prior to the scheduled necropsy on study day 28. The liver, spleen, and thymus were weighed. Spleen cell suspensions were prepared, spleen cell counts were performed and the number of specific IgM antibody forming cells directed towards the sRBC was determined. There was no indication that benzovindiflupyr is immunotoxic.

Mechanistic studies included a 28 day study (Robertson, 2010b, 2012a), a 14 day dietary thyroid mode of action study (Robertson, 2012b), an in vitro study for effects on thyroid peroxidase activity (Lake, 2012a) and an investigation of hepatic UDPGT activity towards thyroxine as substrate (Lake, 2012b). In addition, a position paper considering all the available data supporting the weight of evidence arguments for the postulated mode of action was prepared (Green, 2012). All these data were reported in the annex of the CLH report.

Comments received during public consultation

No comments were received during public consultation.

Assessment and comparison with the classification criteria

The data presented in the CLH report showed no indication that benzovindiflupyr has neurotoxic or immunotoxic potential and investigative studies supported the proposed mode of action for thyroid cancer formation (for details see the paragraph on carcinogenicity).

RAC concludes that no classification is necessary for neurotoxicity and immunotoxicity in accordance with CLP.

5 ENVIRONMENTAL HAZARD ASSESSMENT

5.1 Degradation

Table 24: Summary of relevant information on degradation

Method	Results	Remarks	Reference
Hydrolytic stability OECD Guideline 111 GLP	Stable at pH 4, 5, 7 and 9 for up to five days at 50ºC Recovery (in %): pH : 104.4 — 114.1 at 50 °C after 5 d Transformation products: no	Radiochemical purity (if radiolabelling): 98.7% Specific Activity(if radiolabelling): 4.35 MBq/mg; 117.5 µCi/mg	Lowrie C (2009)
Photochemical degradation in water (direct and indirect photolysis) OECD Guideline draft (Phototransformation of Chemicals in Water - Direct and Indirect Photolysis) OECD Guidelines for the Testing of Chemicals, Guideline 316 GLP	Rapid photo-degradation in natural water (DegT50: 5.0 days, indirect photolysis), but slower in buffer solution (DegT50; 44.2 days, direct photolysis) Transformation products: yes	[Phenyl-U-14C]-SYN545192 Radiochemical Purity by HPLC: 98.5%; Specific Activity: 5.31 MBq/mg [Pyrazole-5-14C]-SYN545192 Radiochemical Purity by HPLC: 98.6%; Specific Activity: 5.38 MBq/mg Light source: Xenon lamp Light spectrum: 290 — 800	Wardrope L (2011)
Ready biodegradability OECD Guideline 301 F/ EU Method C.4-D (Ready Biodegradability: Manometric Respirometry Test) GLP	Under test conditions no biodegradation observed	Test material purity: 97.0% Activated sludge, domestic, non-adapted	Eisner G (2010)
Water sediment study OECD Guideline 308 (Aerobic and Anaerobic Transformation in Aquatic Sediment Systems) GLP	Swiss Lake (aerobic & anaerobic): DT50 water: 19 and 20 d DT90 water: 63 and 67 d DT50 whole system: 616 and 767 d DT90 whole system: >1000 d Calwich Abbey (aerobic & anaerobic): DT50 water: 18 and 11 d DT90 water: 58 and 35 d DT50 whole system: 427 and 620 d DT90 whole system: >1000 d	[Phenyl-U-14C]-SYN545192 Radiochemical purity (if radiolabelling): 99.0% Specific Activity: 5.38 MBq/mg; 145.3 µCi/mg Degradation was significantly faster under more realistic conditions where light is provided and phototrophic organisms such as algae and aquatic macrophytes are present.	Ferguson H & Lowrie C (2012)
Water sediment study OECD Guideline 308 (Aerobic and Anaerobic Transformation in Aquatic Sediment Systems) GLP	Swiss Lake (aerobic & anaerobic): DT50 water: 25 and 21 d DT90 water: 83 and 71 d DT50 whole system: 742 and 934 d DT90 whole system: >1000 d Calwich Abbey (aerobic &	[Pyrazole-5-14C]-SYN545192 Radiochemical purity (if radiolabelling): 99.7% Specific Activity: 5.42 MBq/mg; 146.4 µCi/mg Degradation was significantly faster under more realistic	Lowrie C & Ferguson H (2012)

Method	Results	Remarks	Reference
	anaerobic): DT50 water: 14 and 16 d DT90 water: 48 and 54 d DT50 whole system: 502 and 436 d DT90 whole system: >1000 d	conditions where light is provided and phototrophic organisms such as algae and aquatic macrophytes are present.	
Adsorption/desorption (soil) OECD Guideline 106 (Adsorption - Desorption Using a Batch Equilibrium Method) GLP	Strongly adsorbed to all soils with the average K_F and K_{FOC} values ranging from 31.6-93.3 and 3172-4507 g/g, respectively.		Wardrope L (2010)

5.1.1 Stability

Hydrolytic degradation

Under sterile aqueous conditions, the substance (benzovindiflupyr or SYN545192) was found to be hydrolytically stable at pH 4, 5, 7 and 9 for up to five days at 50ºC and showed no degradation. Benzovindiflupyr accounted for 100% of applied radioactivity in every sample analysed. No transformation products were observed in the HPLC analysis of any of the samples.

Photochemical degradation in water

The substance degrades by aqueous photolysis to form the pyrazole half molecule moieties, NOA449410 and SYN508272 at up to 8.5% and 2.6% AR, respectively, by direct photolysis in sterile buffer and 36.4% and 23.5% AR respectively as a result of indirect photolysis in sterile natural water.

5.1.2 Biodegradation

5.1.2.1 Biodegradation estimation

Not relevant as a screening study is available.

5.1.2.2 Screening tests

Ready biodegradability

The ready biodegradability of benzovindiflupyr was determined by observing the BOD (biochemical oxygen demand) using manometric methods, according to OECD 301F. An inoculum control and a procedure control as well as toxicity controls were incubated for 28 days in the darkness at 22°C. Aerobic activated sludge from a wastewater treatment plant treating predominantly domestic wastewater was used as the inoculum. As a procedure control, the reference item sodium benzoate was tested. The toxicity control contained both test material and the reference item sodium benzoate.

The percentage biodegradation of test material and of the reference item sodium benzoate was calculated based on their biochemical oxygen demand (BOD) and theoretical oxygen demand (ThOD). Since the test item contains nitrogen, the % biodegradation was calculated based on the $ThOD_{NH4}$ (considering that nitrification is absent) and $ThOD_{NO3}$ (considering that nitrification is complete).

Biodegradation in sludge exposed to the test item

The BOD of the test item, SYN545192, in the test media was in the normal range found for the inoculum controls throughout the study period. The test item was degraded by an average of 1% only by day 28; hence the test material was not biodegradable under the conditions of the test within 28 days.

Biodegradation of the reference item in the procedure controls
In the procedure controls, the reference item was degraded by an average of 99% by day 14, confirming the suitability of the activated sludge.

Biodegradation in the toxicity control
In the toxicity control, the course of biodegradation over the 28-day exposure period was similar to that in the two procedure controls containing the reference item. Within 14 days of exposure, biodegradation of 48% and 42% was observed based on the $ThOD_{NH4}$ and $ThOD_{NO3}$ respectively. Thus, according to the test guidelines, the test item had no inhibitory effect on activated sludge microorganisms at the tested concentration of 98 mg/L because biodegradation in the toxicity control was >25% within 14 days.

5.1.2.3 Simulation tests

Biodegradation in water/sediment systems

The rate and route of degradation of [^{14}C]- benzovindiflupyr has been investigated in laboratory water-sediment systems with two different sediments (Calwich Abbey, a silt loam and Swiss Lake, a sandy loam) with both ^{14}C-pyrazol labelled benzovindiflupyr (Lowrie & Ferguson, 2012) and ^{14}C-phenyl labelled benzovindiflupyr (Ferguson & Lowrie, 2012). In both laboratory studies degradation was investigated under standard aerobic and anaerobic conditions in the dark and additionally in water-sediment systems incubated in a light/dark cycle in the presence of either naturally abundant algae, which proliferated under the incubation conditions, or an aquatic macrophyte that was introduced to the water-sediment system.

In the dark aerobic systems 83-94 % of applied benzovindiflupyr remained in the total systems after 100 days and no metabolites were observed at levels above 3% AR. In the water-sediment systems incubated under light/dark conditions (both with algae and with macrophytes), where there was rapid degradation of benzovindiflupyr and a number of metabolites were identified. Only two metabolites were found at levels >10% AR in the total test systems under light/dark cycles: SYN546039 at a maximum of 19.5 % AR and SYN546040 at a maximum level of 11.8% AR. Further details on these studies follow:

In standard water-sediment systems incubated in the dark with ^{14}C-phenyl labelled benzovindiflupyr (Ferguson & Lowrie, 2012), the dissipation rate (DT_{50}) of ^{14}C- benzovindiflupyr from the water column was 19 and 20 days for Swiss Lake samples under aerobic and anaerobic incubations, respectively. The corresponding values for Calwich Abbey were 18 and 11 days, respectively. Levels of benzovindiflupyr declined slowly in the total systems (sum of surface water and sediment extract), with >85% of the applied radioactivity remaining as the parent molecule at the end of the study, and no significant degradation products were formed. The total system degradation rates ($DegT_{50}$) of 616 and 767 days were obtained for Swiss Lake samples incubated under aerobic and anaerobic conditions, respectively. Corresponding values for Calwich Abbey were 427 and 620 days, respectively.

The dissipation from the water column and total system degradation rates in the modified incubations systems containing algae and macrophytes and incubated under light/dark conditions were significantly faster than the corresponding dark aerobic incubations. The dissipation rate (DT50) of benzovindiflupyr from Swiss Lake water in algal and macrophyte incubations was 9 and 8 days, respectively. The corresponding values for Calwich Abbey were 4 and 3 days, respectively. The total system DegT50 for the Swiss Lake incubation group containing algae was 81 days and in systems containing macrophytes was 46 days. The $DegT_{50}$ for the Calwich Abbey incubation group containing algae was 52 days and in systems containing macrophytes was 28 days.

In modified systems, biotransformation proceeded by hydroxylation of the alicyclic ring to yield the metabolites SYN546039 and SYN546040. N-demethylation of the pyrazole ring occurred to form

SYN546206 with subsequent hydroxylation of the alicyclic ring to give the metabolites SYN546041 and SYN546042. In Swiss Lake sediment only, the metabolite SYN546648 was formed through further oxidation of the alicyclic hydroxy metabolites SYN546039 and SYN546040. SYN546039 was the most significant metabolite formed, reaching 16.5% AR in systems containing algae and 16.8% AR in systems containing macrophytes. SYN546040 was also present at 1.2% AR in systems containing algae and 8.7% in systems containing macrophytes. All other metabolites were detected at levels of <5% AR.

In the second study with ^{14}C-pyrazole labelled benzovindiflupyr (Lowrie & Ferguson, 2012), in the, standard water-sediment systems incubated in the dark the dissipation rate (DT_{50}) of ^{14}C benzovindiflupyr from the water column was 25 and 21 days for Swiss Lake samples under aerobic and anaerobic incubations, respectively. The corresponding values for Calwich Abbey were 14 and 16 days, respectively. Levels of benzovindiflupyr declined slowly in the total systems (sum of surface water and sediment extract), with >82% of the applied radioactivity remaining as the parent molecule at the end of the study, and no significant degradation products were formed. Degradation rates (DegT50) of 742 and 934 days were obtained for Swiss Lake samples incubated under aerobic and anaerobic conditions, respectively. Corresponding values for Calwich Abbey were 502 and 436 days, respectively.

The dissipation from the water column and total system degradation rates in the modified systems containing algae and macrophytes and incubated under light/dark conditions were significantly faster than the corresponding dark aerobic incubations. The dissipation rate (DT50) of benzovindiflupyr from Swiss Lake water in algal and macrophyte incubations was 7 and 5 days, respectively. The corresponding values for Calwich Abbey were 4 and 3 days, respectively. The total system $DegT_{50}$ for the Swiss Lake incubation group containing algae was 91 days and in systems containing macrophytes was 40 days. The $DegT_{50}$ for the Calwich Abbey incubation group containing algae was 68 days and in systems containing macrophytes was 19 days.

In modified systems, biotransformation proceeded by hydroxylation of the alicyclic ring to yield the metabolites SYN546039 and SYN546040. N-demethylation of the pyrazole ring occurred to form SYN546206 with subsequent hydroxylation of the alicyclic ring to give the metabolites SYN546041. Cleavage of the bond between the pyrazole and phenyl rings occurred to give the pyrazole carboxylic acid NOA449410. In Swiss Lake sediment only, the metabolite SYN546648 was formed through further oxidation of the alicyclic hydroxy metabolite SYN546039 and SYN546040. SYN546039 was the most significant metabolite formed, reaching 11.3% AR in systems containing algae and 19.5% AR in systems containing macrophytes. SYN546040, NOA449410 and SYN546648 were also present at 6.6% AR, 8.7% AR and 3.6% AR, respectively in systems containing algae and 11.8% AR, 4.9% AR and 5.4% AR, respectively in systems containing macrophytes. All other metabolites were detected at levels <5% AR.

Both studies demonstrated that benzovindiflupyr is not significantly labile under standard aerobic or anaerobic study conditions. However, under more realistic conditions, where light is provided and phototrophic organisms such as algae and aquatic macrophytes can grow, degradation of benzovindiflupyr was significantly faster.

5.1.3 Summary and discussion of degradation

Benzovindiflupyr is stable to hydrolysis under acidic, neutral and alkaline conditions (DT_{50} > 1 year at 25 °C). However it degrades by aqueous photolysis to form the pyrazole half molecule moieties, NOA449410 and SYN508272 at up to 8.5% and 2.6% AR respectively by direct photolysis in sterile buffer and 36.4% and 23.5% AR respectively as a result of indirect photolysis in sterile natural water.

The rate and route of degradation of [^{14}C]- benzovindiflupyr has been investigated in water-sediment systems with two different sediments (Calwich Abbey, a silt loam and Swiss Lake, a sandy loam) laboratory conditions (Lowrie & Ferguson, 2012 and Ferguson & Lowrie, 2012). In the laboratory studies degradation was investigated under standard aerobic and anaerobic conditions in the dark and additionally in water-sediment systems incubated in a light/dark cycle in the presence of either naturally abundant algae, which proliferated under the incubation conditions, or an aquatic macrophyte that was introduced to the water-sediment system.

In the dark aerobic systems 83-94 % of applied benzovindiflupyr remained in the total systems after 100 days and no metabolites were observed at levels above 3% AR. In the water-sediment systems incubated under light/dark conditions (both with algae and with macrophytes), there was faster degradation of SYN545192 and a number of metabolites were identified. Only two metabolites were found at levels >10% AR in the total test systems under light/dark cycles: SYN546039 at a maximum of 19.5 % AR and SYN546040 at a maximum level of 11.8% AR.

5.2 Environmental distribution

5.2.1 Adsorption/Desorption

Adsorption K_{FOC} values determined for benzovindiflupyr in five soils (Wardrope, 2010) ranged from 3172 to 4507 g/g with a mean K_{FOC} for SYN545192 of 3697 g/g. K_{OC} values for ranged from 4499 to 6995 g/g, with the mean K_{OC} calculated as 5578 g/g.

Using the McCall Classification scale the potential mobility of SYN545192 in soil can be classified "slight" to "immobile" based on these data. There was an increase in the Kd, K_{OC}, K_F and K_{FOC} between the adsorption and desorption steps suggesting that the adsorption of ^{14}C-labelled benzovindiflupyr was not fully reversible in these soils. There is a significant correlation between adsorption of benzovindiflupyr and the percentage organic matter of the soil.

5.2.2 Volatilisation

The vapour pressure for technical SYN545192 is 3.2×10^{-9} Pa at 25°C and the Henry's law constant is 1.3×10^{-6} Pa.m^3.mol^{-1} at 25ºC. Given these properties, SYN545192 has a low potential for volatilisation and volatilisation studies (laboratory or wind tunnel) are not required.

5.2.3 Distribution modeling

Not relevant.

5.3 Aquatic Bioaccumulation

Table 25: Summary of relevant information on aquatic bioaccumulation

Method	Results	Remarks	Reference
Bluegill sunfish (*Lepomis macrochirus*) (freshwater) flow-through test system uptake duration: 23 d; depuration duration: 7 d Analytical purity: 96.8 % Radiochemical purity: 98.7 % Specific activity: 1737 MBq/mmol (46.95 mCi/mmol), 4.35 MBq/mg (117.5 µCi/mg), 260,850 dpm/µg OECD Guideline 305 GLP	Whole body BCF benzovendiflupyr: 76 L/kg (whole body w.w.) (Time of plateau: 10 d)(steady state) (Based on concentration of test substance in fish tissue at 23 days) Whole body BCF: 407 L/kg (whole body w.w.) (Time of plateau: 10 d)(steady state) (Based on 14C-residues) Whole body BCF: 374 dimensionless (whole body w.w.) (Time of plateau: 10 d)(kinetic) (Based on 14C-residues) Whole body BCF lipid benzovindiflupyr: 2447 L/kg, based on wet weight and 492 L/kg, based on dry weight (whole body lipid w.w.) (Time of plateau: 10 d) (steady state) (Based on lipid-normalised concentration of test substance	Transformation products: [14C] test substance was extensively metabolized in fish. The test substance concentration was 0.113 µg [14C] test substance/g tissue, 18.56% of the total radioactive residue (TRR) in the whole fish at day 23. Two unidentified components were detected in the whole fish extractable fraction at 75.57 – 77.60 % TRR by HPLC-RAM on day 10 and day 23 of exposure. No detectable levels of metabolites were found in the exposure water. The mean lipid content of fish on day 23 was 3.09 % and 15.35 %, based on wet weight and dry weight, respectively.	York DO, Lentz NR (2010)

Method	Results	Remarks	Reference
	in fish tissue at 23 days) Whole body lipid BCF: 13186 L/kg, based on wet weight and 2654 L/kg, based on dry weight (whole body lipid w.w.) (Time of plateau: 10 d) (steady state) (Based on lipid-normalised 14C-residues)		

5.3.1 Aquatic bioaccumulation

5.3.1.1 Bioaccumulation estimation

The substance has a log Kow of 4.3. Since this value is >3 the potential for bioaccumulation has been measured (see below).

5.3.1.2 Measured bioaccumulation data

The bioconcentration and subsequent depuration of [^{14}C] test substance in bluegill sunfish (*Lepomis macrochirus*) was determined in a GLP-compliant test performed to standard guidelines. Calculated bioconcentration factors (BCF) were based on analyses of water and fish tissues for total radioactive residues and SYN545192 residues. The study was run with nominal concentration of 0.26 µg ^{14}C labelled test substance/L and a solvent control. The mean measured steady state concentration was 0.291 µg [^{14}C] test substance/L. The measured BCF_{ss} value obtained for whole fish tissues was 407 L/kg. The calculated kinetic BCF_k based on the uptake (K_u was 479.3079) and depuration constants (K_d was 1.2802) was 374 for whole fish tissues. The BCF test substance based on characterisation of the residues in whole fish tissues was 76 L/kg. The plateau concentration of radioactivity in whole fish (µg [^{14}C] test substance equivalents/kg) was attained within 10 days of exposure. The depuration of accumulated residue from the whole body was rapid with the depuration half-life of [^{14}C] test substance being 0.54 days. By day 8 of depuration, 96.9% of the accumulated whole body residues were eliminated in 0.26 µg/L exposure.

5.3.2 Summary and discussion of aquatic bioaccumulation

The whole body BCF for fish exposed to a mean measured steady state concentration of 0.291 µg [14C] benzovindiflupyr /L was 76 L/kg based on SYN545192 and 407 L/kg based on [14C] residues. The depuration of accumulation residue was rapid with approximately 96.9% depuration by day 8. The depuration half-life was 0.54 days.

Benzovindiflupyr has a log Kow of 4.3. However, the experimentally derived steady state BCF value of 76 L/kg for SYN545192 is below the trigger of 100 (criterion for bioaccumulating potential conform Directive 67/548/EEC) and is also below the trigger of 500 (criterion for bioaccumulating potential conform Regulation EC 1272/2008).

5.4 Aquatic toxicity

Table 26: Summary of relevant information on aquatic toxicity for benzovindiflupyr

Method	Results	Reference
Cyprinus carpio Freshwater; flow-through Test material purity: 98.3% OECD Guideline 203 (Fish, Acute Toxicity Test); GLP	**LC50 (96 h): 3.5 µg/L** test mat. (meas. (arithm. mean)) based on: mortality	Fournier AE (2010a)
Pimephales promelas Freshwater; flow-through Test material purity: 98.3% OECD Guideline 203 (Fish, Acute Toxicity Test) ; GLP	LC50 (96 h): 4.7 µg/L test mat. (meas. (arithm. mean)) based on: mortality	Fournier AE (2010b)
Oncorhynchus mykiss Freshwater; flow-through Test material purity: 98.3% OECD Guideline 203 (Fish, Acute Toxicity Test) ; GLP	LC50 (96 h): 9.1 µg/L test mat. (meas. (arithm. mean)) based on: mortality	Fournier AE (2010c)
Lepomis macrochirus Freshwater; flow-through test system Analytical purity: 96.8 % OECD Guideline 305 (preliminary study for selection of definitive study concentration) and in line with OECD Guidelines 203 (Fish, Acute Toxicity Test) Not GLP but conducted in a GLP facility so all procedures performed in line with facilities standard practises.	LC50 (96 h): 26 µg/L test mat. (meas. (arithm. mean)) based on mortality	York D0 (2013)
Cyprinodon variegatus Saltwater; flow-through Test material purity: 98.3% OECD Guideline 203 (Fish, Acute Toxicity Test) ; GLP	LC50 (96 h): 27 µg/L test mat. (meas. (arithm. mean)) based on: mortality	Fournier AE (2010d)
Pimephales promelas Freshwater; flow-through Test material purity: 98.3% embryo and larval development: (sub)lethal effects OECD Guideline 210 (Fish, Early-Life Stage Toxicity Test) ; GLP	**NOEC (32 d): 0.95 µg/L** test mat. (meas. (arithm. mean)) based on larvae length and weight	York DO (2010a)
Daphnia magna Freshwater; static Test material purity: 98.3% OECD Guideline 202 (Daphnia sp. Acute Immobilisation Test) ; GLP	EC50 (48 h): 85 µg/L test mat. (meas. (geom. mean)) based on: mobility (95% CL: 56 to 130 µg test item/L.)	Fournier AE (2010e)
Americamysis bahia Saltwater; static Test material purity: 98.3% EPA OPPTS 850.1035 (Mysid Acute Toxicity Test) ; GLP	LC50 (96 h): 56 µg/L test mat. (meas. (arithm. mean)) based on: mortality (95% CL: 17 to 120 µg test item/L.)	Fournier AE (2010f)
other aquatic mollusc: Crassostrea virginica	EC50 (96 h): 160 µg/L act. ingr. (meas. (arithm.	York DO

Method	Results	Reference
saltwater; flow-through Test material purity: 98.3% EPA OPPTS 850.1025 (Bivalve Acute Toxicity (shell deposition test)) ; GLP	mean)) based on: New shell growth (95 % confidence intervals: 120 to 210 µg a.i./L)	(2010b)
Daphnia magna Freshwater; semi-static Test material purity: 98.3% OECD Guideline 211 (Daphnia magna Reproduction Test) ; GLP	NOEC (21 d): 15 µg/L test mat. (meas. (arithm. mean)) based on: reproduction; growth and dry weight NOEC (21 d): 34 µg/L test mat. (meas. (arithm. mean)) based on: mortality	Fournier AE (2010g)
Americamysis bahia Saltwater; flow-through Test material purity: 97.0% EPA OPPTS 850.1350 (Mysid Chronic Toxicity Test) ; GLP	NOEC (28 d): 7.4 µg/L act. ingr. (meas. (arithm. mean)) based on: reproduction	Lee MR (2011)
Pseudokirchnerella subcapitata (algae) Freshwater; static Test material purity: 98.3% OECD Guideline 201 (Alga, Growth Inhibition Test) ; GLP	EC50 (72 h): > 890 µg/L test mat. (meas. (arithm. mean)) based on: biomass, growth rate and yield NOEC (72 h): 420 µg/L test mat. (meas. (arithm. mean)) based on: biomass NOEC (72 h): 890 µg/L test mat. (meas. (arithm. mean)) based on: growth rate and yield	Softcheck KA (2010)
Skeletonema costatum (algae) Saltwater; static Test material purity: 97.0% OECD Guideline 201 (Alga, Growth Inhibition Test) ; GLP	EC50 (72 h): 450 µg/L test mat. (meas. (arithm. mean)) based on: biomass (area under growth curve) EC50 (72 h): 550 µg/L test mat. (meas. (arithm. mean)) based on: growth rate EC50 (72 h): 470 µg/L test mat. (meas. (arithm. mean)) based on: biomass (yield) NOEC (72 h): 100 µg/L test mat. (meas. (arithm. mean)) based on: biomass (area under growth curve) NOEC (72 h): 400 µg/L test mat. (meas. (arithm. mean)) based on: growth rate and biomass (yield)	Softcheck KA (2011a)
Lemna gibba (aquatic plants) Freshwater; semi-static Test material purity: 97.0% OECD Guideline 221 (Lemna sp. Growth Inhibition test) ; GLP The EC values were empirically estimated, therefore corresponding 95 % confidence limits could not be calculated	EC50 (7 d): > 880 µg/L test mat. (meas. (arithm. mean)) based on: Frond density and yield based on frond density; growth rate; biomass and yield and growth rate based on frond dry weight NOEC (7 d): 880 µg/L test mat. (meas. (arithm. mean)) based on: Frond density and yield based on frond density; growth rate NOEC (7 d): 430 µg/L test mat. (meas. (arithm. mean)) based on: biomass ; yield and growth rate based on frond dry weight	Softcheck KA (2011b)
Chironomus dilutus Freshwater; semi-static Test material purity: 97.0% long-term toxicity (laboratory study) US EPA. Test method 100.5, Methods for Measuring the Toxicity and Bioaccumulation of Sediment-Associated Contaminants with Freshwater Invertebrates, 2nd Edition. Office	NOEC (56 d): 48 mg/kg sediment dw test mat. (meas. (arithm. mean)); based on: – emergence rate (The NOEC for both male and female midges was the same.); – days to death (The NOEC for both male and female midges was the same.); – days to Oviposition NOEC (56 d): 6.7 mg/kg sediment dw test mat.	Picard RP (2012a)

Method	Results	Reference
of Research and Development. EPA/600/R-99/064 (2000) US EPA. Ecological Effects Test Draft Guideline OPPTS 850.1760. Whole Sediment Life Cycle Toxicty Test with Chironomus spp. EPA 712-C-08-068 (September 2009) ; GLP	(meas. (arithm. mean)) based on: – egg masses per mated female; – number of eggs per mated female NOEC (56 d): 24 mg/kg sediment dw test mat. (meas. (arithm. mean)) based on: – number of eggs per egg mass; – percent hatch	
Leptocheirus plumulosus Saltwater; semi-static long-term toxicity (laboratory study) Test material purity: 97.0% U.S. EPA, 2001. Methods for Assessing the Chronic Toxicity of Marine and Estuarine Sediment-Associated Contaminants with the Amphipod Leptocheirus plumulosus. EPA/600/R-01/020; GLP	NOEC (28 d): 4.1 mg/kg sediment dw test mat. (meas. (arithm. mean)) based on: mortality; growth rate and reproduction	Picard RP (2012b)

Table 27: Summary of relevant information on aquatic toxicity for benzovindiflupyr metabolites

Group (species)	Metabolite an Time scale	Endpoint	Value (mg test substance/L)	Expressed as	Reference
Fish					
Oncorhynchus mykiss	SYN546039 (static)	96 h LC_{50}	2.4 mg/L	Mean measured	Liedtke (2012) SYN546039_10004
	NOA449410 (M700F001) (static)		> 100 mg/L	Nominal	Nierzedska (2009) CA4312_10909
	SYN508272 (M700F007) (static)		> 100 mg/L	Nominal	Rzodeczko (2009) SYN508272_10893
Invertebrates					
Daphnia magna	SYN546039 (static)	48 h EC_{50}	5.2 mg/L	Mean measured	Liedtke (2012) SYN546039_10003
	SYN546040 (static)		>0.88 mg/L[a]	Mean measured	Liedtke (2012) SYN546040_10000
	NOA449410 (M700F001) (static)		> 100 mg/L	Nominal	Nierzedska (2009) CA4312_10908
	SYN508272 (M700F007) (static)		> 100 mg/L	Nominal	Rzodeczko (2009) SYN508272_10894
Algae					
Pseudokirchneriella subcapitata	SYN546039 (static)	72 h EC_{50}	EC50 (b,r,y) > 6.4 mg/L	Nominal	Liedtke (2012) SYN546039_10002
	NOA449410 (M700F001) (static)		EyC50 = 26.42 mg/L ErC50 = 36.31	Nominal	Nierzedska (2009) CA4312_10906

Group (species)	Metabolite an Time scale	Endpoint	Value (mg test substance/L)	Expressed as	Reference
			mg/L		
	SYN508272		EyC50 > 100 mg/L ErC50 > 100 mg/L	Nominal	Rzodeczko (2009) SYN508272_10895

[a] 0.88 mg/L was the practical solubility limit of SYN546040 in the exposure medium

Benzovindiflupyr metabolites are significantly less toxic than the active substance, and would therefore not influence the classification and labeling proposals. In these conditions, only summaries of relevant information on aquatic toxicity for benzovindiflupyr are reported below.

5.4.1 Fish

5.4.1.1 Short-term toxicity to fish

Four guidelines, GLP studies (three with a freshwater and one with a saltwater species) are available for this endpoint. In addition to these, range finding in the fish bioaccumulation study gave a 96 hour LC50 for bluegill sunfish. This study has been presented as a separate non-GLP study but has been considered as acceptable as conducted in a GLP facility so all procedures were performed in line with facilities standard practices. All are considered to be relevant, reliable and adequate for classification purposes.

The sensitivity of common carp, *Cyprinus carpio*, to benzovindiflupyr was determined in a GLP-compliant flow-through test performed to standard guidelines (Fournier, 2010a). The 96-hour LC50 for *C. carpio* exposed to the test substance was determined to be 3.5 µg/L with 95% confidence interval of 2.3 - 5.4 µg/L. The 96-hour NOEC, based on mortality and sub-lethal effects, was determined to be 2.3 µg/L. Analytical monitoring of the exposure concentrations was undertaken and the results are based on the mean measured test substance concentrations measured at the start and end of the test period.

In a second study, the sensitivity of the fathead minnow, *Pimephales promelas*, to benzovindiflupyr was determined in a GLP-compliant test performed to standard guidelines (Fournier, 2010b). The 96-hour LC50 for *P. promelas* exposed to the test substance was determined to be 4.7 µg/L with 95% confidence interval of 2.2 to 9.4 µg/L. The 96-hour NOEC, based on mortality and sub-lethal effects, was determined to be 2.2 µg/L. Analytical monitoring of the exposure concentrations was undertaken and the results are based on the mean measured test substance concentrations measured at the start and end of the test period.

The sensitivity of rainbow trout, *Oncorhynchus mykiss*, to benzovindiflupyr was determined in a GLP-compliant test performed to standard guidelines (Fournier, 2010c). The 96-hour LC50 for *O. mykiss* exposed to the test substance was determined to be 9.1 µg/L with 95% confidence interval of 4.7 – 18 µg/L. The 96-hour NOEC, based on mortality and sub-lethal effects, was determined to be 2.3 µg/L. Analytical monitoring of the exposure concentrations was undertaken and the results are based on the mean measured test substance concentrations measured at the start and end of the test period.

In the range finding study (York 2013) the sensitivity of bluegill sunfish (*L. macrochirus*), was determined with a 96 hour acute exposure to benzovindiflupyr at nominal test concentrations of 6.3, 13, 25, 50 and 100 µg/L. The 96-hour LC50 estimated by binominal probability was 26 µg test substance/L with 95 % confidence intervals of 11 to 47 µg test substance/L. The NOEC was determined to be 11 µg/L.

In the final study, the sensitivity of the saltwater species, sheepshead minnow (*Cyprinodon variegatus*) to benzovindiflupyr was determined in a GLP-compliant test performed to standard guidelines (Fournier, 2010d).

The 96-hour LC50 for *C. variegatus* exposed to the test substance was determined to be 27 µg/L with 95% confidence interval of 16 to 70 µg/L. The 96-hour NOEC, based on mortality and sub-lethal effects, was determined to be 16 µg/L. Analytical monitoring of the exposure concentrations was undertaken and the results are based on the mean measured test substance concentrations measured at the start and end of the test period.

The lowest LC50 result for freshwater species, the 96-h LC50 of 3.5 µg/L in carp (Fournier, 2010a), is carried forward for classification purposes. Therefore, a full summary for this study is reported below:

- **Reference:** IIA 8.2.1.2/01
- **Author(s); year:** Fournier, A. E. (2010)
- **Title:** SYN545192- Acute Toxicity to Carp (*Cyprinus carpio*) Under Flow-Through Conditions
- **Report No:** 1781.6721
- **Guidelines:** OECD Guidelines for Testing of Chemicals, Section 2 - Effects on Biotic Systems, No. 203: Fish, Acute Toxicity Test (1992). US EPA Ecological Effects Test Guidelines, OPPTS 850.1075: Fish Acute Toxicity Test, Freshwater and Marine (1996)
- **GLP:** Yes
- **Executive summary**

The acute toxicity of SYN545192 to carp *Cyprinus carpio* was determined under flow-through conditions. Fish were exposed to nominal concentrations of 0.63, 1.3, 2.5, 5.0 and 10 µg a.i./L (0.61, 1.1, 2.3, 5.4, and 10 µg a.i./L mean measured), alongside a dilution water control and solvent control. Based on mean measured concentrations, the 96 hour LC_{50} was 3.5 µg a.i./L, with 95% confidence interval of 2.3 to 5.4 µg a.i/L.

- **Materials and methods:**

Materials

Test material	SYN545192 CSCD064398
Lot/Batch #:	TE-6341
Purity:	98.3%
Description:	White solid
Stability of test compound:	Stable under test conditions
Reanalysis/expiry date:	31 August 2010
Treatments	
Test concentrations:	Dilution water control, solvent control (0.10 mL DMF/L), and nominal SYN545192 concentrations of 0.63, 1.3, 2.5, 5.0 and 10 µg a.i./L
Solvent:	Dimethylformamide (DMF, CAS No. 68-12-2)
Analysis of test concentrations:	At 0 and 96 hours using LC-MS/MS analysis
Test organisms	
Species:	Carp, *Cyprinus carpio*
Source:	Osage Catfisheries, Osage Beach, Missouri, USA
Acclimatisation period:	14 days

Treatment for disease:	None
Weight and length of test population fish at start of exposure period:	Mean length: 45 mm (range: 39 to 54 mm) Mean weight: 1.4 g (range:1.0 to 2.1 g)
Feeding:	None during test

Test design

Test vessels:	Glass vessels (30 x 15 x 20 cm) - total test solution volume maintained at 6.8 L
Test medium:	Well water
Replication:	None
No of fish per tank:	7
Exposure regime:	Flow-through, using an intermittent-flow proportional diluter
Duration:	96 hours

Environmental conditions

Test temperature:	21 – 23 °C
pH:	6.9 – 7.4
Dissolved oxygen:	7.0 – 9.2 mg/L (>60%)
Hardness of dilution water:	54 - 58 mg/L as $CaCO_3$
Lighting:	510 to 690 Lux. 16 hours fluorescent light and 8 hours dark with 30 minute transition periods

Study Design and Methods

Experimental dates: 26 November 2008 to 30 November 2008.

A flow-through test system was employed. A 0.10 mg/mL diluter stock solution was prepared by placing 0.0200 g of test substance in a 200 mL volumetric flask and bringing it to volume with dimethylformamide (DMF). This stock solution was delivered at 0.0768 mL/cycle into the diluter system's chemical mixing chamber which also received 0.768 L of dilution water per cycle. The mixing chamber, holding a stir bar, was positioned over a magnetic stirrer and was also partially submerged in an ultrasonic water bath to ensure continuous mixing. The concentration of SYN545192 in the solution contained within the mixing chamber was equivalent to that of the highest nominal test concentration (10 µg a.i./L) and was proportionally diluted (50%) to produce the remaining nominal test concentrations.

At the start of the test seven fish were randomly allocated to each of the test concentrations, the solvent control and the dilution water control. The test was conducted in a temperature controlled water-bath. Observations for mortalities and symptoms of toxicity were made at 0, 24, 48, 72 and 96 hours.

Daily measurements of the test solutions were undertaken throughout the 96 hour period for pH, temperature and dissolved oxygen concentration.

The test concentrations were verified by chemical analysis of SYN545192 at 0 and 96 hours using an LC/MS/MS method.

- **Findings:**

Results and Discussion

Mean measured concentrations ranged from 86 to 110% of nominal values (see table below).

Table IIA 8.2.1.2-1: Analytical results

Nominal Concentration (µg a.i./L)	Measured Concentration 0 hours (µg a.i./L)	Measured Concentration 96 hours (µg a.i./L)	Mean measured concentration (µg a.i./L)[a]	Percent (%) of nominal [a]
Dilution water control	< LOQ[b]	< LOQ	N/A	N/A
Solvent control	< LOQ	< LOQ	N/A	N/A
0.63	0.59	0.64	0.61	97
1.3	1.2	1.0	1.1	86
2.5	2.2	2.3	2.3	91
5.0	4.7	6.1	5.4	110
10	9.0	11	10	100

[a] Mean and percent of nominal are based on the original raw data and not the rounded results presented in this table

[b] LOQ = the limit of quantification. The LOQ for each analysis is dependent upon the linear regression, the area of the low standards and the dilution factor of the controls. At both 0 and 96 hours the LOQ was 0.022 µg a.i./L

NA = Not Applicable

Mean measured concentrations were used for the calculation and reporting of results.

The median lethal concentration (LC_{50}) was defined as the concentration resulting in 50% mortality of the fish in the time period specified and was calculated by binomial probability. The NOEC (No Observed Effect Concentration) is defined as the highest tested concentration which did not produce toxic-related mortalities or physical and behavioural abnormalities, when compared to the control organisms, and was determined by visual inspection of the data.

The mortality data and LC_{50} values are shown in the table below:

Table IIA 8.2.1.2-2 Effects of SYN545192 on the survival of *Caprinus carpio*

Mean Measured Concentration (µg a.i./L)	Cumulative Percent Mortality (Number of Dead Fish)[a]			
	24 hours	48 hours	72 hours	96 hours
Dilution water control	0 (0)	0 (0)	0 (0)	0 (0)
Solvent control	0 (0)	0 (0)	0 (0)	14(1)
0.61	0 (0)	0 (0)	0 (0)	0 (0)
1.1	0 (0)	0 (0)	0 (0)	0 (0)
2.3	0 (0)	0 (0)	0 (0)	0 (0)
5.4	0 (0)[b]	29 (2)[cd]	71 (5)[ef]	100 (7)
10	57 (4)[g]	100 (7)	100 (7)	100 (7)
LC_{50} (µg a.i./L)	9.4	6.3	4.4	3.5

95% confidence interval (µg a.i./L)	5.4 - 10	2.3 - 10	2.3 - 10	2.3 – 5.4
NOEC (µg a.i./L)	2.3	2.3	2.3	2.3

[a] The actual number of mortalities is presented in parentheses.
[b] Two fish exhibited a partial loss of equilibrium and were dark in coloration
[c] Two fish exhibited a partial loss of equilibrium
[d] Several fish exhibited a complete loss of equilibrium
[e] One fish was observed to be lethargic
[f] One fish exhibited a complete loss of equilibrium and was on the bottom of the test vessel
[g] Several fish exhibited a complete loss of equilibrium and were on the bottom of the test vessel

Validity criteria

The test was considered to be valid, since no mortality in the control was observed and the validity criterion of at least 60% oxygen saturation was fulfilled.

- **Conclusion:**

The acute toxicity of SYN545192 to carp *Cyprinus carpio* was determined under flow-through conditions. Based on mean measured concentrations, the 96-hour LC_{50} to carp (*Cyprinus carpio*) was 3.5 µg a.i./L with 95% confidence interval of 2.3 to 5.4 µg a.i./L.

5.4.1.2 Long-term toxicity to fish

A fish early life-stage toxicity study with the fathead minnow (*Pimephales promelas*) was conducted according to standard guidelines and GLP (York, 2010a). Fertilized eggs and subsequently larvae were exposed to five concentrations of test substance plus a dilution water control and a solvent control for 32 days (28 days post-hatch). The endpoints were egg hatchability, survival and growth (length and dry weight). Egg hatching success was unaffected at all concentrations of the test substance tested. Larval survival was significantly different for organisms exposed to 3.6 µg/L compared to control survival. Based on growth (length and dry weight), the most sensitive indicator of toxicity, the 32-day NOEC for benzovindiflupyr was determined to be 0.95 µg/L and the 32-day LOEC was determined to be 1.8 µg/L. Analytical monitoring of the exposure concentrations was undertaken and the results are based on the mean measured test substance concentrations measured throughout the exposure period.

This study is considered to be relevant and reliable and is carried forward for classification purposes with a NOEC of 0.95 µg/L. Therefore, a full summary for this study is reported below:

- **Reference:** IIA 8.2.4/01
- **Author(s); year:** York D.O. (2010)
- **Title:** SYN545192 – Early Life-Stage Toxicity Test with Fathead Minnow (*Pimephales promelas*)
- **Report No:** 1781.6718

  Guidelines: OECD Guidelines for Testing of Chemicals, Section 2 - Effects on Biotic Systems, No. 210: Fish, Early-life Stage Toxicity Test (1992). U.S. EPA Ecological Effects Test Guidelines, OPPTS 850.1400: Fish, Early-life Stage Toxicity Test (1996)
- **GLP:** Yes
- **Executive summary :**

The effects of SYN545192 on the embryos and larvae of fathead minnow (*Pimephales promelas*) were determined under flow-through conditions. Fish were exposed to nominal concentrations of 0.25, 0.50, 1.0, 2.0 and 4.0 µg a.i./L, a solvent control and a dilution water control. Results were based on the mean measured concentrations of 0.29, 0.49, 0.95, 1.8 and 3.6 µg a.i./L.

Hatching success was unaffected compared to the controls at all concentrations of SYN545192 tested. The 28-day post-hatch survival was unaffected compared to the controls at all treatment levels except the highest concentration of 3.6 µg a.i./L. Length and dry weight were unaffected compared to the controls up to the treatment level of 0.95 µg a.i./L.

Based on growth (length and dry weight), the 32-day NOEC for SYN545192 was determined to be 0.95 µg a.i./L, and the 32-day LOEC was determined to be 1.8 µg a.i./L.

- **Materials and methods:**

Materials

Test material	SYN545192 CSCD064398
Lot/Batch #:	TE-6341
Purity:	98.3%
Description:	White solid
Stability of test compound:	Stable under test conditions
Reanalysis/expiry date:	31 August 2010
Treatments	
Test concentrations:	Dilution water control, solvent control (0.01 mL DMF/L) and nominal SYN545192 concentrations of 0.25, 0.50, 1.0, 2.0 and 4.0 µg a.i./L
Solvent:	Dimethylformamide (DMF, CAS No. 68-12-2)
Analysis of test concentrations:	Yes on Days 0, 5, 13, 21, 27 and 32 using LC/MS/MS analysis
Test organisms	
Species:	Fathead minnow *Pimephales promelas*
Source:	Springborn Smithers Laboratory brood stock, originating from the U.S. Environmental Research Laboratory, Duluth, Minnesota, U.S.A.
Treatment for disease:	None
Feeding:	Live brine shrimp nauplii (*Artemia salina*) three times daily from Day 4 (Day 0 post-hatch). No food given during last 24 hours of the study.
Test design	
Exposure regime:	Flow-through using a proportional diluter system
Aeration:	Dilution water aerated in storage reservoir
Replication:	4
Test vessels:	Glass aquaria measuring 60 x 30 x 30 cm, with a high side drain maintaining a solution volume of approximately 27 L. Embryo incubation cups: round glass jars with Nitex® screen bottoms with 475-µm screen openings.
No of eggs per tank:	30
Duration:	28 days post-hatch (32 days exposure)
Environmental conditions	

Test temperature:	24 - 25°C continuously monitored in one tank
pH:	7.0 – 7.6
Dissolved oxygen:	5.7 – 9.0 mg/L Mean dissolved oxygen concentrations ranged from 69 to 109% saturation
Hardness of dilution water:	44 - 56 mg/L as $CaCO_3$
Lighting:	16 hours fluorescent light and 8 hours dark with 30 minute transition periods, 50-120 footcandles

Study Design and Methods

Experimental dates: 12 March 2009 to 13 April 2009.

A flow-through test system was employed. A 0.400 mg/mL diluter stock solution was prepared twice weekly by placing approximately 0.0200 g of SYN545192 in a 50-mL volumetric flask and bringing it to volume with DMF. This stock solution, together with appropriate volumes of dilution water, were delivered to the diluter's mixing chamber so that the mixing chamber solution constituted the highest nominal concentration of 4.0 µg a.i./L. This was sequentially diluted (50%) to provide the remaining exposure concentrations.

At the start of the test 30 eggs, approximately six hours old, were randomly allocated to egg cups and one egg cup suspended in each of four replicate test vessels at each test and control treatment. Hence, 120 eggs were exposed at each treatment. The test was undertaken in a temperature controlled water-bath.

The concentrations were verified by chemical analysis of SYN545192 on days 0, 5, 13, 21, 27 and 32 using an LC-MS/MS method.

- **Findings:**

Results and Discussion

The mean measured concentrations ranged from 88% to 120% of their nominal concentrations (see table below). Mean measured concentrations were used for the calculation and reporting of the results.

Table IIA 8.2.4-1: Analytical results

Sample	**Nominal Concentration (µg a.i./L)**						
	Control	Solvent Control	0.25	0.5	1.0	2.0	4.0
	Measured Concentration[a] (µg SYN545192/L)						
Day 0	<LOQ[a]	<LOQ	0.25	0.59	1.0	2.1	4.1
Day 5	<LOQ	<LOQ	0.21	0.44	0.88	1.8	3.4
Day 13	<LOQ	<LOQ	0.28	0.50	0.74[c]	1.3[c]	3.6
Day 21	0.021[b]	0.018[b]	0.35	0.53	1.1	1.8	3.5
Day 27	<LOQ	<LOQ	0.29	0.43	1.0	1.8	3.6
Day 32	<LOQ	<LOQ	0.39	0.44	1.0	1.8	3.5
Mean [c]	NA (NA)	NA (NA)	0.29	0.49	0.95	1.8	3.6
	% of Nominal Measured						

Mean Measured Values [c]	NA	NA	120	98	95	88	90

[a] LOQ = the limit of quantification. The LOQ for each analysis is dependent upon the linear regression, the area of the low standards and the dilution factor of the controls. At days 0, 5, 13, 27 and 32, the LOQ was 0.0096, 0.0088, 0.0098, 0.0085 and 0.0087 µg a.i./L, respectively
[b] Analytical result is due to contamination in the control samples as a result of sample processing outside of the exposure system and is not representative of actual test conditions
[c] Analytical result presented in the table for this sample is from the resampling on day 14.
NA = Not Applicable

At test termination, data on embryo hatching success, the percentage of embryos producing live normal fry at hatch and survival, total length and dry weight at test termination were analysed to identify significant reductions in the treatment organisms compared to the control organisms.

The Student's t-Test was used to compare the performance of the dilution water control organisms with the solvent control organisms, the Shapiro-Wilks' Test was used to determine sample distribution normality, homogeneity of variance was evaluated using Bartlett's Test, and the Williams' Test and the Kruskal-Wallis' Test were used to establish treatment effects.

The biological data are presented in the table below.

Table IIA 8.2.4-2: Effects of SYN545192 on the hatch success, larval survival and growth of *Pimephales promelas*

Mean Measured Concentration (µg a.i./L)	**Embryo Hatching Success (Mean %)**	**Mean Normal Fry at Hatch (Mean %)**	**28 Days Post-Hatch**		
			Larval Survival (Mean %)	**Mean Length (mm)**	**Mean dry weight (mg)**
Control	91	99	73	26.4	0.0332
Solvent control	88	99	82	26.2	0.0326
0.29	91	100	83	25.7	0.0308
0.49	88	100	74	26.2	0.0329
0.95	**89**	**100**	**75**	**26.0**	**0.0330**
1.8	89	99	80	24.4[a]	0.0270[a]
3.6	87	100	40[ab]	19.6[b]	0.0176[b]

[a] Statistically reduced compared to the control, based on Williams' Test.

[b] Treatment level was excluded from statistical analysis of growth (total length and dry weight) due to the survival effect observed.

Validity criteria

Average control hatchability was > 66% and larval survival (post-hatch) was > 70%, therefore this study is considered acceptable.

- **Conclusion:**

Hatching success was unaffected compared to the controls at all concentrations of SYN545192 tested. The 28-day post-hatch survival was unaffected compared to the controls at all treatment levels except the highest concentration of 3.6 µg a.i./L. Length and dry weight were unaffected compared to the controls up to the treatment level of 0.95 µg a.i./L.

Based on growth (length and dry weight), the 32-day NOEC for SYN545192 was determined to be 0.95 µg a.i./L, and the 32-day LOEC was determined to be 1.8 µg a.i./L.

5.4.2 Aquatic invertebrates

5.4.2.1 Short-term toxicity to aquatic invertebrates

Three studies (one with a freshwater and two with a saltwater species) are available for this endpoint. All are considered to be relevant, reliable and adequate for classification purposes, with the results for the freshwater *Daphnia magna* being most relevant for classification purposes.

The sensitivity of *D. magna* to benzovindiflupyr was determined in a GLP-compliant test performed to standard guidelines (Fournier, 2010e). The *Daphnia* were exposed for 48 hours to 5 concentrations of benzovindiflupyr. Analytical monitoring of the exposure concentrations was undertaken and the results based on the geometric mean of the concentrations measured for each test treatment at the start and end of the test period. The 48 hour EC50 for *Daphnia magna* exposed to the test substance was determined to be 85 µg/L with 95% confidence limits of 56 to 130 µg/L. The 48 hour NOEC was determined to be 21 µg/L.

The sensitivity of saltwater mysid (*Americamysis bahia*) to the test substance was determined in a GLP-compliant test performed to standard guidelines (Fournier, 2010f). The mysids were exposed for up to 96 hours to 5 concentrations of benzovindiflupyr. Analytical monitoring of the exposure concentrations was undertaken and the results based on the mean of the concentrations measured for each test treatment at the start and end of the test period. The 96-hour LC50 for *A. bahia* exposed to the test substance was determined to be 56 µg/L with 95% confidence limits of 17 to 120 µg/L. The 96-hour NOEC was determined to be 7.4 µg/L.

The sensitivity of eastern oysters, *Crassostrea virginica*, to benzovindiflupyr was determined in a GLP-compliant test performed to standard guidelines (York, 2010b). The oysters were exposed for 96 hours to five concentrations of the test substance, a control and a solvent control. Analytical monitoring of the exposure concentrations was undertaken and the results based on the arithmetic mean of the concentrations measured for each treatment at the start and end of the test period. Shell growth was the most sensitive endpoint measured. The 96 hour EC50 for *C. virginica* exposed to benzovindiflupyr was determined to be 160 µg/L, with a 95 % confidence interval of 120 - 210 µg/L. The NOEC was determined to be 67 µg/L.

The EC50/LC50 results considered for classification purposes are the 48-h EC50 for *D. magna* of 85 µg/L, the 96-h LC50 for *A. bahia* of 56 µg/L and the 96-h EC50 for *C. virginica* of 160 µg/L. However all these species are less sensitive than fish (acute LC50 3.5 µg/L; see 5.4.1.1 above) and therefore the results do not determine the classification for benzovindiflupyr.

5.4.2.2 Long-term toxicity to aquatic invertebrates

Two studies (one with a freshwater and one with a saltwater species) are available for this endpoint. Both are considered to be relevant, reliable and adequate for classification purposes.

The toxicity of the test item to the chronic survival and reproduction of aquatic invertebrate, *Daphnia magna*, was determined in a GLP-compliant test performed to OECD guideline 211(Fournier, 2010g). *D. magna* were exposed for 21 days to six concentrations of benzovindiflupyr, a control and a solvent control. Analytical monitoring of the exposure concentrations was undertaken and the results were based on the arithmetic mean of the concentrations measured for each treatment at the start and end of each renewal period. The 21 day EC50 for survival and reproduction were determined to be 65 and 23 µg/L, respectively. Based on reproduction and growth (the most sensitive indicators of toxicity), the 21 day NOEC was determined to be 15 µg/L and the 21 day LOEC was determined to be 34 µg/L.

The chronic survival and reproduction of the saltwater invertebrate, mysid (*Americamysis bahia*), was determined in a GLP-compliant flow-through test performed to standard guidelines (Lee, 2011). The mysids were exposed for 28 days to five concentrations of benzovindiflupyr, and a control. Analytical monitoring of

the exposure concentrations was undertaken and the results were based on the mean of the concentrations measured for each treatment at 7 day intervals during the exposure priority. The 28-day LC50 for benzovindiflupyr to the mysid was calculated to be 28 µg/L, with 95% confidence interval of 23 – 30 µg/L. The 28-day NOEC, based on reproduction was determined to be 7.4 µg/L, and the 28-day LOEC was determined to be 15 µg/L based on mean measured concentrations.

The results considered for classification purposes are the 21-d NOEC for *D.magna* of 15 µg/L and the 28-d NOEC for *A. bahia* of 7.4 µg/L. Both these species are less sensitive than fish (NOEC 0.95 µg/L; see 5.4.1.2 above) and therefore the results do not determine the classification for benzovindiflupyr.

5.4.3 Algae and aquatic plants

Two algal studies (one with a freshwater and one with a saltwater species) are available for this endpoint. Both are considered to be relevant, reliable and adequate for classification purposes. The EC50 and NOEC results for freshwater species are carried forward for classification purposes. These tests with algae are also considered to be long-term tests.

The toxicity of benzovindiflupyr to the green algae *Pseudokirchneriella subcapitata* was tested in a GLP compliant, guideline study (Softcheck, 2010). Following a preliminary exposure test, a definitive test was carried out with control, solvent control and test concentrations 500 and 1000 µg/L. Analytical monitoring of the exposure concentrations was undertaken and the results are expressed as the mean measured concentration from the start and end of the exposure period. The 72-hour EC50 values were > 890 µg/L based on biomass (AUC), yield and growth rate. The 72-hour NOEC were 420 µg test substance/L for biomass, growth rate and yield.

The toxicity of benzovindiflupyr to the marine diatom, *Skeletonema costatum*, was tested in a GLP compliant, guideline study (Softcheck, 2011a). Following a preliminary exposure test, a definitive test was carried out with a control, solvent control and nominal test concentrations of 0.063, 0.13, 0.25, 0.50 and 1.0 mg benzovindiflupyr /L. Analytical monitoring of the exposure concentrations was undertaken and the results are expressed as mean measured concentrations from the start and end of the exposure period. The 72 hour ErC50 was 0.55 mg/L, the EyC50 was 0.47 mg/L and the EbC50 was 0.45 mg/L based on growth rate, yield and biomass (area under growth curve) respectively. The 72 hour NOEC is 0.4, 0.1 and 0.1 mg/L for growth rate, yield and biomass (area under growth curve) respectively. Overall the lowest EC50 (72 h) was 0.45 mg/L and the overall NOEC (72 h) was 0.1 mg/L.

A study is available for aquatic plants and this is considered to be relevant, reliable and adequate for classification purposes. The toxicity of benzovindiflupyr to duckweed (*Lemna gibba*) was tested in a GLP-compliant, guideline study (Softcheck, 2011b). Following a preliminary exposure test, a definitive test was carried out with control, solvent control and mean measured test concentrations 58, 110, 230, 430 and 880 µg/L. Analytical monitoring of the exposure concentrations was undertaken and the results are expressed as the mean measured concentration between test solution renewals. The 7-day EC50 values were > 880 µg/L for yield and growth rate based on frond number and dry weight. The 7 -day NOECs were 880 µg/L based on frond number and 430 µg/L based on dry weight.

The results considered for classification purposes are the lowest 72 hour EC50 value for algae (0.45 mg/L for *S. costatum*) and the lowest NOEC values for algae and *Lemna* (NOEC 0.1 mg/L, also for *S. costatum*). These species are all less sensitive than fish (96-h LC50 3.5 µg/L and NOEC 0.95 µg/L; see 5.4.1.1 & 2 above) and therefore the results do not determine the classification for benzovindiflupyr.

5.4.4 Other aquatic organisms (including sediment)

Two chronic studies (one with a freshwater and one with a saltwater species) are available for this endpoint. Both are considered to be relevant, reliable and adequate for classification purposes.

The effects of benzovindiflupyr on the life cycle of *Chironomus dilutus* were determined under static-renewal conditions in a GLP-compliant test performed to EPA guidelines (Picard, 2012a). Organisms were exposed to nominal concentrations of 3.1, 6.3, 13, 25 and 50 mg/kg dry weight in sediment alongside a dilution water control and a solvent control.

Based on mean measured sediment concentrations, the 20-day LC50 and EC50 for larval survival and growth, respectively, were >48 mg/kg dry weight, and the corresponding 20-day NOECs were 48 mg/kg dry weight. The 56-day EC50for emergence, emergence rate and days to death were >48 mg/kg dry weight, and the corresponding 56-day NOECs were 48 mg/kg dry weight.

For reproductive endpoints, the 56-day EC50 for percent hatch and days to oviposition were 39 and >48 mg/kg dry weight, respectively, with corresponding NOECs of 24 and 48 mg/kg dry weight, respectively. The EC50 for egg masses per mated female, eggs per egg mass and eggs per mated female were not determined. The NOECs for these endpoints were 6.7, 24 and 6.7 mg/kg dry weight, respectively. The overall study NOEC was 6.7 mg/kg dry weight, based on egg masses per mated female and the number of eggs per mated female.

In the second GLP, guideline study (Picard, 2012b), the toxicity of benzovindiflupyr to the amphipod *Leptocheirus plumulosus* was determined under static renewal conditions in a 28-day chronic toxicity test. The amphipods were exposed to six concentrations of benzovindiflupyr, a control and a solvent control.

Based on mean measured concentrations the 28-day LC50 for survival was determined to 9.6 mg/kg, and the 28-day EC50 values for growth and reproduction were determined to be >8.7 and 7.0 mg/kg, respectively. The NOEC and LOEC values for all endpoints were determined to be 4.1 and 8.7 mg/kg, respectively.

These sediment toxicity study results have not been considered for classification purposes since reliable aquatic toxicity studies for all required tropic levels are available and are sufficient to conclude on the appropriate classification of benzovindiflupyr.

5.5 Comparison with criteria for environmental hazards (sections 5.1 – 5.4)

Summary of acute and long term toxicity endpoints of benzovindiflupyr to aquatic organisms is reported in previous table (table 26).

In aquatic toxicity studies the relevant (lowest) acute LC50 value for fish, EC50 value for invertebrates, and ErC50 value for algae were all < 1 mg/L. The lowest acute endpoint for technical benzovindiflupyr was observed for *C. carpio* with an acute 96 hr LC50 of 0.0035 mg a.s./L.

In long- term toxicity studies NOEC values < 0.1 mg/L for fish, invertebrates, algae and aquatic plants were determined. The lowest NOEC (32 d) of 0.00095 mg/L was for the fish, *P. promelas*.

Benzovindiflupyr is not readily biodegradable. Based on the findings from water/sediment simulation tests benzovindiflupyr is not significantly labile under normal aerobic or anaerobic study conditions. Under more realistic conditions, where light is provided and phototrophic organisms such as algae and aquatic macrophytes can grow, degradation of benzovindiflupyr was significantly faster. However, considering the levels of mineralisation in all these simulation studies, benzovindiflupyr is considered not readily/ rapidly biodegradable (a degradation > 70 % within 28 days) for purposes of classification and labeling.

Although benzovindiflupyr has a log Kow of 4.3, the experimentally derived steady state BCF value of 76 L/kg ww is below the trigger of 500 criterion for bioaccumulating potential conform Regulation EC 1272/2008).

5.6 Conclusions on classification and labelling for environmental hazards (sections 5.1 – 5.4)

On the basis of its acute and long-term toxicity for fish, and based on the fact that benzovindiflupyr is not readily biodegradable, the following provisional classification and labelling of benzovindiflupyr is proposed according to CLP Regulation.

Hazard symbol:	Warning
Risk phrases:	Aquatic Acute 1 H400 (M-factor: 100) Aquatic Chronic 1 H410 (M-factor: 100)
Justification:	Acute LC50, fish 0.0035 mg/L Chronic NOEC, fish 0.00095 mg/L Not rapidly biodegradable

Since the lowest L(E)C50 is between 0.001 and 0.01 mg/L, the acute M-factor is 100. Since the lowest chronic NOAEC of this not rapidly degradable substance is between 0.0001 and 0.001 mg/L, the chronic M-factor is 100.

CLP: Aquatic Acute 1 H400 (M-factor: 100) ; Aquatic Chronic 1 H410 (M-factor: 100)

RAC evaluation of environmental hazards

Summary of the Dossier submitter's proposal

Benzovindiflupyr has no environmental classification and labelling in Annex VI of CLP Regulation.

The DS proposed to classify benzovindiflupyr as Aquatic Acute 1 (H400) with an M-factor = 100 and Aquatic Chronic 1 (H410) with an M-factor = 100. The classification was based on the substance being not rapidly degradable, non-bioaccumulative and very toxic to fish. The relevant lowest acute toxicity value was an LC_{50} (96h) of 0.0035 mg/L on *Cyprinus carpio* and the lowest chronic toxicity value was a NOEC (32d) of 0.00095 mg/L on *Pimephales promelas*.

Degradation

The substance degrades by indirect aqueous photolysis (DT_{50} of 5.0 days) and slower by direct aqueous photolysis in sterile buffer (DT_{50} of 44.2 days) according to OECD TG 316.

The substance was found to be hydrolytically stable for up to five days at pH 4, 5 and 7 after the preliminary test carried out according to OECD TG 111. No hydrolysis products were detected.

Regarding biodegradation, one study on ready biodegradability and another study on biodegradation in water/sediment tests were included in the CLH report. The ready biodegradability test was performed according to OECD TG 301F and GLP. Benzovindiflupyr was degraded by an average of 1% after 28 days and was considered not-readily biodegradable under these test conditions. The substance did not show inhibitory effects on the activated sludge microorganisms at the tested concentration.

The degradation of benzovindiflupyr was also investigated in a standard water/sediment system with two different sediments (silt loam and sandy loam) according to OECD TG 308 under GLP conditions. In this study standard aerobic and anaerobic degradation was investigated under dark conditions, which resulted in c.a. 90% of the applied benzovindiflupyr remaining not degraded after an exposure period of 100 days. Both aerobic and anaerobic mineralization ranged between 0.1-0.3% after 100 days. No metabolites were observed above 3% of applied radioactivity. The total system degradation rates (DT_{50} and DT_{90}) were extrapolated to >500 and 1000 days, respectively.

The DS concluded that benzonvidiflupyr is considered not rapidly biodegradable.

Bioaccumulation
The substance has a measured log Kow of 4.3, according to OECD TG 107. A BCF was determined in bluegill sunfish (*Lepomis machrochirus*) in a GLP study conducted according to OECD TG 305. The BCF of benzovindiflupyr based on the characterisation of the residues in the whole fish tissues was 76 L/kg. After normalisation for lipid content of the test organisms (3.09%) the final experimentally normalised steady state BCF was estimated to be 123 L/kg based on an assumed 5% lipid content. This value was used for normalisation. The clearance of accumulated residue from the whole body was rapid, with a depuration half-life of 0.54 days. The DS concluded that based on experimental results accumulation of the substance in fish is not expected.

Aquatic Toxicity
Acute and chronic aquatic toxicity data are available for the three trophic levels (fish, aquatic invertebrate and algae) resulting in fish being shown to be the most sensitive species from both acute and chronic tests.

Acute toxicity

Five acute toxicity tests on **fish** were included in the CLH dossier, all of which were carried out according to OECD TG 203: four with freshwater fish and one with a saltwater species (*Cyprinodon variegatus*). The substance was less toxic to saltwater fish than freshwater fish species by one order of magnitude. Studies were reliable and considered adequate for classification purposes: an LC_{50} (96h) for *C. carpio* of 3.5 µg/L, LC_{50} (96h) for *P. promelas* of 4.7 µg/L, LC_{50} (96h) for *Oncorhynchus mykiss* of 9.1 µg/L, LC_{50} (96h) for *L. machrochirus* of 26 µg/L and LC_{50} (96h) for *C. variegatus* of 27 µg/L. The lowest acute toxicity value for freshwater species, the LC_{50} (96h) of 3.5 µg/L in carp (*C. carpio*) was selected by the DS for classification purposes. The study was carried out under GLP and under flow-through conditions and the test substance concentrations were analytically monitored during the course of the study.

Three reliable acute toxicity tests on aquatic **invertebrates** were included in the CLH dossier: one with the freshwater *Daphnia magna* (EC_{50} (48h) of 85 µg/L) carried out according to OECD TG 202 and two tests with a saltwater species: the mysid *Americamysis bahia* (LC_{50} (96h) of 56 µg/L), conducted according to EPA OPPTS 850.1035 and the bivalve *Crassostrea virginica* (EC_{50} (96h) of 160 µg/L), conducted according to the EPA OPPTS 850.1025.

Regarding the acute information for **algae and aquatic plants**, two algal studies were included in the dossier carried out according to OECD TG 201: one with *Pseudokirchneriella subcapitata* (ErC_{50} (72h) >890 µg/L) and other with *Skeletonema kostatum* (ErC_{50} (72h) of 550 µg/L). Additionally, an aquatic plant study with *Lemna gibba* was included in the dossier resulting in a EC_{50} (7d) value >880 µg/L based on the change in frond number produced.

Chronic toxicity

One **fish** early life-stage toxicity study with *P. promelas* was conducted according to OECD TG 210 under GLP conditions. This study resulted in a NOEC (32d) of 0.95 µg/L, which was considered by the DS for classification purposes. The study, carried out under GLP, was a flow-through and the test substance concentrations were analytically monitored.

Regarding the chronic information for **aquatic invertebrates**, two chronic toxicity tests were included in the dossier: one with the freshwater *Daphnia magna* (NOEC (21d) of 15

µg/L) conducted according to OECD TG 211 and another test with a saltwater species: the mysid *A. bahia* (NOEC (28d) of 7.4 µg/L), according to EPA OPPTS 850.1350. Both studies are reliable and showed that aquatic invertebrates are less sensitive than fish to benzovindiflupyr.

Regarding the chronic information for **algae and aquatic plants**, two algal studies were included in the dossier carried out according to OECD TG 201: one with *P. subcapitata* (NOErC (72h) of 890 µg/L) and other with *S. kostatum* (NOErC (72h) of 400 µg/L). Additionally, an aquatic plant study (*Lemna gibba*) was included in the dossier (NOEC (7d) >880 µg/L).

Comments received during public consultation

Comments from six Member States (MS) were received during the public consultation (PC). All of them agreed with the classification proposed by the DS.

Regarding bioaccumulation, two MS indicated the need for lipid normalization of the BCF. The initial BCF of the test substance, based on the characterization of the residues in the whole fish tissues was estimated as 76 L/kg. After the normalization for lipid content of the test fish, the final experimentally steady state BCF is 123 L/kg. This correction has no effect on the proposed classification and labelling.

Also regarding bioaccumulation, one MS noted that there is no explanation why the BCF test was carried out with only one test concentration instead of two test concentrations. The DS explained that according to OECD TG 305, one test concentration can be considered sufficient when it is likely that the BCF is independent of the test concentration and the test concentration is as low as technically feasible. In the case of benzovindiflupyr, the concentration for the BCF test was 0.26 µg/L which is well below the solubility limit of the substance (water solubility of 0.98 mg/L).

Regarding ecotoxicity, one MS requested a clarification on two endpoints (larval survival and growth) mentioned in the CLH report which had only one value. The DS clarified this in their response in the RCOM.

Assessment and comparison with the classification criteria

Degradation
RAC evaluated the information in the CLH report and RCOM and agrees with the DS's proposal to consider benzovindiflupyr as a non-rapidly degradable substance based on 1% degradation in the OECD TG 301F study and 0.1-0.3% aerobic and anaerobic mineralization in the OECD TG 308 study. Both studies were performed under GLP.

Bioaccumulation
Despite the measured log Kow of 4.3 (OECD TG 117), RAC evaluated the information in the CLH report and RCOM and agrees with the DS that benzovindiflupyr has a low potential to bioaccumulate based on rapid metabolism/depuration rates and a 5% lipid normalized BCF of 123 L/kg in the OECD TG 305 study which was performed under GLP.

Aquatic Toxicity
RAC evaluated the information in the CLH report and RCOM. Both acute and chronic aquatic toxicity data on fish, aquatic invertebrate and algae were available. All studies are reliable, carried out under GLP and are appropriate for classification purposes. Fish was determined to be the most sensitive species in both acute and chronic tests.

RAC agrees with the DS´s proposal to classify benzovindiflupyr as aquatic acute 1 and aquatic chronic 1 based on an LC_{50} (96h) in *C. carpio* of 3.5 µg/L (OECD TG 203) and a NOEC

(32d) in *P. promelas* of 0.95 µg/L (OECD TG 210), respectively.

Conclusion on Classification

Based on the observed acute toxicity ($0.001 < LC_{50} \leq 0.01$) in fish and chronic toxicity ($0.0001 < NOEC \leq 0.001$) also in fish and non rapid degradation of the substance, RAC agrees with the DS's proposal to classify benzovindiflupyr as:

Aquatic Acute 1 (H400), M=100 and
Aquatic Chronic 1 (H410), M=100.

This classification was based on the substance being not rapidly degradable, non-bioaccumulative and very toxic to aquatic organisms.

6 OTHER INFORMATION

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8 ANNEX

PART 1: POSTULATED MODE OF ACTION FOR THYROID TUMOURS IN MALE RATS

It is proposed that benzovindiflupyr-induced thyroid tumours in male rats are attributable to induction of hepatic UDP-glucuronsyltransferase (UDPGT), which results in a series of downstream events, ultimately leading to tumourgenesis. This mode of action is not relevant for human hazard/risk assessment purposes due to qualitative and quantitative differences in response to UDPGT induction and increased clearance of thyroid hormones (T_3/T_4) between rats and humans. Benzovindiflupyr does not pose a carcinogenic hazard to humans. The assessment within this document uses the framework developed by the International Programme on Chemical Safety (IPCS) (Sonich-Mullin *et al*, 2001) and the International Life Sciences Institute (ILSI) (Meek *et al*, 2003). The framework aims to answer three questions sequentially: (i) Has a mode-of-action (MOA) been established in the test species?; (ii) based on qualitative assessment of the differences between species in terms of toxicokinetics and toxicodynamics, is the MOA plausible in humans?; (iii) based on an assessment of the quantitative differences between species in terms of toxicokinetics and toxicodynamics, is the MOA plausible in humans?

Overview of carcinogenicity data for benzovindiflupyr

In a combined chronic toxicity and carcinogenicity study, Han Wistar rats were treated with benzovindiflupyr at dietary inclusion levels of 0, 25, 100 and 400(♀)/600(♂) ppm. The only treatment-related neoplastic finding was a dose dependant increase in thyroid follicular cell adenomas [1/52 (2%), 4/52 (7.6%), 5/52 (9.6%), 9/52* (17.3%) at 0, 25, 100, 600 ppm, respectively], but not carcinomas [1/52, 0/52. 1/52, 0/52 at 0, 25, 100, 600 respectively] in males (Mackay, 2012a).

There was no evidence for a treatment-related effect on the incidence of thyroid tumours, nor any other tumour type in an 80 week study in CD-1 mice treated at 0, 20, 60 and 200 ppm (Mackay, 2012b).

The proposed MOA involves a number of causal and associated key events. Treatment of male Han Wistar rats with benzovindiflupyr results in the induction of hepatic UDPGT. UDPGT is a phase II liver enzyme that catalyses the glucuronidation of endogenous circulating triiodothyronine (T_3) and thyroxine (T_4). These glucuronide conjugates are readily excreted via the bile, and so induction of hepatic UDPGT results in lower circulating T_3 and/or T_4 due to their increased clearance. The reduced concentration of circulating T_3/T_4 is detected in the hypothalamus. In order to maintain homeostasis, the secretion of thyrotropin-releasing hormone [TRH] (by the hypothalamus) is triggered, consequently increasing the secretion of thyroid stimulating hormone (TSH) by the pituitary. Increased circulating TSH results in thyroid follicular cell hypertrophy and proliferation (resulting in increased thyroid weight) in order to increase the capacity for production of T_3/T_4 by the thyroid and thereby return circulating T_3 and T_4 to basal levels. Chronically-increased circulating TSH results in persistent proliferative stimulation of thyroid follicular cells, which eventually results in the formation of thyroid follicular cell adenomas. This MOA can be described as a perturbation of the hypothalamus-pituitary-thyroid (HPT) axis as a secondary consequence of liver xenobiotic metabolising enzyme induction and has been well characterised and described for a number of compounds.

Relevant data for benzovindiflupyr

Male Han Wistar rats (15/group/time point) were treated with benzovindiflupyr at 0, 100, 600 or 1200 ppm for 1, 3, 7 or 14 days before termination and a number of liver- and thyroid-related parameters measured (Robertson, 2012b). The reversibility of benzovindiflupyr -related effects was assessed by including additional groups of animals treated with either 0 or 1200 ppm benzovindiflupyr for 14 days, followed by a 63 day recovery period. In addition to treatment with benzovindiflupyr, further animals were treated with 1200 ppm phenobarbital sodium salt (PB). PB was included as a positive control as it is a known promotor of thyroid follicular cell adenomas in the rat by a MOA similar to that proposed for benzovindiflupyr (Meek *et al*, 2003). The data from these studies are summarized in Tables 8-1, 8-2 and 8-3.

Table 8-1: Summary of selected data from a 14 day mode of action study with benzovindiflupyr and PB - Liver-related parameters

	Mean adjusted liver wt (g)				Hepatocellular centrilobular hypertrophy (incidence/15)				Mean hepatic UDPGT activity (mmol/min/liver wt/kg bw)			
Days of treatment	**1**	**7**	**14**	**14R^**	**1**	**7**	**14**	**14R^**	**1**	**7**	**14**	**14R^**
Control	9.31	9.91	10.66	13.07	0	0	0	0	48.85	52.59	32.27	35.50
Benzovindiflupyr 100ppm	9.18	10.29	10.99	N/A	0	0	0	N/A	50.53	56.62	34.47	N/A
Benzovindiflupyr 600ppm	8.46*	10.77*	11.88**	N/A	0	10**	13**	N/A	38.07	59.69	49.82**	N/A
Benzovindiflupyr 1200ppm	8.03*	11.38**	12.31**	13.14	0	15**	15**	0	45.43	96.99**	76.43**	35.00
PB 1200ppm	8.92	12.36**	14.26**	N/A	0	15**	15**	N/A	57.20	134.92**	122.44**	N/A

^ 14 days of treatment + 63 days recovery

* and **: Statistically-significantly different from control with $p<0.05$ and $p<0.01$, respectively. From Robertson, 2012b.

Table 8-2: Summary of selected data from a 14 day mode of action study with benzovindiflupyr and PB - Hormones

	Mean serum T_3 (ng/dL)				Mean serum T_4 (ng/dL)				Mean serum TSH (ng/dL)			
Days of treatment	**1**	**7**	**14**	**14R^**	**1**	**7**	**14**	**14R^**	**1**	**7**	**14**	**14R^**
Control	138	118	119	84	5.8	5.9	6.0	4.8	5.3	5.4	4.6	4.5
Benzovindiflupyr 100ppm	126	121	103*	N/A	6.0	6.4	5.8	N/A	4.9	6.1	4.3	N/A
Benzovindiflupyr 600ppm	107**	107	92**	N/A	5.6	6.4	5.6	N/A	4.8	5.7	6.0	N/A
Benzovindiflupyr 1200ppm	95**	103	78**	90	5.1**	6.2	5.4	5.2	4.7	4.8	7.5*	4.9
PB 1200ppm	94**	101	85**	N/A	4.8**	5.9	5.0**	N/A	4.7	5.8	7.8**	N/A

^ 14 days of treatment + 63 days recovery

* and **: Statistically-significantly different from control with $p<0.05$ and $p<0.01$, respectively. From Robertson, 2012b.

Table 8-3: Summary of selected data from a 14 day mode of action study with benzovindiflupyr and PB - Thyroid-related parameters

	Mean adjusted thyroid wt (g)				Thyroid follicular cell hypertrophy (incidence/15)				Mean thyroid follicular cell proliferation (labelling index [%]+)			
Days of treatment	**1**	**7**	**14**	**14R^**	**1**	**7**	**14**	**14R^**	**1**	**7**	**14**	**14R^**
Control	0.0136	0.0142	0.0138	0.0191	0	0	0	0	1.29	0.69	0.46	0.36
Benzovindiflupyr 100ppm	0.0138	0.0147	0.0160	N/A	0	0	0	N/A	2.21**	2.47**	0.70	N/A
Benzovindiflupyr 600ppm	0.0117	0.0149	0.0144	N/A	0	0	0	N/A	1.81	0.87	0.60	N/A
Benzovindiflupyr 1200ppm	0.0136	0.0158	0.0171	0.0180	0	0	0	0	1.61	1.88**	2.18**	0.30
PB 1200ppm	0.0124	0.0166	0.0175**	N/A	0	0	8**	N/A	2.40**	1.56**	2.15**	N/A

+ Thyroid follicular cell proliferation was assessed using the 5-bromo-2'-deoxyuridine (BrdU)-incorporation technique
^ 14 days of treatment + 63 days recovery
* and **: Statistically-significantly different from control with $p<0.05$ and $p<0.01$, respectively. From Robertson, 2012b.

Benzovindiflupyr results in dose- and time-related effects on the proposed causal and associative key events that are consistent with the hypothesized MOA and these effects are reversible following cessation of treatment. Benzovindiflupyr induces hepatic UDPGT, reduces circulating T_3 and T_4 and increases circulating TSH. Data for thyroid follicular cell proliferation were highly variable, with a number of statistically significant increases of up to 2 times the concurrent control observed at all time points. Due to the lack of a dose-response relationship and no corresponding increases in TSH, any differences observed after 1, 3 or 7 days of treatment with either benzovindiflupyr or PB were considered to reflect normal variability. The only effects on thyroid follicular cell proliferation considered to be biologically plausible were the >4 fold statistically significant increases observed following 14 days treatment with 1200 ppm benzovindiflupyr or 1200 ppm PB as these changes were associated with corresponding increases in serum TSH levels and thyroid weight and were of a greater magnitude than earlier apparent differences in labelling index. The data obtained for PB were similar to the data obtained for benzovindiflupyr for liver parameters, thyroid hormone changes and cell proliferation.

Relevant liver and thyroid data generated in a 28 day study (Robertson, 2010b), 90 day study (Robertson, 2010a) and the combined chronic toxicity and carcinogenicity study in rats are presented in Tables 8-4, 8-5, 8-6 and 8-7.

Table 8-4: Summary of data from a 28 day study with benzovindiflupyr - Liver-related parameters from male rats

	Mean adjusted liver weight (g)					Mean hepatic UDPGT activity (mmol/min/liver wt/kg bw)				
Days of treatment	**2**	**3**	**7**	**14**	**28**	**2**	**3**	**7**	**14**	**28**
Control	9.32	8.81	9.77	10.39	10.86	-	21.31	16.71	19.91	17.65
Benzovindiflupyr 100ppm	10.40	9.98	10.98*	10.24	12.37	-	27.69	19.05	27.64	19.18
Benzovindiflupyr 750ppm	10.02	10.28*	10.82*	12.03	12.03	-	38.95*	33.55*	39.70**	28.95
Benzovindiflupyr 1500ppm	10.99**	10.34*	12.34**	13.56**	13.85**	-	39.31*	52.74**	40.04**	42.95*

* and **: Statistically-significantly different from control with p<0.05 and p<0.01, respectively. – Data not collected. From Robertson, 2010b and Lake, 2012a.

Table 8-5: Summary of data from a 28 day study with benzovindiflupyr - Thyroid-related parameters from male rats

	Mean adjusted thyroid wt(g)					Thyroid follicular cell hypertrophy (incidence/5)				
Days of treatment	**2**	**3**	**7**	**14**	**28**	**2**	**3**	**7**	**14**	**28**
Control	0.0146	0.0162	0.0155	0.0169	0.0178	-	0	0	0	0
Benzovindiflupyr 100ppm	0.0182	0.0163	0.0188	0.0189	0.0226	-	0	0	0	0
Benzovindiflupyr 750ppm	0.0174	0.0147	0.0187	0.0221	0.0173	-	0	0	0	2(/4)
Benzovindiflupyr 1500ppm	0.0207	0.0153	0.0170	0.0220	0.0221	-	0	1	2	1

No statistically-significant differences were noted. – Data not collected. From Robertson, 2010b; Robertson, 2012a.

Table 8-6: Summary of data from a 90 day study with benzovindiflupyr – Liver- and thyroid-related parameters from male rats

	Mean adjusted liver weight (g)	Hepatocellular centrilobular hypertrophy (incidence/10)	Mean adjusted thyroid weight (g)	Thyroid follicular cell hypertrophy (incidence/10)
Control	13.56	0	0.0231	0
Benzovindiflupyr 100ppm	13.84	0	0.0239	0
Benzovindiflupyr 750ppm	15.04	4	0.0247	0
Benzovindiflupyr 1500ppm	17.15**	10***	0.0240	0

** and ***: Statistically-significantly different from control with p<0.01 and p<0.001, respectively. From Robertson, 2010a

Table 8-7: Summary of data from a 2 year study with benzovindiflupyr – Liver- and thyroid-related parameters from male rats

	Mean adjusted liver weight (g)		Hepatocellular centrilobular hypertrophy (group incidence)		Thyroid follicular cell hypertrophy (group incidence)	
Weeks of treatment	**52**	**104**	**52**	**104**	**52**	**104**
Control	15.05	16.89	0/12	0/52	0/12	5/52
Benzovindiflupyr 25ppm	14.31	17.24	0/12	1/52	0/12	2/52
Benzovindiflupyr 100ppm	14.86	17.81	2/12	8/5288	0/12	6/52
Benzovindiflupyr 600ppm	16.02	18.15*	9/12**	13/52**	1/12	5/52

* and **: Statistically-significantly different from control with $p<0.05$ and $p<0.01$, respectively. From MacKay 2012a.

Dose-concordance of key events

For thyroid follicular cell adenomas in male Han Wistar rats 25 and 100 ppm can be considered non-tumourigenic doses and 600 ppm can be considered a tumourigenic dose. For dose-concordance analysis, all dose levels ≤100 ppm are considered to be non-tumourigenic and all dose levels ≥600 ppm are considered to be tumourigenic (Table 8-8).

Table 8-8: Summary of dose-concordance of associative and causal key events

Dietary level of benzovindiflupyr (ppm)	25	100	600	750	1200	1500
Hepatic UDPGT induction (Causal)	No data	No	Yes	Yes	Yes	Yes
Increased hepatocellular hypertrophy (Associative)	No	Yes	Yes	Yes	Yes	Yes
Increased liver weight (Associative)	No	No	Yes	Yes	Yes	Yes
Reduced circulating T_3/T_4 (Causal)	No data	No	Yes	No data	Yes	No data
Increased circulating TSH (Causal)	No data	No	No	No data	Yes	No data
Increased thyroid follicular cell hypertrophy (Associative)	No	No	No	Yes	No	Yes
Increased thyroid weight (Associative)	No	No	No	No	No	No
Increased thyroid follicular cell proliferation (Causal)	No data	No	No	No data	Yes	No data
Increased thyroid follicular cell adenoma (Outcome)	No	No	Yes	Yes (assumed)	Yes (assumed)	Yes (assumed)

Overall, there is good dose concordance of the proposed key events with tumour outcome. The dose concordance is strong for UDPGT induction, with tumourigenic dose levels (≥600 ppm) resulting in dose-dependent increases in hepatic UDPGT that was consistent across studies and with non-tumourigenic doses (≤100 ppm) having no effect. Similarly for reduced circulating T_3 / T_4, dose related reductions were seen at tumourigenic dose levels (≥600 ppm) but not at non-tumourigenic doses (≤100 ppm). As the first and second causal key events are directly related (induction of hepatic UDPGT results in increased clearance and reduced circulating levels of T_3 and T_4), the strong dose-concordance for these effects is supportive of the proposed MOA.

Increased circulating TSH was observed at the high dose of 1200 ppm only. The lack of effect at 600 ppm is most likely due to the fact that this parameter was only assessed in the 14 day study and the low statistical power to detect the expected minimal change in this highly variable parameter. After 14 days treatment, circulating T_3 levels had not returned to control levels at either 600 or 1200 ppm, suggesting that the peak increase in TSH

occurs sometime after 14 days. Increased thyroid follicular cell proliferation was also seen at the high dose of 1200 ppm only. This is in agreement with the fact that it was only at this dose level and only after treatment for 14 days that an increase in circulating TSH, the agent proposed to promote thyroid follicular cell proliferation, was observed.

For the associative key events, the expected increases in liver centrilobular hypertrophy and weight were observed in a dose-responsive manner consistent with the proposed MOA. These liver parameters were affected consistently across multiple studies, and they occurred at dose levels at or below the tumourigenic dose level of 600 ppm. Thyroid follicular cell hypertrophy occurred at low incidence at the higher doses of 750 and 1500 ppm only. At tumourigenic dose levels the observed effects on parameters associated with the key events occur in a logical, time-dependent manner consistent with the proposed MOA (Table 8-9).

Table 8-9: Summary of temporal-concordance of associative and causal key events

Time	1-7 days	14 days	28 days	90 days	1-2 years
Hepatic UDPGT induction (Causal)	Yes	Yes	Yes	No data	No data
Increased hepatocellular hypertrophy (Associative)	Yes	Yes	Yes	Yes	Yes
Increased liver weight (Associative)	Yes	Yes	Yes	Yes	Yes
Reduced circulating T_3/T_4 (Causal)	Yes	Yes	No data	No data	No data
Increased circulating TSH (Causal)	No	Yes	No data	No data	No data
Increased thyroid follicular cell hypertrophy (Associative)	Yes	Yes	Yes	No	No
Increased thyroid weight (Associative)	No	No	No	No	No
Increased thyroid follicular cell proliferation (Causal)	No	Yes	No data	No data	No data
Increased thyroid follicular cell adenoma (Outcome)	No	No	No	No	Yes

The observed steps occur in a logical sequence, with the key events preceding the time of occurrence of thyroid follicular adenomas:

- Induction of hepatic UDPGT, increased liver hypertrophy and increased liver weight occurred early (within 1-7 days) and remained consistently affected over time;
- Decreases in circulating T_3/T_4 occurred early (within 1-7 days), and T_4 levels, but not T_3 levels, had returned to control levels by the last time of measurement (14 days);
- TSH was unaffected after 1-7 days, but showed an increase at 14 days, reflecting a response of the HPT axis to lower T_3 / T_4 levels;
- Thyroid cell proliferation was unaffected at 1-7 days, but it matched the time of observed increases in TSH levels at 14 days;
- Thyroid follicular cell hypertrophy was observed at 1-7 days, and at 14 and 28 days;
- An increase in adenomas of the thyroid required 1-2 years before it was observed.

Reproducibility and concordance

Where parameters were measured in multiple studies, there is a high degree of reproducibility between studies and consistency between key events. The induction of hepatic UDPGT, showed a high degree of consistency and reproducibility in two independent studies. Hepatomegaly and hepatocellular hypertrophy were observed in every study in the rat. Thyroid follicular cell hypertrophy was seen at low incidence in one study at the presumed carcinogenic doses of 750 and 1500 ppm (after 7, 14 and/or 28 days of treatment) but not in other studies/time

points. This is consistent with a weak stimulation of this MOA leading to minimal increases in thyroid adenomas after 2 years of treatment.

The concordance analyses have established that the proposed key events resulting in the induction of thyroid tumours in male rats exhibit good dose- and temporal-concordance with the tumour endpoint. There can be confidence that the hypothesised MOA was responsible for the induction of thyroid tumours in male rats following dietary exposure to 600 ppm benzovindiflupyr.

Biological plausibility

The induction of thyroid follicular cell adenomas in rats is a common finding in chronic toxicity and carcinogenicity studies (Wilson *et al*, 1996; Hurley *et al*, 1998; Finch *et al*, 2006). The proposed MOA can be described as a perturbation of the HPT axis secondary to induction of hepatic UDPGT and is well described for a number of compounds, including the archetypal UDPGT inducer, PB (Finch *et al*, 2006).

Alternative mode of action hypotheses

In addition to the MOA described, alternative modes of action for the induction of thyroid tumours exist. Genotoxicity can be excluded as benzovindiflupyr has been demonstrated to be negative for genotoxicity in a comprehensive package of *in vitro* and *in vivo* assays (see Table 17).

A second MOA is inhibition of the organification of iodine. Organification of iodine is the first step in the synthesis of T_3 and T_4 and is catalysed by the enzyme thyroid peroxidase (TPO). Inhibition of TPO, in order to reduce circulating T_3/T_4, by compounds such as propylthiouracil (PTU) is exploited as a treatment for hyperthyroidism in humans, such as in Graves' disease. PTU has also been shown to induce thyroid follicular cell adenomas in rats (IARC, 2001). This MOA can be excluded for benzovindiflupyr as it was found not to be an inhibitor of male rat thyroid-derived TPO *in vitro*, whereas PTU was shown to be a potent inhibitor (Lake, 2012b).

Uncertainties, inconsistencies and data gaps

The available data support the proposed hypothesized MOA for the minimally increased incidence of rat thyroid tumours by benzovindiflupyr, whilst excluding the alternative MOAs. Some minor uncertainties and data gaps remain.

Neither the induction of thyroid follicular cell proliferation, nor elevated TSH were observed at the lowest carcinogenic dose (600 ppm) in the 14 day mode of action study; however, under the conditions of this study, PB and 1200 ppm benzovindiflupyr did not stimulate increased TSH and follicular cell proliferation until day 14. Examination of the individual animal data for TSH levels for rats treated with 600 pm (Robertson, 2012a) indicates that at day 14 a few rats had elevated TSH similar to PB and 1200 ppm benzovindiflupyr; therefore, it is reasonable to assume that the 600 ppm dose of benzovindiflupyr would respond if observations had been taken at later time points.

The hypothesised consequence of UDPGT induction, increased clearance of T_3/T_4 from the blood into the bile, has not been directly demonstrated; however, the consequence of this increased clearance, namely decreased T_3/T_4 in the serum was demonstrated and was associated with the increased hepatic UDPGT. It is therefore reasonable to infer that all of the intermediate key events are operating.

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

Assessment of the Postulated Mode of Action

The concordance analyses have established that the proposed key events resulting in the induction of thyroid tumours in male rats exhibit good dose- and temporal-concordance with the tumour endpoint. This is a well described MOA for the induction of thyroid tumours in rats and the parameters essential for describing the MOA have been presented for benzovindiflupyr. Therefore, there is a high level of confidence that the hypothesised MOA was responsible for the induction of thyroid tumours in male rats following dietary exposure to 600 ppm benzovindiflupyr.

Part 2: THE RELEVANCE TO HUMANS OF TUMOURS INDUCED BY BENZOVINDIFLUPYR IN RATS

The framework developed by the IPCS and ILSI/HESI has been used to determine the human relevance of the identified mode of action in rats.

In contrast to rats, serum TSH levels in humans are more stable following exposure to hepatic enzyme inducers (Meek *et al*, 2003; Dellarco *et al*, 2006). The human HPT axis is qualitatively very similar to that of rats and it has been demonstrated that human administration of pharmaceuticals that result in the induction of UDPGT (including PB, phenytoin and carbamazepine) also result in reduced circulating T_3/T_4. However, despite the reduced T_3/T_4 levels, TSH levels in humans remain largely unaffected, whereas in the rat TSH levels increase in order to compensate (Curran and DeGroot, 1991). Therefore, although the HPT axis is responsible for homeostatic control of thyroid hormones in both species, there is a large difference in their sensitivity to perturbation, with the human considerably less susceptible (Dellarco *et al*, 2006).

In addition, the half-life of T_3 and T_4 in humans (5-9 days) is considerably longer than that in rats (12 hours for T_4) (Dohler *et al*, 1979; US EPA, 1998). The substantially longer half-life in humans is a result of binding to a high-affinity thyroid-binding globulin, which binds T_4 (and T_3 to a lesser degree), and is not present in rats (Hill *et al*, 1998; US EPA, 1998). These differences mean that rats have a higher rate of turnover of T_3/T_4. As a result of this higher turnover, rats have a much higher (approximately 25-fold) basal level of TSH when compared to humans (Dohler *et al*. 1979). This means that the compensatory reaction in rats towards a T_3/T_4 deficiency is much more pronounced than in humans.

Finally, it has been suggested that interspecies differences in thyroid histology play a role in the differential sensitivity. In humans, the thyroid follicular cell epithelium is composed of short, cuboidal cells, indicative of their quiescent nature. In rats, however, the thyroid follicular cells are tall and cuboidal and appear to be continually active in synthesis. It appears that the rodent thyroid gland is chronically stimulated by TSH levels to compensate for the increased clearance of thyroid hormones. It follows that increases in TSH levels above basal levels in rats more readily moves that gland towards increased growth and potential neoplastic change than in humans (Dellarco *et al*, 2006; US EPA, 1998). Interestingly, adult male rats have higher serum TSH levels than female rats (Chen, 1984), and they are often more sensitive to stimulation of thyroid growth and carcinogenesis. Overall, the histological differences in thyroid follicular cells between rats and humans is related to a higher rate of production of T_4 to maintain a consistent serum concentration, thus making the rat thyroid more "functionally active" than primates including humans (McClain, 1995; Dellarco *et al*, 2006).

Even though certain agents can cause a reduction in T_3/T_4 levels in humans, there is no evidence that these agents can induce an increased susceptibility to thyroid cancer in humans (Ron *et al*, 1987; Dellarco *et al*, 2006). Epidemiology studies with phenobarbital have not shown any increased risk for thyroid cancer in humans (Olsen *et al*, 1993). As a result, the only known human thyroid carcinogen is radiation, which is a mutagenic mode of action.

In summary, a wealth of information in the literature has established a lack of susceptibility of humans to thyroid hormone alterations, resulting changes in TSH, and thyroid tumour responses that are initiated in rats by induction of UDPGT and increased T_3/ T_4 clearance (e.g. Dellarco *et al*, 2006; Dohler *et al*, 1979; US EPA, 1998). Therefore, based on qualitative differences (presence of high-affinity thyroid binding globulin in humans but not rodents) and quantitative differences (including lower responsiveness to fluctuations in thyroid hormone levels), the MOA established in rats with benzovindiflupyr is not relevant to humans.

Overall conclusion

Benzovindiflupyr-induced thyroid tumours in male rats are attributable to induction of hepatic UDPGT, resulting in a series of downstream events, ultimately leading to tumourgenesis. This mode of action is not relevant for human hazard/risk assessment purposes due to qualitative and quantitative differences in response to UDPGT induction and increased clearance of thyroid hormones (T_3/T_4) between rats and humans. Benzovindiflupyr does not pose a carcinogenic hazard to humans.

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