Committee for Risk Assessment

RAC

Annex 1

Background document

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

methyl *N*-(isopropoxycarbonyl)-L-valyl-(3*RS*)-3-(4-chlorophenyl)-β-alaninate; valifenalate

EC Number: -

CAS Number: 283159-90-0

CLH-O-0000006928-58-01/F

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

Adopted

10 December 2020

CLH report

Proposal for Harmonised Classification and Labelling

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

Substance Name: methyl N-(isopropoxycarbonyl)-L-valyl-(3*RS*)-3-(4-chlorophenyl)-β-alaninate; valifenalate

EC Number: -

CAS Number: 283159-90-0

Index Number: -

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ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON METHYL *N*-(ISOPROPOXYCARBONYL)-L-VALYL-(3*RS*)-3-(4-CHLOROPHENYL)-B-ALANINATE; VALIFENALATE

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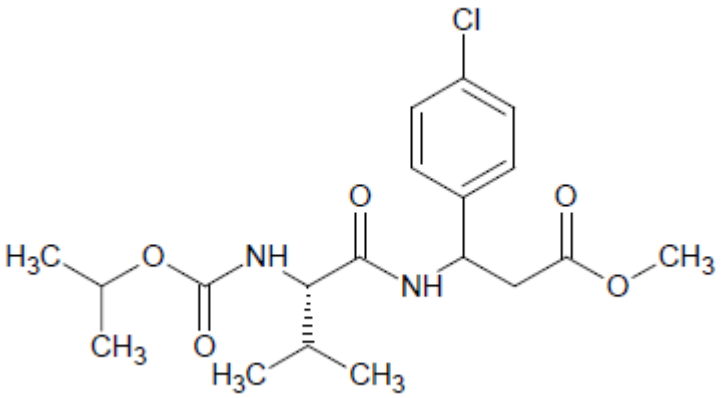
1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

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

Name(s) in the IUPAC nomenclature or other international chemical name(s)	Methyl N-(isopropoxycarbonyl)-L-valyl-(3*RS*)-3-(4-chlorophenyl)-β-alaninate
Other names (usual name, trade name, abbreviation)	IR5885
ISO common name (if available and appropriate)	
EC number (if available and appropriate)	Not available
EC name (if available and appropriate)	Not available
CAS number (if available)	283159-90-0
Other identity code (if available)	CIPAC number 857
Molecular formula	$C_{19}H_{27}ClN_2O_5$
Structural formula	
SMILES notation (if available)	Not available
Molecular weight or molecular weight range	398.89 g/mol
Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	Not Applicable
Description of the manufacturing process and identity of the source (for UVCB substances only)	Not Applicable
Degree of purity (%) (if relevant for the entry in Annex VI)	≥98 % w/w

1.2 Composition of the substance

Table 2: Constituents (non-confidential information)

Constituent (Name and numerical identifier)	Concentration range (% w/w minimum and maximum in multi-constituent substances)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)
Valifenalate	≥ 980 g/kg	No current entry	None. No classification warranted according to CLP

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

Impurity (Name and numerical identifier)	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)	The impurity contributes to the classification and labelling
Confidential data	≥ 1 g/kg	No current entry	No classification	There are no impurities of toxicological or environmental concern in valifenalate technical.

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

Additive (Name and numerical identifier)	Function	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)	The additive contributes to the classification and labelling
None					

2 PROPOSED HARMONISED CLASSIFICATION AND LABELLING

2.1 Classification and labeling in accordance with the CLP regulation (regulaton (EC) 1272/2008

Table 5: Proposed harmonised classification and labelling

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	No current Annex VI entry										
Dossier submitters proposal	TBD	methyl N-(isopropoxycarbonyl)-L-valyl-(3*RS*)-3-(4-chlorophenyl)-β-alaninate; valifenalate	-	283159-90-0	Aquatic Chronic 2	H411	GHS09	H411			
Resulting Annex VI entry if agreed by RAC and COM	TBD	methyl N-(isopropoxycarbonyl)-L-valyl-(3*RS*)-3-(4-chlorophenyl)-β-alaninate;; valifenalate	-	283159-90-0	Aquatic Chronic 2	H411	GHS09	H411			

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

Hazard class	Reason for no classification	Within the scope of public consultation
Explosives	Data conclusive but not sufficient for classification	Yes
Flammable gases (including chemically unstable gases)	Hazard class not applicable (solid)	No
Oxidising gases	Hazard class not applicable (solid)	No
Gases under pressure	Hazard class not applicable (solid)	No
Flammable liquids	Hazard class not applicable (solid)	No
Flammable solids	Data conclusive but not sufficient for classification	Yes
Self-reactive substances	Data lacking	No
Pyrophoric liquids	Hazard class not applicable (solid)	No
Pyrophoric solids	Data lacking	No
Self-heating substances	Data conclusive but not sufficient for classification	Yes
Substances which in contact with water emit flammable gases	Data lacking	No
Oxidising liquids	Hazard class not applicable (solid)	No
Oxidising solids	Data conclusive but not sufficient for classification	Yes
Organic peroxides	Data lacking	No
Corrosive to metals	Not assessed in this dossier	No
Acute toxicity via oral route	Data conclusive but not sufficient for classification	Yes
Acute toxicity via dermal route	Data conclusive but not sufficient for classification	Yes
Acute toxicity via inhalation route	Data conclusive but not sufficient for classification	Yes
Skin corrosion/irritation	Data conclusive but not sufficient for classification	Yes
Serious eye damage/eye irritation	Data conclusive but not sufficient for classification	Yes
Respiratory sensitisation	Data lacking	No
Skin sensitisation	Data conclusive but not sufficient for classification	Yes
Germ cell mutagenicity	Data conclusive but not sufficient for classification	Yes
Carcinogenicity	Data conclusive but not sufficient for classification	Yes
Reproductive toxicity	Data conclusive but not sufficient for classification	Yes
Specific target organ toxicity-single exposure	Data conclusive but not sufficient for classification	Yes
Specific target organ toxicity-repeated exposure	Data conclusive but not sufficient for classification	Yes
Aspiration hazard	Data lacking	No
Hazardous to the aquatic environment	**Aquatic Chronic 2** **H411 Toxic to aquatic life with long lasting effects**	Yes
Hazardous to the ozone layer	Data conclusive but not sufficient for classification	Yes

3 HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING

Valifenalate is a new active substance developed as a fungicide. There is no previous classification and labelling.

RAC general comment
Valifenalate (methyl *N*-(isopropoxycarbonyl)-L-valyl-(3*RS*)-3-(4-chlorophenyl)-β-alaninate) is a new active substance in the meaning of Regulation (EU) No 1107/2009 developed as fungicide. It has no previous entry in Annex VI of Regulation EC 1272/2008.

4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

Valifenalate is an active substance in the meaning of Regulation (EU) No 1107/2009, therefore there is no requirement for justification that action is needed at Community level.

5 IDENTIFIED USES

This substance is approved as a fungicide.

6 DATA SOURCES

Draft Assessment Report (DAR) for methyl N-(isopropoxycarbonyl)-L-valyl-(3*RS*)-3-(4-chlorophenyl)-β-alaninate prepared under Regulation 1107/2009.

7 PHYSICOCHEMICAL PROPERTIES

Table 7: Summary of physicochemical properties (as reported in DAR Vol. 3 B.2.1)

Property Method	**Value**	**Reference**	**Comment (e.g. measured or estimated)**
Physical state at 20°C and 101.3 kPa OPPTS 830.6302; visual	White, opaque solid in the form of a free flowing fine powder, containing a small number of soft aggregates at 20 ± 0.5°C. White, fine powder with a tendency to form clumps, weak, characteristic hint of antiseptic	*See Annex conf. 1.* *See Annex conf. 55-57.*	Determined on a valifenalate analytical standard purity 99.6% Technical, purity 98.36%
Melting/freezing point EEC A.1	147°C at 101.74 kPa	*See Annex conf. 2.*	Determined on a valifenalate analytical standard purity 99.6%
Boiling point EEC A.2	367 ± 0.5°C at 101.83 to 102.16 kPa with minor decomposition	*See Annex conf. 3.*	Determined on a valifenalate analytical standard purity 99.6% In the study results a minor decomposition is mentioned. A different interpretation of data was proposed: endotherms at 320 and 367°C correspond to decomposition events occurring at these high temperatures and not to boiling point of the two diastereoisomers. In fact it is very unlikely to determine a boiling point of dipeptides, as they decompose before having reached it. Therefore the observed and measured events at 320-367°C were decomposition, so boiling point for IR5885 resulted not measurable.
Relative density EEC A.3 pycnometer	1.25 at 21 ± 0.5°C	*See Annex conf. 4.*	Determined for valifenalate analytical standard purity 99.6%
Vapour pressure EEC A.4 vapour pressure balance	9.6×10^{-8} Pa at 20°C; 2.3×10^{-7} Pa at 25°C	*See Annex conf. 42.*	Determined for valifenalate analytical standard purity 99.6%
Surface tension EEC A.5 ring method OECD 115	66.0 mN/m (1.89×10^{-2} g/L solution) at 20 ± 0.5°C	*See Annex conf. 58.*	Determined for valifenalate technical material purity 98.36% The test material is considered not to be a surface active material.

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Property Method	**Value**	**Reference**	**Comment (e.g. measured or estimated)**
Water solubility EEC A.6 flask method	At ambient conditions (pH): 2.41×10^{-2} g/L measured pH: 4.9 to 5.9 At basic pH: 4.55×10^{-2} g/L pH: 9.5 to 9.8	*See Annex conf. 5.*	Determined for valifenalate analytical standard purity 99.6% Due to known hydrolysis under basic conditions the conclusive value was determined from a short term assessment of solubility, using reduced saturation and equilibrium time periods.
Partition coefficient n-octanol/water EEC A.8 OECD 107 shake flask OEECD 117 HPLC	pH4 I° 3.07 ± 0.03 Log(P) II° 3.04 ± 0.02 pH7 I° 3.11 ± 0.07 Log(P) II° 3.05 ± 0.03 pH9 I° 3.08 ± 0.02 Log(P) II° 3.06 ± 0.03	*See Annex conf. 28.*	Determined for valifenalate analytical standard purity 99.6% A preliminary measurement of Log P by HPLC method (OECD 117) with 60% CH_3OH confirmed the obtained values higher than 3.00 (3.07) for the 1° component and 3.19 for the II° component.
Flash point	Not required		Valifenalate is not a liquid at temperature <40°C
Flammability EEC A.10	No ignition under test conditions. Technical grade valifenalate determined to be not highly flammable.	*See Annex conf. 42.*	Determined for valifenalate technical material purity 98.36%
Explosive properties EEC A.14	The substance is not sensitive to heat, shock or friction. Valifenalate is not considered to be explosive under the test conditions.	*See Annex conf. 43.*	Determined for valifenalate technical material purity 98.36%
Self-ignition temperature EEC A.16	The test substance has been determined not to have a relative self-ignition temperature below its melting temperature	*See Annex conf. 44.*	Valifenalate is not considered as auto-flammable under the test conditions
Oxidising properties EEC A.17	No oxidising properties	*See Annex conf. 45.*	Determined for valifenalate technical material purity 98.36% Valifenalate is not considered as oxidising under the test conditions.
Granulometry	Not relevant for CLP		
Stability in organic solvents and identity of relevant degradation products	Not relevant for CLP		

Property Method	**Value**	**Reference**	**Comment (e.g. measured or estimated)**
Dissociation constant OECD 112	Functional group: Predicted value of pKa Amide group 1: -1.78 ± 0.70 proton accepted; 11.35 ± 0.46 proton donated Amide group 2: -1.08 ± 0.70 proton accepted; 14.88 ± 0.46 proton donated	*See Annex conf. 6.*	Valifenalate analytical standard purity 99.6%
Viscosity	Not required		Not relevant for a solid

8 EVALUATION OF PHYSICAL HAZARDS

8.1 Explosives

Table 8: Summary table of studies on explosive properties

Method	**Results**	**Remarks**	**Reference**
Explosive properties EEC A.14	The substance is not sensitive to heat, shick or friction. Valifenalate is not considered to be explosive under the test conditions.	Measured on technical (98.36%)	*See Annex conf. 43.*

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

Based on the study (*See Annex conf. 43.*), valifenalate technical is not sensitive to heat, shock or friction. Valifenalate is not considered to be explosive under the test conditions.

8.1.2 Comparison with the CLP criteria

Valifenalate does not meet the criteria for classification for explosive properties under CLP.

8.1.3 Conclusion on classification and labelling for explosive properties

Valifenalate is not explosive and does not warrant classification for explosive properties.

8.2 Flammable gases (including chemically unstable gases)

Not applicable as valifenalate is not a gas.

8.3 Oxidising gases

Not applicable as valifenalate is not a gas.

8.4 Gases under pressure

Not applicable as valifenalate is not a gas.

8.5 Flammable liquids

Not applicable as valifenalate is not a liquid.

8.6 Flammable solids

Table 9: Summary table of studies on flammable solids

Method	Results	Remarks	Reference
Flammability EEC A.10	Not highly flammable No ignition under test conditions.	Measured on technical purity 98.36%	*See Annex conf. 42.*

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

Valifenalate is not flammable and there was no ignition under test conditions.

8.6.2 Comparison with the CLP criteria

Valifenalate does not meet the criteria for classification of flammable solids under CLP.

8.6.3 Conclusion on classification and labelling for flammable solids

Valifenalate is not flammable and does not warrant classification for flammable solids.

8.7 Self-reactive substances

Not evaluated

8.8 Pyrophoric liquids

Not applicable as valifenalate is not a liquid

8.9 Pyrophoric solids

Not evaluated

8.10 Self-heating substances

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

Method	Results	Remarks	Reference
Auto-flammability EEC A.16	The test substance has been determined not to have a relative self-ignition temperature below its melting temperature	Measured on technical purity 98.36%	*See Annex conf. 44.*

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

Valifenalate has been determined not to have a relative self-ignition temperature below its melting temperature. Valifenalate is not considered as auto-flammable under the test conditions.

8.10.2 Comparison with the CLP criteria

Valifenalate does not meet the criteria for classification for self heating substance under CLP.

8.10.3 Conclusion on classification and labelling for self-heating substances

Valifenalate is not auto-flammable and does not warrant classification for self heating substance.

8.11 Substances which in contact with water emit flammable gases

Not evaluated.

8.12 Oxidising liquids

Not applicable as valifenalate is not a liquid.

8.13 Oxidising solids

Table 11: Summary table of studies on oxidising solids

Method	Results	Remarks	Reference
Oxidising properties EEC A.17	Valifenalate is not considered as oxidising since there are no chemical groups in the molecule that would imply oxidising properties.	The chemical structure of valifenalate was examined for groups that would infer that the material could possess oxidising properties.	*See Annex conf. 46.*

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

Valifenalate is not considered as oxidising under the test conditions.

8.13.2 Comparison with the CLP criteria

Valifenalate does not meet the criteria for classification for oxidising properties under CLP

8.13.3 Conclusion on classification and labelling for oxidising solids

Valifenalate is not oxidising and does not warrant classification for oxidising properties.

8.14 Organic peroxides

Not applicable as valifenalate is not a peroxide.

8.15 Corrosive to metals

Not evaluated.

RAC evaluation of physical hazards

Summary of the Dossier Submitter's proposal

The Dossier Submitter (DS) proposed no classification of valifenalate for physical hazards based on the following facts:

- Negative results with an EEC A.14 assay for testing explosive properties;
- Negative results with an EEC A.10 assay for testing flammability;
- Negative results with an EEC A.16 assay for testing self-heating; and,
- Negative results with an EEC A.17 assay for testing oxidising properties.

No data for the following hazards were provided by the DS:

- self-reactivity,
- pyrophoricity,
- capability to emit flammable gases and
- corrosivity to metals.

Comments received during consultation

No comments were received during consultation.

Assessment and comparison with the classification criteria

RAC notes that no test for explosivity was found in the CLH-report since Annex I shows that the A.14 test report was limited to a prediction based on structure. Nevertheless, the molecule of valifenalate does not contain groups associated with explosive properties and therefore no test is needed. Thus, **RAC supports no classification for explosivity due to lack of data.**

With regard to flammability, RAC notes that a preliminary test according to A.10 (equivalent to a preliminary test according to UN N.1) was negative. Thus, RAC supports **no classification for flammability.**

The result of the A.16 test was negative. However, RAC notes that the A.16 test is not the same as that required under CLP criteria (UN N.4) for testing self-heating. Therefore, RAC supports **no classification for self-heating but in this case, due to inconclusive data.**

No test was available for assessing the oxidising capability of valifenalate. However, RAC notes that the molecule contains oxygen and chlorine, but these are bonded only to carbon and therefore no test is need. Thus, **RAC supports no classification for oxidising properties.**

9 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

Table 12: Summary table of toxicokinetic studies

Method	Results	Remarks	Reference
US EPA. OPPTS 870.7485	Rapid excretion (95%; 24hr), mainly via faeces; blood levels peaked at 1-2 hrs post administration	Preliminary disposition in rat: single oral dose: C14 (label in phenyl ring) valifenalate (250mg/kg) male and female	*See Annex conf. 8.*
US EPA. OPPTS 870.7485	Profile of metabolites the same in male and female. Some quantitative differences but major metabolite was valifenalate acid, R2	Preliminary profile of metabolites *See Annex conf. 8.*	*See Annex conf. 36.*
US EPA. OPPTS 870.7485	Confirm main route of rapid excretion is in faeces, via bile. Relatively small amount (15% of radiolabel) unabsorbed parent chemical in faeces at low dose. Excretion routes remain somewhat similar low and high doses and with repeated low-dose Cmax only 2 or 3-fold greater at 10-fold higher external dose, but occurring at 2h post-dose at both low and high dose-levels. Low carcass residue by 72 hours.	Disposition following single and repeat (100mg/kg) and single (1000mg/kg) oral administration to male and female rats Blood and excretion kinetics. Tissue distribution and biliary excretion Large, 10-phase study	*See Annex conf. 22.*
US EPA. OPPTS 870.7485	Metabolic profile same cross gender with some quantitative differences All metabolites present in faeces found in urine. 3 additional metabolits in urine. Unchanged valifenalate only in faeces – male and female Valifenalate acid main metabolite Other metabolites identified <6% Urine and bile from bile-duct-cannulated animals contained 77% of administered dose in male and female and did not contain parent chemical.	Metabolite profiling: main study using samples *See Annex conf. 22.*	*See Annex conf. 37.*

Profiling of metabolites of [^{14}C-U-phenyl] IR5885 in urine and faeces of male and female rats was carried out on samples generated in single and repeated oral administration studies.

The excretion of radioactivity following single administration at low (100 mg/kg) and high (1000 mg/kg) doses and repeated administration at low dose was mainly *via* faeces for both male and female animals. Radioactivity was almost completely excreted *via* urine within 24 hours and *via* faeces within 48-72 hours. The excretion patterns following the three administration doses were not markedly different except for the radioactivity eliminated in faeces which was higher in male than in female rats.

Following single administration at low dose the radioactivity was eliminated mainly in faeces (86.23% of administered dose [AD] for males and 50.48% AD for females) with an appreciable amount excreted *via* urine (9.21% AD for males and 40.59% AD for females). Following single administration at high dose the radioactivity was eliminated mainly in faeces (76.22% AD for males and 64.52% AD for females) and in lower amount *via* urine (14.42% AD for males and 24.77% AD for females). The recovery of radioactivity following repeated oral administration was very similar to that obtained following a single low administration. The greater proportion of the administered dose was excreted in faeces (82.94% and 56.46% AD for males and females, respectively) while a considerable amount was eliminated in urine (8.18% and 32.25% AD).

In bile-duct-cannulated male and female rats, the radioactivity was eliminated mainly *via* urine and bile (77.00% AD for male and 77.63% AD for female animals). Bile was an important route of elimination for radioactivity with a mean of 64.27% AD (males) and 48.02% AD (females) within 24 h from dose administration. Excretion in urine accounted for 12.73% AD (males) and 29.61% AD (females) and in faeces accounted for 15.54% AD (males) and 12.54% AD (females) always by 24 h post dose.

Following low, high and repeated oral administrations, the only tissues containing significant radioactivity, besides gastrointestinal tract, were liver and kidneys for both male and female rats.

The urine samples of the same sex up to 24 hours were pooled per time interval, relatively to each administration dose, and analysed for radioactivity content by LSC. Aliquots of pooled urine samples were analysed directly by TLC and HPLC for radioactivity distribution.

The faeces samples of the same sex up to 48-72 hours were pooled per time interval, relatively to each administration dose, and extracted twice with acetone and then once with acetone-H_2O (1-1). The extracts were analysed for radioactivity content by LSC and the profile of the metabolites was obtained by TLC and HPLC. The dried faeces residues were oxidized to determine the non-extractable radioactivity content by LSC. The non-extractable radioactivity was always lower than 2% AD.

Chromatographic analyses established that IR5885 (R1) was extensively metabolized and six compounds were characterised: R2, R3, R4, R5, R6, and R7. Study results showed that the metabolic profile was almost the same following single oral (low and high) administration and repeated oral administration although the amounts of some compounds were different especially between low and high doses. The metabolic profile was the same in male and female rats treated at the same dose, although the amounts of some compounds were slightly different in the two sexes. Three metabolites were found both in urine and faeces, while three compounds were observed only in urine. IR5885 (R1) was largely degraded following single low administration (it amounted to 5.30% AD in males and 6.17% AD in females) and repeated administration at low dose (7.80% AD in males and 5.47% AD in females) while it was less degraded in rats administered with single high dose (40.41% AD in males and 9.50% AD in females). Compound R2 was the major metabolite following all administration doses: it amounted to 75.88% AD in males and 76.67% AD in females at low dose, 42.04% and 72.55% AD at high dose, 68.46% and 74.12% AD at repeated administration. None of the other metabolites reached 6% AD in the excreta. Among these, compound R3 was the main reaching 5.14% AD and 2.45% AD in the excreta of male and female rats administered with single low dose, 3.18% AD and 3.20% AD in male and female rats administered with single high dose and 5.90% AD and 3.28% AD in males and females administered with repeated dose.

TLC analysis of biliary excretion phase (from rats administered with single low dose) established that IR5885 was largely degraded (8.08% AD and 6.65% AD in male and female rats, respectively) producing mainly IR5885 acid (R2, 70.73% AD in male and 76.93% AD in female rats).

Other metabolites never reached 6% AD in the excreta, with compound R3 as main product reaching 5.67% AD in males and 2.46% AD in females.

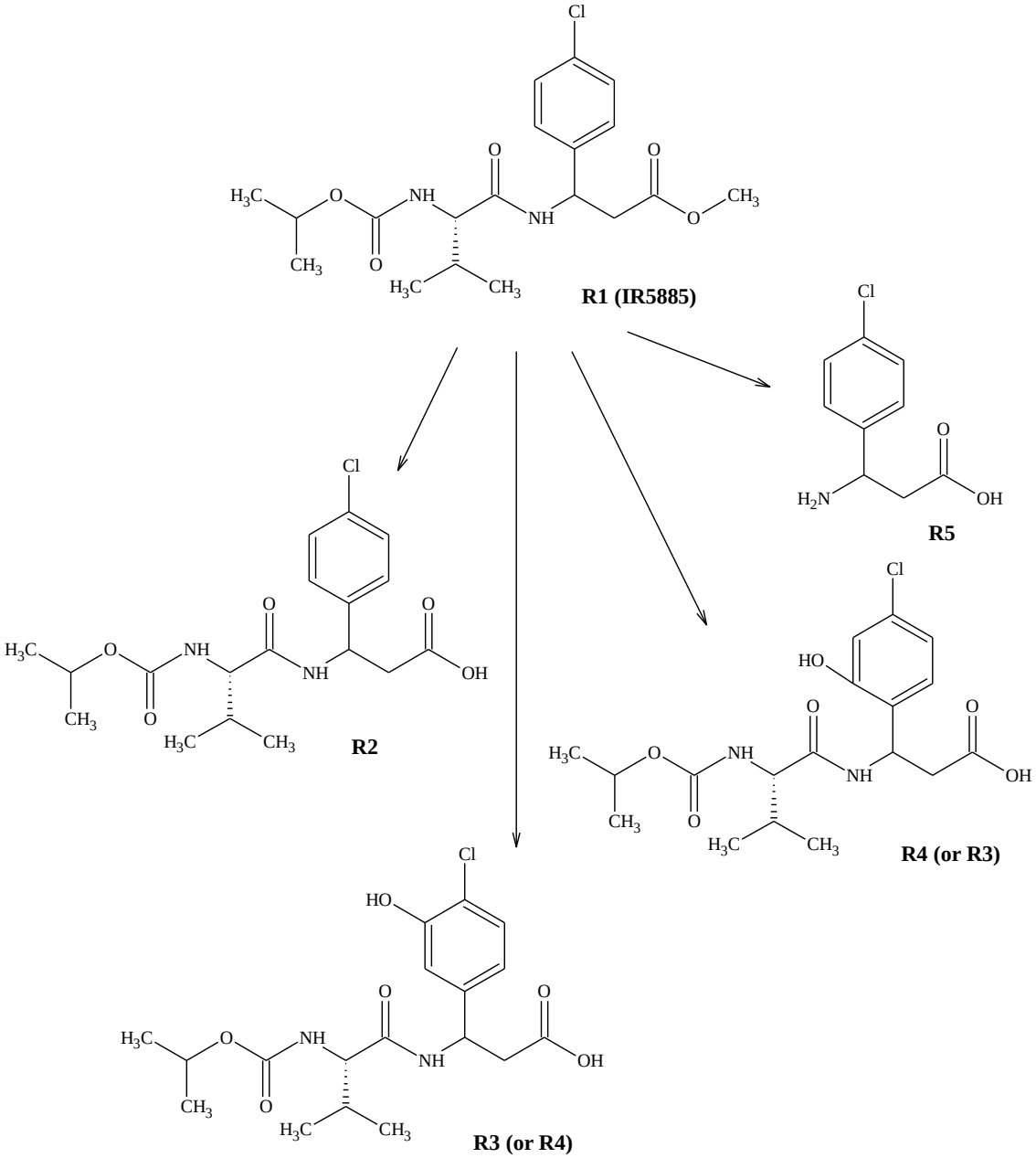
Liver and kidneys from each animal of the same sex administered with the same dose were pooled and extracted twice with acetone and then once with acetone-H_2O (1-1). The extracts were analysed for radioactivity content by LSC and the profile of the metabolites was obtained by TLC and HPLC. The dried residues were oxidized to determine the non-extractable radioactivity content by LSC.

Chromatographic analyses established that compounds found in extracts of liver and kidneys were the same as found in urine. In liver almost only IR5885 acid (R2) was found, both in male and female rats, following all doses. In kidneys the principal compounds found was IR5885 acid (R2) both in male and female rats following all administration doses while other compounds (already found in urine) were present only as traces.

In conclusion, study results showed that:

- the metabolic profile was the same in male and female rats, although the amounts of some compounds were slightly different in the two sexes;
- all the compounds identified in faeces were observed in urine but three compounds were found only in urine (all lower than 4%);
- unchanged IR5885 (R1) was only found in faeces both for male and female rats;
- R2 was the main degradation products found both in faeces and urine; it was identified as IR5885 acid;
- none of the other metabolites, reached 6% AD in the excreta; among these R3 (or R4) was identified RS-β-alanine, N-[(1-methylethoxy)carbonyl]-L-valyl-3-(2-hydroxy-4-chlorophenyl), R4 (or R3) was identified as RS-β-alanine, N-[(1-methylethoxy)carbonyl]-L-valyl-3-(3-hydroxy-4-chlorophenyl), and R5 was identified as 3-amino-3-(4-chlorophenyl) propionic acid;
- the sum of metabolites found in bile and urine from bile-duct-cannulated rats was ≥ than 77% both in male and female and it was exclusively represented by degradation compounds.

Figure 1: Proposed degradation pathway of IR5885 in rat



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

At 100mg/kg (with single or repeated administration) valifenalate (R1) appears to be well-absorbed, rapidly excreted (about 95%) and extensively metabolised (about 80%) in both male and female rats. At the higher dose-level (1000mg/kg) valifenalate was less well metabolised, particularly in males (about 60% in males; 90% in females).

Excretion of radioactivity was mainly via the faeces (100mg/kg: 86.23% of administered dose for males and 50.48% administered dose for females within 24 hours) and urine (100mg/kg: 9.21% of administered dose for males and 40.59% of administered dose for females).

A biliary excretion study, with rats administered a single dose of 100mg/kg established that valifenalate was extensively metabolised producing mainly valifenalate acid, R2 (about 75% of administered dose both in males and females) which was mainly excreted in bile (64.27% of administered dose in males and 48.02% of

administered dose in females and urine (12.73% of administered dose in males and 29.61% of administered dose in females). A relatively small amount (about 15% of administered dose) of valifenalate (parent chemical) was detected in faeces and assumed to represent unabsorbed valifenalate.

Valifenalate acid, R2, was the major metabolite in all studies: it amounted to about 75% of administered dose in both males and females at the lower dose-level, changing little if any (68.46% and 74.12% of administered dose) with repeated administration. At the higher dose-level R2 accounted for 42.04% and 72.55% of administered dose in males and females respectively. None of the other metabolites reached 6% of administered dose in the excreta.

Following low, high and repeated (low) oral administrations, the only tissues containing significant radioactivity, besides gastrointestinal tract, were liver and kidneys for both male and female rats.

In short, the metabolic profile (mainly de-esterification) was similar for male and female rats treated at the same dose-level. Faecal excretion was the predominant route of excretion in each gender, although the contribution of urinary excretion was greater in females than males. The extent of metabolism at the high dose was lower, particularly in males, with greater amounts of valifenalate excreted in faeces, possibly as unabsorbed material.

In the absence of other information it is assumed that the disposition of valifenalate in mice will be similar to that of the rat.

10 EVALUATION OF HEALTH HAZARDS

Acute toxicity

10.1 Acute toxicity - oral route

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

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels, duration of exposure	Value LD_{50}	Reference
Acute Oral Toxicity OECD 401 GLP	Rat Sprague Dawley (Crl: CD (SD) BR). 5/sex/group	Valifenalate (IR5885) Purity: 98.9%	5000 mg/kg bw Single dose followed by 14 days observation.	LD_{50} > 5000 mg/kg bw	*See Annex conf. 62.*

In an acute oral toxicity study in Sprague Dawley rats (*See Annex conf. 62.)* 5000 mg/kg bw was administered orally (by gavage) and was well tolerated by males and females. No mortalities occurred at 5000 mg/kg bw, the only dose level tested. Transient piloerection was observed in all animals the day after the treatment. No abnormalities were found thereafter. Normal weight gain was recorded in the animals during the study. At autopsy carried out at the end of the observation period, no appreciable macroscopic findings were evident in any treated rat. The acute oral LD_{50} of valifenalate was found to be higher than 5000 mg/kg bw..

Table 14: Summary table of human data on acute oral toxicity

Type of data/report	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
No human data available on acute oral toxicity				

Table 15: Summary table of other studies relevant for acute oral toxicity

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
No other studies available on acute oral toxicity				

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

The acute oral LD50 of valifenalate was found to be higher than 5000 mg/kg bw. Valifenalate does not warrant classification as being toxic or harmful on the basis of its acute oral toxicity.

10.1.2 Comparison with the CLP criteria

Valifenalate does not meet the criteria for classification for acute oral toxicity under CLP.

10.1.3 Conclusion on classification and labelling for acute oral toxicity

CLP: **Not classified (conclusive but not sufficient for classification).**

10.2 Acute toxicity - dermal route

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

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels duration of exposure	Value LD_{50}	Reference
Acute Dermal Toxicity OECD 402 GLP	Rat Sprague Dawley rats (Strain: Crl: CD (SD) BR). 5/sex/group	Valifenalate (IR5885) Purity: 98.6%	2000 mg/kg bw. 24 h dermal exposure followed by 14 days observation.	LD_{50} > 2000 mg/kg bw	*See Annex conf. 63.*

In an acute dermal toxicity study in Sprague Dawley rats (*See Annex conf. 63.*) , valifenalate was applied dermally at the limit dose of 2000 mg/kg bw for 24 hours. There were no mortalities and there were no clinical effects or signs of local irritation. Body weights of both males and females were found to be unaffected by the test item administration. At autopsy carried out at the end of observation period no appreciable macroscopic findings were evident in any treated rat. The acute dermal LD_{50} of valifenalate was found to be higher than 2000 mg/kg bw.

Table 17: Summary table of human data on acute dermal toxicity

Type of data/report	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
No human data available on acute dermal toxicity				

Table 18: Summary table of other studies relevant for acute dermal toxicity

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
No other studies available on acute dermal toxicity				

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

The acute dermal LD50 of valifenalate was found to be higher than 2000 mg/kg bw. Valifenalate does not warrant classification as being toxic or harmful on the basis of its acute dermal toxicity.

10.2.2 Comparison with the CLP criteria

Valifenalate does not meet the criteria for classification for acute dermal toxicity under CLP.

10.2.3 Conclusion on classification and labelling for acute dermal toxicity

CLP: Not classified (conclusive but not sufficient for classification).

10.3 Acute toxicity - inhalation route

Table 19: Summary table of animal studies on acute inhalation toxicity

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance, , form and particle size (MMAD)	Dose levels, duration of exposure	Value LC_{50}	Reference
Acute Inhalation Toxicity OECD 403 GLP	Rat Wistar Han-Ibm 5/sex/group	Valifenalate (IR5885), purity 98.6% MMAD 2.42, 2.45 GSD: 2.95, 2.89	Gravimetric concentration: 3.118 mg/L 4 hour nose-only exposure followed by 14 days observation.	LC_{50} > 3.118 mg/L air (gravimetric mean aerosol concentration)	*See Annex conf. 11.*

In an acute inhalation study in rats (*See Annex conf. 11.*), rats were exposed (nose-only) to an aerosol of valifenalate at a gravimetric concentration of 3.118 mg/L. There were no mortalities and no significant signs of toxicity. There was a slight reduction in body weight gain between days 1 and 4 but no effects thereafter. There were no macroscopic findings at termination. The acute inhalation LC_{50} of valifenalate was found to be greater than 3.118 mg/L, the highest technically achievable concentration.

Table 20: Summary table of human data on acute inhalation toxicity

Type of data/report	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
No human data available on acute inhalation toxicity				

Table 21: Summary table of other studies relevant for acute inhalation toxicity

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
No other studies available on acute inhalation toxicity				

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

The acute inhalation LC_{50} of valifenalate was found to be greater than 3.118 mg/L, the highest technically achievable concentration. Valifenalate does not warrant classification as being toxic or harmful on the basis of its acute inhalation toxicity.

10.3.2 Comparison with the CLP criteria

Valifenalate does not meet the criteria for classification for acute inhalation toxicity under CLP.

10.3.3 Conclusion on classification and labelling for acute inhalation toxicity

CLP: Not classified (conclusive but not sufficient for classification).

RAC evaluation of acute toxicity

Summary of the Dossier Submitter's proposal

The DS proposed no classification of valifenalate based on OECD-guideline and GLP compliant tests showing an LD_{50} higher than 5000 mg/kg bw for the oral route and higher than 2000 mg/kg bw for the dermal route, and an LC_{50} higher than 3.1 mg/l for the inhalation route.

Comments received during consultation

One manufacturer/company agreed with the DS's proposal for no classification.

Assessment and comparison with the classification criteria

Table 1 summarised all the available studies for assessment of acute toxicity of valifenalate.

***Table 1**: Summary of animal studies on acute toxicity with valifenalate.*

Study	Dose level	Results	Reference
Acute oral toxicity OECD TG 401 GLP Sprague Dawley rats (Crl: CD (SD) BR) 5/sex/group	Valifenalate (IR5885) Purity: 98.9% 5000 mg/kg bw Single dose followed by 14 days observation	No mortalities Transient piloerection in all animals the day after treatment No appreciable macroscopic changes in necropsies of treated animals LD_{50} > 5000 mg/kg bw	Confidential study number 62
Acute dermal toxicity OECD TG 402 GLP Sprague Dawley rats (Crl: CD (SD) BR) 5/sex/group	Valifenalate (IR5885) Purity: 98.6% 2000 mg/kg bw 24 h dermal exposure followed by 14 days observation	No mortalities No clinical effects No local irritation No appreciable macroscopic changes in necropsies of treated animals LD_{50} > 2000 mg/kg bw	Confidential study number 63
Acute inhalation toxicity OECD TG 403 GLP Wistar Han-Ibm rats 5/sex/group	Valifenalate (IR5885) Purity: 98.6% MMAD: 2.42, 2.45 µm GSD: 2.95, 2.89 Gravimetric	No mortalities No significant signs of toxicity Slight reduction in body weight between days 1 and 4 No macroscopic changes at termination LC_{50} > 3.118 mg/L air (gravimetric mean aerosol concentration) (highest technically achievable concentration)	Confidential study number 11

	concentration: 3.118 mg/l 4 hour nose-only exposure of an aerosol followed by 14 days observation	

Comparison with the criteria

The cut-off point for triggering classification for both acute oral and acute dermal toxicity is 2000 mg/kg bw. Table 1 shows as two reliable OECD-guideline studies conducted observing GLP procedures yielded LD_{50} values higher than 5000 and 2000 mg/kg bw for oral and dermal toxicity; respectively. Thus, RAC supports the DS's proposal for **no classification of valifenalate for acute oral and dermal toxicity.**

The cut-off point for triggering classification for acute inhalation toxicity of dusts and aerosols is 5 mg/l. Table 1 shows as one reliable OECD-guideline study conducted observing GLP procedures yielded an LC_{50} higher than the maximum achievable concentration (3.1 mg/L). Thus, RAC supports the DS's proposal for **no classification of valifenalate for acute inhalation toxicity.**

10.4 Skin corrosion/irritation

Table 22: Summary table of animal studies on skin corrosion/irritation

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels duration of exposure	Results -Observations and time point of onset -Mean scores/animal -Reversibility	Reference
Acute dermal irritation OECD 404 GLP	Rabbit New Zealand White 3 males	Valifenalate (IR5885), 98.6% purity	0.5g / animal Single 4 hour application Application sites scored at: 1, 24, 48 and 72 hours after patch removal (Draize scheme).	No signs of irritation <u>Mean scores / animal (24, 48 & 72 hours):</u> Erythema: 0, 0, 0 Oedema: 0, 0, 0	*See Annex conf. 33.*

In a primary dermal irritation study in New Zealand White rabbits (*See Annex conf. 33.*) there were no signs of skin irritation in 3/3 rabbits and no signs of toxicity. Valifenalate was non irritating to rabbit skin.

Table 23: Summary table of human data on skin corrosion/irritation

Type of data/report	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
No human data available on skin corrosion/irritation				

Table 24: Summary table of other studies relevant for skin corrosion/irritation

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
No other studies available on skin corrosion/irritation				

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

No signs of dermal irritation were observed in any rabbit during the study period. There were no deaths or overt signs of toxicity during the study. Valifenalate did not irritate the skin of rabbits.

10.4.2 Comparison with the CLP criteria

No signs of erythema or oedema were observed, therefore, valifenalate does not meet the criteria for classification according to the CLP Regulation.

10.4.3 Conclusion on classification and labelling for skin corrosion/irritation

CLP: Not classified (conclusive but not sufficient for classification).

RAC evaluation of skin corrosion/irritation

Summary of the Dossier Submitter's proposal

The DS proposed no classification for skin irritation based on a dermal irritation study showing no signs of irritation in 3/3 New Zealand rabbits.

Comments received during consultation

One manufacturer/company agreed with the DS's proposal for no classification.

Assessment and comparison with the classification criteria

Table 2 summarises the findings in the skin corrosion/irritation study available in the CLH-report.

Table 2: *Summary of the animal study on skin corrosion/irritation with valifenalate.*

Study	Dose level	Results	Reference
Acute dermal irritation OECD TG 404 GLP New Zealand White rabbits 3 males	Valifenalate (IR5885) Purity: 98.6% 0.5 g/animal Single 4 hour application Application sites scored at: 1, 24, 48 and 72 hours after	No signs of irritation <u>Mean scores / animal (24, 48 & 72 hours):</u> Erythema: 0, 0, 0 Oedema: 0, 0, 0	Confidential study number 33

	patch removal (Draize scheme)

Comparison with the criteria

RAC notes that the skin irritation study performed according to OECD TG 404 and GLP showed that valifenalate was not able to irritate skin of rabbits since no erythema and no oedema was found in any of the three treated New Zealand White rabbits (Table 2). Thus, RAC supports the DS proposal for **no classification of valifenalate for skin irritation/corrosion.**

10.5 Serious eye damage/eye irritation

Table 25: Summary table of animal studies on serious eye damage/eye irritation

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels duration of exposure	Results - Observations and time point of onset - Mean scores/animal - Reversibility	Reference
Acute eye Irritation OECD 405 GLP	Rabbit New Zealand White 3 males	Valifenalate (IR5885), 98.6% purity	0.1g / animal Single instillation. Eyes scored at: 1, 24, 48 and 72 hours after instillation.	Slight, conjunctival redness was seen at the 1 hour examination in 3/3 rabbits. Mean Scores / animal (24, 48 & 72 hours): Cornea:- 0, 0, 0, Iris - 0, 0, 0, Conjunctiva: redness – 0, 0, 0, Conjunctiva: chemosis - 0, 0, 0, All symptoms had fully reversed by 24 hours.	*See Annex conf. 34.*

In a rabbit eye irritation study (*See Annex conf. 34.*), slight conjunctival redness (grade 1) was noted in all rabbits 1 hour after instillation. All symptoms had fully reversed in all animals at the 24 hour observation. No clinical signs of systemic toxicity were observed in the animals during the study. Valifenalate was non irritating to rabbit eyes.

Table 26: Summary table of human data on serious eye damage/eye irritation

Type of data/report	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
No human data available on eye damage/irritation				

Table 27: Summary table of other studies relevant for serious eye damage/eye irritation

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
No other studies available on eye damage/irritation				

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

In a rabbit eye irritation study, slight conjunctival redness (grade 1) was noted in all rabbits at the reading carried out 1 hour after application and subsided within 24 hours of treatment. No other concomitant or subsequent ocular changes were noted.

10.5.2 Comparison with the CLP criteria

No effects were observed on the cornea or the iris. All average eye irritation scores were <2, therefore, no classification is required in accordance with CLP.

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

CLP: Not classified (conclusive but not sufficient for classification).

RAC evaluation of serious eye damage/irritation

Summary of the Dossier Submitter's proposal

The DS proposed no classification for eye damage/irritation based on an eye damage study showing light conjunctival redness 1 hour after instillation (fully reversible by 24 hours) but no signs of corneal or iris damage and no signs of conjunctival redness or chemosis by 24 hours and thereafter.

Comments received during consultation

One manufacturer/company agreed with the DS's proposal for no classification.

Assessment and comparison with the classification criteria

Table 3 summarises the findings in the acute eye irritation/corrosion study available in the CLH-report.

Table 3: *Summary of the animal study on eye irritation/corrosion with valifenalate.*

Study	Dose level	Results	Reference
Acute Eye Irritation/Corrosion OECD TG 405 GLP New Zealand White rabbits 3 males	Valifenalate (IR5885) Purity: 98.6% 0.1 g/animal Single instillation Eyes scored at: 1, 24, 48 and 72 hours after instillation	Slight (grade 1), conjunctival redness was seen at the 1 hour examination in 3/3 rabbits (fully reversed by 24 hours) <u>Mean Scores / animal (24, 48 & 72 hours):</u> Cornea: 0, 0, 0, Iris: 0, 0, 0, Conjunctiva redness: 0, 0, 0. Conjunctiva chemosis: 0, 0, 0.	Confidential study number 34

Comparison with the criteria

RAC notes that only grade 1 conjunctival redness was seen 1 hour after instillation while

no signs of eye damage was seen by 24 hours and thereafter in an OECD TG 405 study conducted observing GLP (Table 3). Thus, RAC supports the DS proposal for **no classification of valifenalate for eye damage/irritation.**

10.6 Respiratory sensitisation

Table 28: Summary table of animal studies on respiratory sensitisation

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels, duration of exposure	Results	Reference
No relevant studies.					

Table 29: Summary table of human data on respiratory sensitisation

Type of data/report	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
No human data available on respiratory sensitisation				

Table 30: Summary table of other studies relevant for respiratory sensitisation

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

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

No formally recognised and validated animal or in vitro tests currently exist for respiratory sensitisation. However, data from some animal studies may be indicative of the potential of a substance to cause respiratory sensitisation in humans. There are no data to indicate evidence of respiratory tract irritation with valifenalate. The acute inhalation study showed no evidence for impairment of the respiratory system up to the limit dose. Both the rabbit dermal and eye irritation studies indicated a lack of irritant potential on the dermis and mucosal membranes.

10.6.2 Comparison with the CLP criteria

Because of the lack of data, a definitive conclusion on respiratory sensitisation cannot be made.

10.6.3 Conclusion on classification and labelling for respiratory sensitisation

CLP: Data lacking

RAC evaluation of respiratory sensitisation

Summary of the Dossier Submitter's proposal

The DS proposed no classification of valifenalate for respiratory sensitisation based on lack of data.

Comments received during consultation

One company manufacturer commented that the conclusion of lack of data is not correct since test for respiratory sensitisation cannot be provided because no formally recognised and validated animal test currently exists. The DS thanked the comment and replied that this hazard was not in the scope of the public consultation, although the provided comments will be brought to consistency with the conclusion.

Assessment and comparison with the classification criteria

Comparison with the criteria

RAC notes that: i) there are no data indicating evidence of respiratory tract irritation with valifenalate; ii) the acute inhalation study showed no evidence of respiratory system impairment; and iii) rabbit dermal and eye irritation studies indicated lack of irritant potential on skin and mucosal membranes. Overall, RAC supports the DS's proposal for **no classification of valifenalate for respiratory sensitisation.**

10.7 Skin sensitisation

Table 31: Summary table of animal studies on skin sensitisation

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels duration of exposure	Results	Reference
Maximisation test OECD 406 GLP	Guinea pig Dunkin Hartley 17 males (10 test, 5 controls, 2 preliminary test)	Valifenalate (IR5885), 98.6% purity Vehicle: corn seed oil	**Induction:** Intradermal: 1% in corn seed oil, 1% in Freund's Complete Adjuvant (FCA) and FCA emulsion (1:1 v/v FCA/water) – day 0. Topical : pre-treatment with 0.5mL 10% sodium lauryl sulfate in Vaseline oil - day 5. Test article (10%) or vehicle applied under an occlusive dressing	Induction: Slight, swollen reddish are as seen 24 hours after the intradermal injections with FCA and /or test material. There were no signs of irritation observed following the topical induction. Challenge: Challenge sites assessed at 24 and 48 hours. No dermal reaction following challenge in test or control animals. % positive reactions at 24 and 48 hours	*See Annex conf. 47.*

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels duration of exposure	Results	Reference
			for 48 hours. **Challenge:** Test article (10%) and vehicle applied to the flanks of all animals under an occlusive dressing for 24 hours.	Control group : Valifenalate 0%, 0% Vehicle 0%, 0% Test group : Valifenalate 0%, 0% Vehicle 0%, 0% **Sensitisation rate = 0%.**	

In a Maximisation skin sensitisation study in guinea pigs (*See Annex conf. 47.*), there were no signs of irritation or oedema in any of the test or control group animals. No deaths occurred and no signs of general toxicity were observed in any animal. No animals showed positive reactions to either the induction or challenge application. No skin reactivity was observed in the negative control group.

Table 32: Summary table of human data on skin sensitisation

Type of data/report	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
No human data available on skin sensitisation				

Table 33: Summary table of other studies relevant for skin sensitisation

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
No other studies available on skin sensitisation				

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

In a guinea pig Maximisation study, the highest concentrations selected for induction and challenge were based on results from a preliminary test. There were no signs of irritation or oedema in any of the test or control group animals and the sensitisation rate was 0%. In a positive control study with 2-mercaptobenzothiazole (R12330), 2/5 test animals exhibited signs of sensitisation (sensitisation rate of 40%, proving the sensitivity of the test system. Therefore, valifenalate is not considered to be a dermal sensitiser.

10.7.2 Comparison with the CLP criteria

Classification is not required as there is no evidence that valifenalate is a dermal sensitiser.

10.7.3 Conclusion on classification and labelling for skin sensitisation

CLP: Not classified (conclusive but not sufficient for classification).

RAC evaluation of skin sensitisation

Summary of the Dossier Submitter's proposal

The DS proposed no classification of valifenalate for skin sensitisation based on the negative result of a guinea pig maximisation test conducted following OECD TG 406 and observing GLP.

Comments received during consultation

One manufacturer/company agreed with the DS's proposal for no classification.

Assessment and comparison with the classification criteria

Table 4 summarises the findings in the skin sensitisation study available in the CLH-report.

***Table 4**: Summary of the animal study on skin sensitisation with pyridalyl*.

Study	Dose level	Results	Reference
Maximisation test OECD TG 406 GLP Dunkin Hartley guinea pigs 17 males (10 test, 5 controls, 2 preliminary test)	Valifenalate (IR5885) Purity: 98.6% Vehicle: corn seed oil **Induction:** Intradermal: 1% in corn seed oil, 1% in Freund's Complete Adjuvant (FCA) and FCA emulsion (1:1 v/v FCA/water)-day 0. Topical: Pre-treatment with 0.5 ml 10% sodium lauryl sulfate in Vaseline oil-day 5. Test article (10%) or vehicle applied under an occlusive dressing for 48 hours. **Challenge** Test article (10%) and vehicle applied to the flanks of all animals under an occlusive dressing for 24 hours.	**Induction** Slight, swollen reddish seen 24 hours after the intradermal injections with FCA and /or test material. There were no signs of irritation observed following the topical induction. **Challenge:** Challenge sites assessed at 24 and 48 hours. No dermal reaction following challenge in test or control animals. **No positive reactions at 24 and 48 hours. Sensitisation rate: 0%** **Positive control (2-mercaptobenzothiazole):** Sensitisation rate 40%.	Confidential study number 47

Comparison with the criteria

The guinea pig maximisation test conducted according to OECD TG 406 Guideline and observing GLP showed no evidence that valifenalate is a dermal sensitiser. RAC notes that the question whether higher concentrations could have been tested using other vehicles remains unresolved and gives uncertainties for the assessment. Overall, RAC supports the DS's proposal for **no classification of valifenalate for skin sensitisation.**

10.8 Germ cell mutagenicity

Table 34: Summary table of mutagenicity/genotoxicity tests *in vitro*

Method, guideline, deviations if any	Test substance,	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
In vitro bacterial gene mutation Ames test OECD 471 GLP	Valifenalate (IR5885), Purity 98.9% Positive controls: sodium azide; 4-nitro-o-phenylene-diamine; methyl methane sulfonate and 2-aminoanthracene Solvent: Dimethyl sulfoxide (DMSO)	Strains: TA98, TA100, TA102, TA1535, TA1537 of *Salmonella typhimurium* Concentrations : 33, 100, 333, 1000, 2500 and 5000 valifenalate µg/plate Limit dose.	Negative +/- S9	*See conf. Annex 53.*
In vitro clastogenicity in mammalian cells Chromosome aberration test OECD 473 GLP	Valifenalate (IR5885), Purity 98.9% Positive controls: ethylmethane sulfonate and cyclophosphamide Solvent: Dimethylsulfoxide (DMSO)	Chinese Hamster Ovary (CHO/D1) cells Concentrations : Expt 1: Concentrations of up to 1600 µg /mL (with and without S9 mix) tested, selected on the basis of the pre-test for toxicity. Expt 2: Concentrations of up to 200 µg /mL (without S9 mix) and up to 1600µg /mL (with S9 mix) tested, selected on the basis of the pre-test for toxicity.	Negative +/- S9	*See Annex conf. 41.*
In vitro mammalian gene mutation OECD 476 GLP	Valifenalate (IR5885), Purity 98.9% Positive controls: 3-methyl chloranthracene and methyl methane sulfonate Solvent: Dimethyl sulfoxide (DMSO)	L5178Y mouse lymphoma cells Concentrations : Expt 1: 12.5, 25, 50, 100, 200 and 400 µg/mL (with and without S9 mix). Expt 2: 25, 50, 100, 200, 400 & 800 µg/mL (without S9 mix). Concentrations selected from a pre-test for toxicity.	Negative +/- S9	*See Annex conf. 54.*

Table 35: Summary table of mutagenicity/genotoxicity tests in mammalian somatic or germ cells *in vivo*

Method, guideline, deviations if any	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
In vivo mouse micronucleus OECD 474 GLP	Valifenalate (IR5885) Purity 99.56% . Positive control cyclophosphamide Vehicle: corn oil	NMRI mouse 6/sex/group 24 hour preparation interval groups dosed at:0, 500, 1000 or 2000 mg/kg bw valifenalate plus positive control group. 48 hours preparation interval : an additional group dosed at 2000 mg/kg bw. Preliminary experiment: 2/sex dosed at 2000 mg/kg bw.	Negative	*See Annex conf. 20.*

Table 36: Summary table of human data relevant for germ cell mutagenicity

Type of data/report	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
No human data available on germ cell mutagenicity				

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

Valifenalate did not induce gene mutations by base pair changes or frameshifts in the genome in a reverse mutagenicity test in bacteria (*Salmonella typhimurium* strains). In a mammalian cell mutation assay using L5178Y mouse lymphoma cells, valifenalate did not induce mutations in the thymidine kinase locus. In a bone marrow micronucleus assay using NMRI mice, valifenalate did not induce micronuclei and is therefore considered to be non-mutagenic. In a chromosome aberration test in Chinese Hamster Ovary cells, valifenalate did not induce structural chromosome aberrations *in vitro* and is considered to be non-clastogenic. Valifenalate is therefore considered non-mutagenic in bacteria and in cultured mammalian cells.

An *in vivo* genotoxicity test in somatic cells (e.g. an unscheduled DNA synthesis assay or a mouse spot test) was considered not required as none of the *in vitro* tests nor the *in vivo* mouse micronucleus test were positive. Similarly, an *in vivo* study in germ cells was considered not required on the basis of the results from the studies presented.

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

10.8.2 Comparison with the CLP criteria

The genotoxicity of valifenalate was tested in three *in vitro* and one *in vivo* test. The results of all studies were negative with positive and negative controls demonstrating the validity of the tests. Valifenalate can be considered not to be genotoxic and no classification is proposed.

10.8.3 Conclusion on classification and labelling for germ cell mutagenicity

CLP: Not classified (conclusive but not sufficient for classification).

RAC evaluation of germ cell mutagenicity

Summary of the Dossier Submitter's proposal

The DS proposed no classification of valifenalate for germ cell mutagenicity based on three *in vitro* and one *in vivo* negative studies.

Comments received during consultation

One company-manufacturer agreed with the DS's proposal for no classification.

Assessment and comparison with the classification criteria

Tables 9 and 10 summarise the results of the mutagenicity/genotoxicity assays contained in the CLH-report.

Table 9: *Summary of mutagenicity/genotoxicity in vitro studies with valifenalate***.**

Method	Tested concentrations	Results	Reference
In vitro bacterial gene mutation Ames test OECD TG 471 GLP Strains: TA98, TA100, TA102, TA1535, TA1537 of Salmonella typhimurium	Valifenalate (IR5885) Purity: 98.9% Positive controls: sodium azide; 4-nitro-o-phenylene-diamine; methyl methane sulfonate and 2-aminoanthracene Solvent: Dimethyl sulfoxide (DMSO) Concentrations : 33, 100, 333, 1000, 2500 and 5000 valifenalate µg/plate	**+S9: Negative** **-S9: Negative**	Confidential study number 53
In vitro clastogenicity in mammalian cells Chromosome aberration test OECD TG 473 GLP Chinese Hamster Ovary (CHO/D1) cells	Valifenalate (IR5885) Purity: 98.9% Positive controls: ethylmethane sulfonate and cyclophosphamide Solvent: Dimethylsulfoxide (DMSO) Concentrations : Experiment 1: Concentrations of up to 1600 µg /mL (with and without S9 mix) Experiment 2: Concentrations of up to 200 µg /mL (without S9 mix) and up to 1600 µg /mL (with S9 mix)	**+S9: Negative** **-S9: Negative**	Confidential study number 41
In vitro mammalian gene mutation OECD TG 476 GLP L5178Y mouse lymphoma cells	Valifenalate (IR5885) Purity: 98.9% Positive controls: 3-methyl chloranthracene and methyl methane sulfonate Solvent: Dimethyl sulfoxide (DMSO) Concentrations : Experiment 1: 12.5, 25, 50, 100, 200	**+S9: Negative** **-S9: Negative**	Confidential study number 54

	and 400 µg/mL (with and without S9 mix) Experiment 2: 25, 50, 100, 200, 400 & 800 µg/mL (without S9 mix)		

Table 10: *Summary of the mutagenicity/genotoxicity in vivo study with valifenalate.*

Method	Tested concentrations	Results	Reference
In vivo mouse micronucleus OECD TG 474 GLP NMRI mouse 6/sex/group	Valifenalate (IR5885) Purity: 99.56% Positive control: cyclophosphamide Vehicle: corn oil 24 hours preparation interval groups dosed at: 0, 500, 1000 or 2000 mg/kg bw valifenalate plus positive control group 48 hours preparation interval: an additional group dosed at 2000 mg/kg bw	**Negative**	Confidential study number 20

Comparison with the criteria

The genotoxicity of valifenalate was tested in three *in vitro* and one *in vivo* tests. The results of all studies were negative with positive and negative controls demonstrating the validity of the tests. Thus, RAC supports the DS's proposal for **no classification of valifenalate for germ cell mutagenicity.**

10.9 Carcinogenicity

Table 37: Summary table of animal studies on carcinogenicity

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
2-year combined toxicity and carcinogenicity study OECD 453 GLP Rat (HsdBrl Han Wistar) 50/sex/group (104 weeks) 20/sex/group (52 weeks)	Valifenalate (IR5885) Lot T025/02, purity was 99.56% (weeks 1-103) - 99.63% (weeks 104-106). 0,15,150, 1000 mg/kg bw/day Continuous dietary administration for 104 weeks (carcinogenicity phase) or 52 weeks (toxicity	**Non neoplastic findings** **1000 mg/kg bw/day**: *Body weight*: ↓ 9% in males Carcinogenicity phase weeks 0-104. No effect in females. *Haematology*: Toxicity phase – Low haemoglobin in males in first year; low erythrocyte counts & mean cell haemoglobin concentrations in males in weeks 13 & 26. High platelet counts and prolonged clotting times in males during the first year and in females on occasions. No treatment related changes in Carcinogenicity phase animals. *Urine analysis*: Slightly increased volume and low specific gravity seen in females during the first year. *Liver weights*: ↑ 19.1% and 9.9% relative to body weight in males at 52 and 104 weeks, 12.2% and 7.6% relative to body weights in females at 52 and 104 weeks *Kidney weights*: ↑ 7.6% relative to body weight in males at 52	*See Annex conf. 51.*

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
	phase).	weeks. *Pathology*: ↑ Thyroids follicular cell hypertrophy 11/20 males at 52 weeks only (3/20 controls). ↑ Kidney pelvic/papillary epithelial hyperplasia 25/50 females at 104 weeks (9/50 controls) **150 mg/kg bw/day**: *Body weight*: ↓ Carcinogenicity phase males (8% lower than controls, weeks 0-104). *Pathology*: no treatment-related changes **15 mg/kg bw/day**: No toxicologically significant treatment-related effects. **NOAEL for chronic toxicity 1000 mg/kg/d in females and 150 mg/kg/d in males.** **Neoplastic findings** No treatment-related changes in neoplastic findings at any dose level. **NOAEL for carcinogenicity** 1000 mg/kg in both sexes.	

<table>
<tr><th>Method, guideline, deviations if any, species, strain, sex, no/group</th><th>Test substance, dose levels duration of exposure</th><th>Results</th><th>Reference</th></tr>
<tr>
<td>Carcinogenicity study:
OECD 451
Mouse (Crl: CD-1™ (ICR) BR)
50/sex/group</td>
<td>Valifenalate (IR5885)
Lot T025/02, purity 99.56%
0, 150, 850, 5000 ppm mg/kg bw/day Continuous dietary administration for 78 weeks
Achieved doses 16.8, 97.2 and 657 mg/kg/day for males and 21.6, 124 and 756 mg/kg/day for females.</td>
<td><u>Non-neoplastic findings</u>
<u>5000ppm</u>:
Body weight: ↓ 22 % in males (weeks 0 to 78)
Liver weight: ↑ 97.0 % and 23.1% relative weight in males and females.
Kidney weight: ↑ 11.9 % relative weight in females
Liver pathology: ↑ Centrilobular hepatocyte hypertrophy: 25/50 females (8/50 controls), Generalised hepatocyte hypertrophy: 29/50 males (3/50 controls), Centrilobular hepatocyte vacuolation 32/50 males (11/50 controls), Cytoplasmic eosinophilia 29/50 males (0/50 controls); Pigment in hepatocytes 18/50 males, 13/50 females (0-1/50 controls), Pigment in macrophages 12/50 males, 31/50 females (control 1/50 males and 12/50 females)
Gall bladder pathology: ↑ Choleliths 8/45 females (1/47 controls).
<u>850ppm</u>:
Clinical findings: No adverse effects.
Liver weight: ↑ 28.6% relative weight males
Pathology: ↑ Liver findings Centrilobular hepatocyte vacuolation 33/50 males (11/50 controls)
<u>150ppm</u>:
Clinical findings: No adverse effects.
Organ weights: Increased liver weights (males) .
Pathology: Centrilobular (34/50) and/or generalised liver hypertrophy (6/50) in males (21/50 and 3/50 controls).
<u>Neoplastic findings:</u>
Increased hepatocellular adenoma in both sexes at 850 and 5000 ppm and increased hepatocellular carcinoma in males only at 5000 ppm (Hepatocellular adenoma males 7.8-21.2%, females 0-1.9%
Hepatocellular carcinoma males 1.9-8.0).
<table>
<tr><td rowspan="2">Finding</td><td colspan="8">Dietary concentration (ppm)</td></tr>
<tr><td colspan="4">Males</td><td colspan="4">Females</td></tr>
<tr><td></td><td>0</td><td>150</td><td>850</td><td>5000</td><td>0</td><td>150</td><td>850</td><td>5000</td></tr>
<tr><td>No. Examined</td><td>50</td><td>50</td><td>50</td><td>50</td><td>50</td><td>50</td><td>50</td><td>50</td></tr>
<tr><td>Hepatocellular adenoma</td><td>7</td><td>2</td><td>14</td><td>16*</td><td>0</td><td>0</td><td>2</td><td>5*</td></tr>
<tr><td>Hepatocellular carcinoma</td><td>2</td><td>4</td><td>4</td><td>10*</td><td>0</td><td>1</td><td>0</td><td>0</td></tr>
</table>
* p < 0.05 when compared to control group

NOAEL for carcinogenicity : 150 ppm equivalent to 16.8 mg/kg bw/day in males and 21.6 mg/kg bw/day in females</td>
<td>See Annex conf. 52.</td>
</tr>
</table>

Table 38: Summary table of human data on carcinogenicity

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No evidence of carcinogenicity in humans. Increased incidence of hepatocellular tumours in CD-1 mice was considered secondary to adaptive metabolic changes. Such findings are observed commonly in mice and are generally considered of no significance for man.				

Table 39: Summary table of other studies relevant for carcinogenicity

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
Investigative study no guidelines Non-GLP Mouse: CD1 and C57BL/6 5 males/ group	Valifenalate, batch P/13/024, 99.68% 7000 ppm 7 days in diet	Comparison of C57BL/6 mice and CD1 mice to determine if C57BL/6 mice are a suitable strain for a subsequent study in PPARα knock out mice derived from C57BL/6 strain	**CD1** *Liver weight*: ↑ 19.5% *Liver:bodyweight ratio*: ↑ 21% *PCoA*: ↑ 1.6 fold *Hepatic pentoxyresorufin-O-dependentylation (PROD)*: ↑ 2.1 fold *Hepatic 12-hydroxylauric acid:* ↑ 4.9 fold **C57Bl/6** *Liver weight:*↑ 13.8% *Liver:bodyweight ratio*: ↑ 16% *PCoA*: 1.9 fold *Hepatic pentoxyresorufin-O-dependentylation (PROD):* ↑ 3.4 fold *Hepatic 12-hydroxylauric acid:* ↑7.1 fold **Conclusion:** Overall, the response in both strains was very similar. However, there appeared to be a somewhat increased induction of CAR/PXR in this study. It was concluded that the C57BL/6 mouse strain is an appropriate background strain for further investigations using the PPARα Knockout model	*See Annex conf. 68*
Investigative study no guidelines Non-GLP Mouse: C57BL/6 wild type and PPARα knock out (KO) 10 males/ group	Valifenalate, batch P/13/024, 99.68% 7000 ppm 7 and 14 days in diet	Comparison of response in PPARα knockout mice with wild type controls	**Wild type** *S-phase*: ↑ 8.2 fold day 7, 1.9 fold day 14 *Liver pathology*: ↑ minimal to mild centrilobular hypertrophy 10/10 day 7, moderate centrilobular hypertrophy 10/10 accompanied by increased mitosis 6/10 day 14 *PCoA oxidation*: ↑ 2.0 fold *Acox1 mRNA*: ↑ 1.8 fold 12-hydroxylauric acid levels: ↑ 7.7 fold after 14 days *Cyp2b10 mRNA level*: ↑ 50 fold after 14 days *PROD activity*: ↑ was elevated by 6.0-fold after 14 days *Cyp3a11 mRNA levels*: ↑ 6.3 fold after 14 days	*See Annex conf. 69*

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
			PPARα Knockout *S-phase*: ↑ 5.4 fold day 7, 3.5 fold day 14 *Liver pathology*: ↑ minimal centrilobular hypertrophy: 2/10 day 14 *PCoA oxidation*: ↑ 1.3 fold *Acox1 mRNA*: ↑ 1.3 fold *Cyp4a mRNA levels*: ↑ higher in the KO than in wild type *12-hydroxylauric acid levels*: ↑ 4 fold after 14 days *Cyp2b10 mRNA level*: ↑ 50 fold after 14 days *PROD activity*: ↑ was elevated by 7.1-fold after 14 days *Cyp3a11 mRNA levels*: ↑ 8.5 fold after 14 days **Conclusions** PPARα pathway is responsible for a portion of the hepatic response, additional mechanisms mediated by CAR and PXR activation are also involved	
Investigative study no guidelines Non-GLP Mouse: hepatocytes from CD1 strain	Valifenalate, batch P/13/024, 99.68% 0, 10, 30, 100 & 300 µM valifenalate with phenobarbital (as Na salt at 100 and 1000 µM) and WY-14,643 (50 and 100 µM) as positive controls	Investigate the potential of Valifenalate to activate CAR and/or PPARα nuclear hormone receptors and stimulate cell proliferation in isolated hepatocytes	**Valifenalate** *Cytotoxicity: 300 µM 74% decrease in ATP levels* Essentially no impact on any of the biochemical markers assessed. **Phenobarbital** No effect on replicative DNA synthesis *Cyp2b10*: ↑ *PROD activity*: ↑ *12-OH LA* formation: small increase, not statistically significant *PCoA* oxidation: small increase, not statistically significant **Wy-14,643** *DNA synthesis*: ↑ 1.7 fold *12-OH LA* formation: ↑ 1000 and 100 µM 8.1- and 6.9-fold respectively *PCoA* oxidation: ↑ 1000 and 100 µM 4.9- and 5.4-fold respectively *Cyp2b10 mRNA levels*: ↑ at 100 and 1000 µM by 3.8- and 9.1-fold respectively *Cyp4a10 and Cyp4a14c mRNA levels*: ↑ *Cyp4a10 mRNA levels*: ↑ >298-fold *Cyp4a14 mRNA levels*: ↑ >643-fold *Cyp2b10 and Acox1 mRNA levels*:	*See Annex conf. 70*

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
			↑ **Conclusion** Valifenalate does not activate either mouse CAR or PPARα when assessed in vitro as demonstrated by the lack of hypertrophic and hyperplasic responses in the CD-1 mouse hepatocytes	
Investigative study no guidelines GLP Mouse: Crl:CD-1 (ICR) BR 18 males/group Of which 6/group killed interim and the remainder after 14 days	IR5885, G005/07, purity 97.83% 0, 150, 1750 and 7000 ppm in diet Achieved intake: 0, 21, 249 and 1050 mg/kg bw/day Phenobarbitone positive control 850 ppm 130.mg/kg bw/day 3 (interim kill) or 14 days	Investigation of mechanism of possible liver toxicity. Assessments included cell proliferation, CYP enzymes (activity and/or mRNA expression), peroxisomal β-oxidation, catalase histochemistry and oxidative stress (TBARS).	**7000 ppm, 1050 mg/kg/day** *Cyp4a-1 enzyme sub family (Lauric acid 12-hydroxylase):* ↑ 1106% of control *Peroxisomal β-oxidation*: 308% of control *Liver weight relative to body weight*: ↑ 34% day 3, 35% day 14 *Hepatocellular hypertrophy*: ↑ 6/6 after 3 and 14 days *Catalase area:total nuclear area:* ↑ 16.2% **1750 ppm, 249 mg/kg bw/day** *Cyp4a-1 enzyme sub family (Lauric acid 12-hydroxylase):* ↑ *408*% of control *Peroxisomal β-oxidation*: 208% of control *Liver weight relative to body weight*: ↑ 10% day 3, 13% day 14 *Hepatocellular hypertrophy*: ↑ 3/6 and 4/6 after 3 and 14 days respectively. *Catalase area:total nuclear area:* ↑ 11.5% **150 ppm, 21 mg/kg bw/day** No treatment related effects; marginal increae in *catalase area:total nuclear area:* ↑ 6.0% **Phenobarbitone** *mRNA levels:* ↑ CYP 2B10 (223 fold). ↑ CYP3A11 (12.12 fold) CYP1A1 (3.58 fold) and CYP1A2 (2.96 fold) *Peroxisomal β-oxidation*: no increase *Liver weight relative to body weight*: ↑ 55% day 3, 37% day 14 *Hepatocellular hypertrophy*: ↑ 6/6 after 3 and 14 days, severity more marked after 14 days. *Catalase area:total nuclear area:* no increase. Valifenalate (IR5885) appears as moderate and dose dependent liver enzyme inducer of the	*See Annex conf. 66*

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
			peroxisomal-proliferator type. The mode of action as a liver enzyme inducer of the polycyclic aromatic hydrocarbon-, steroid-, or phenobarbitone-type can be excluded.	

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

Carcinogenicity studies in rats (*See Annex conf. 51.*) and mice (*See Annex conf. 52.*) have been completed for valifenalate. In Han Wistar rats there was no evidence of valifenalate-related carcinogenicity up to and including the limit dose level for carcinogenicity studies of 1000 mg/kg/day.

In CD-1 mice valifenalate induced hepatocellular adenomas and carcinomas in males. Hepatocellular tumours are relatively common in male CD-1 mice, however the incidence of these tumours in males and females given 850 or 5000 ppm exceeded the background range seen in studies of this duration performed recently at this laboratory (see Annex III Historical control data, data from 6 studies performed 1994 to 1998, at most 10 years prior to reported study). For males, at 850 ppm the incidence of adenoma and carcinoma was 28 and 8% respectively, and at 5000 ppm the incidences were 32 and 20%, respectively. The incidences of adenomas exceeded the historical control range at both dose levels. However, the incidence of carcinomas in males at 850m ppm was within the historical control incidence reported by the laboratory and the same as the study with the closest start date to the valifenalate study.

Historical control data for hepatocellular tumours in recent studies performed at the Eye Research Centre (Males)

Code number	cdm094	cdm097	cdm105	cdm107	cdm108	cdm110	Total	Range of
Start date	Jan-94	Jul-94	Sep-96	Sep-97	May-98	Nov-98		percentages*
Study duration (weeks)	78	79	78	78	78	78		
Liver								
Hepatocellular adenoma								
Incidence	6	10	11	4	6	10	47	
Percentage*	11.5%	19.2%	21.2%	7.8%	12.0%	20.0%	15.31%	7.8 - 21.2
Hepatocellular carcinoma								
Incidence	2	3	1	1	1	4	12	
Percentage*	3.8%	5.8%	1.9%	2.0%	2.0%	8.0%	3.91%	1.9 - 8.0
Number of animals examined	52	52	52	51	50	50	307	

Historical control data for hepatocellular tumours in recent studies performed at the Eye Research Centre (Females)

Code number	cdm094	cdm097	cdm105	cdm107	cdm108	cdm110	Total	Range of
Start date	Jan-94	Jul-94	Sep-96	Sep-97	May-98	Nov-98		Percentages*
Study duration (weeks)	78	80	78	78	78	78		
Liver								
Hepatocellular adenoma								
Incidence	1	0	0	0	0	0	1	
Percentage*	1.9%	0.0%	0.0%	0.0%	0.0%	0.0%	0.33%	0.0 - 1.9
Hepatocellular carcinoma								
Incidence	0	0	0	0	0	0	0	
Percentage*	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.00%	0.0 - 0.0
Number of animals examined	52	52	52	51	50	50	307	

In female mice, valifenalate appeared to be less potent with a smaller, but statistically significant, increase in adenomas only being reported at a dose level of 756 mg/kg/day. The incidence of adenoma was 4 and 10% at 850 and 5000 ppm, respectively. At both dose levels this incidence was outside the historical control incidence. The single incidence of hepatocellular carcinoma in a female animal given 150 ppm falls outside the range reported in this data. However, a single incidence of this tumour has been reported in a control group from a study performed at an earlier date (1 out of 672 animals in 13 studies examined; a range of 0.0-2.0%). This indicates that this is a rare tumour, which does however occur spontaneously in CD-1 mice.

Summary of mechanistic studies on liver effects (*further details in Annex II to this report*)

Valifenalate is considered not to be genotoxic. Non-genotoxic modes of action include epigenetic changes, i.e. effects that do not involve alterations in DNA but that may influence gene expression, altered cell-cell communication, or other factors involved in the carcinogenic process. For example, non-genotoxic action can involve specific receptors e.g., peroxisome proliferator-activated receptor-alpha (PPARα) which is associated with liver tumours in rodents. A series of investigative toxicology studies were undertaken in male mice with the aim of, firstly, shedding light on the likely mechanism of formation of hepatocellular carcinomas induced by valifenalate in male CD-1 mice, and secondly, to address the assessment of the relevance of these findings to human health.

In a comparative study in CD-1 and C57BL/6 strains of mouse (*See Annex conf. 68*) increases were seen in liver weights, peroxisome proliferation (PCoA) and the biochemical hepatic markers pentoxyresorufin-O-depentylation (PROD) and 12-hydroxylauric acid. The pattern and extent of the response was similar in both strains. In a further study comparing the response in C57BL/6 wild type mice and C57BL/6 PPARα Knockout mice it was concluded that the PPARα pathway is responsible for a portion of the hepatic response but that additional mechanisms mediated by CAR and PXR activation were also involved.

However in an *in vitro* mouse hepatocyte study (*See Annex conf. 70*), valifenalate had essentially no impact on any of the biochemical markers assessed. This leads to the conclusion that the metabolism of valifenalate is likely to be a key factor in the activation of CAR/PXR and PPARα but that the hepatocyte culture system is incapable of producing the quantity(s) of the metabolite(s) necessary to co-activate CAR/PXR and PPARα. Evidence for this scenario comes from a comparison of the valifenalate-induced induction of the associated mRNAs and Cyp isozymes induced *in vivo*, but the absence, in this mouse (CD-1 strain) hepatocyte culture system, *in vitro* (*See Annex conf. 70*). Unfortunately these factors preclude a study to define a valifenalate-specific lack of induction of replicative DNA synthesis in human hepatocytes.

The time and dose dependency of hepatocellular findings are shown below:

	Time				
Dose ppm (mg/kg bw/day)	**Initiating Event Activation of CAR/PXR/PPARα**	**Key Event 2 Increased replicative DNA synthesis**	**Associated event: Increased hepatocellular hypertrophy**	**Key Event 3 Formation of hepatocellular Carcinoma**	**Reference**
	Measured indirectly from Day 7	*Measured from Day 3*	*Measured from Day 3 to 90*	*Key event: Measured at 78 weeks*	
110 (15.3)			- in CD-1 strain of mouse 90 days		*See Annex conf. 50.*
150 (20.7)	- day 14 in male CD-1 strain of mouse	- day 3 and day 14 in CD-1 strain of mouse	- in CD-1 strain of mouse at 3 & 14 days		*See Annex conf. 66*
150 (16.8)			- in CD-1 strain of mouse at 78 weeks	**- week 78 in CD-1 strain of mouse**	***See Annex conf. 52.***
850 (97.2)			+ in CD-1 strain of mouse at 78 weeks	**- week 78 in CD-1 strain of mouse**	***See Annex conf. 52.***

	Time				
Dose ppm (mg/kg bw/day)	**Initiating Event Activation of CAR/PXR/PPARα**	**Key Event 2 Increased replicative DNA synthesis**	**Associated event: Increased hepatocellular hypertrophy**	**Key Event 3 Formation of hepatocellular Carcinoma**	**Reference**
900 (133.7)			+ in CD-1 strain of mouse at 90 day		*See Annex conf. 50.*
1750 (249)	+ day 14 in male CD-1 strain of mouse	++ day 3 in CD-1 strain of mouse + day 14 in CD-1 strain of mouse	+ in CD-1 strain of mouse at 3 and 14 days		*See Annex conf. 66*
5000 (657)			+ in CD-1 strain of mouse at 78 weeks	**++ week 78 in CD-1 strain of mouse**	***See Annex conf. 52.***
7000 (1049.5)		++ day 3 in CD-1 strain of mouse +(+) day 14 in CD-1 strain of mouse	+ in CD-1 strain of mouse at 3 & 14 days		*See Annex conf. 66*
7000 (995)			+ in CD-1 strain of mouse at 90 days		*See Annex conf. 50.*
7000 (1050)	++ day 14 in male CD-1 strain of mouse	+ day 14 in CD-1 strain of mouse			*See Annex conf 68*
7000 (1324-1636)	++ day 7 in male C57BL/6 and C57BL/6 (PPARα KO) strains of mouse	++ day 7 in C57BL/6 strain of mouse + day 7 in C57BL/6 (PPARα KO) strain of mouse	+ in CD-1 strain of mouse at 7 & 14 days		*See Annex conf. 69*

- represents no response, + represents a positive response and ++ represents a stronger positive response

The data from these studies have been considered in detail (*see Annex II to this report*) and a mode of action for the carcinogenic effects of valifenalate has been determined. The initiating event is the co-activation of multiple nuclear receptors, CAR/PXR/PPARα, and as a direct consequence, the associated induction of gene expression and enzyme activity of Cyp2b10, Cyp3a11 and Cyp4a.

The second key event, increased hepatocellular proliferation, is also initiated in CD-1 mice exposed to valifenalate, on a time scale not dissimilar to the appearance of induction of the hepatic metabolising enzymes.

The final key event is the longer-term formation of carcinomas *via* the development of altered, hyperplastic, hepatic, foci and the subsequent development of benign and, ultimately, malignant hepatocellular neoplasms. This is consistent with information from the 78 week carcinogenicity study in male and female CD-1 mice.

Table 40: Compilation of factors to be taken into consideration in the hazard assessment

Species and strain	Tumour type and background incidence	Multi-site responses	Progression of lesions to malignancy	Reduced tumour latency	Responses in single or both sexes	Confounding effect by excessive toxicity?	Route of exposure	MoA and relevance to humans
Rat Han Wistar	No treatment-related neoplastic findings	n/a	n/a	n/a	n/a	n/a	Oral diet	n/a
Mouse CD1	Hepatocellular adenoma males 7.8-21.2%, females 0-1.9% Hepatocellular carcinoma males 1.9-8.0	No	Yes	No	Both	Yes, high dose male body weight decreased 22%	Oral diet	Initiated by activation of receptors CAR, PXR and PPARα Unlikely to occur in humans on a quantitative basis (Annex II)

10.9.2 Comparison with the CLP criteria

The database for the evaluation of valifenalate carcinogenicity includes two GLP studies conducted to OECD guidelines. The exposure route was oral in both the rat and the mouse studies. Additional mechanistic studies provide insight into the relevance to humans of the neoplastic response in the mouse study.

Classification in category 1A concerns substances known to have carcinogenic potential for humans and is largely based on human evidence. Since there are no human data it cannot be concluded that valifenalate has known carcinogenic potential for humans; therefore Category 1A is not applicable.

Category 1B is for substances presumed to have carcinogenic potential for humans. Classification is largely based on animal evidence. Following an overall evaluation of the human evidence and the tumour data from one rat and one mouse bioassay and mechanistic studies, it is concluded that there is not sufficient evidence for carcinogenicity and a classification of valifenalate in category 1B is thus not warranted. The evaluation of strength of evidence and additional considerations including comparison with historical control data is provided for each tumour type above.

Category 2 substances are suspected human carcinogens. Classification is based on evidence obtained from human and/or animal studies, but which is not sufficiently convincing to place the substance in Category 1A or 1B, based on strength of evidence together with additional considerations. There is no evidence to support a classification in category 2 based on the evaluation of the rat study. After 104 weeks of treatment up to and including a limit dose level of 1000 mg/kg/day, there were no treatment-related changes in neoplastic findings. There was no evidence of significant toxicity or any increase in tumour incidence. The liver, thyroid and kidney were identified as target organs but there was no evidence of a treatment-related increase in tumours in either organ.

In the mouse study, there was an increased incidence of hepatocellular tumours in males and females receiving 850 or 5000 ppm, which were considered secondary to adaptive metabolic changes. A full range of investigative studies was performed to determine the mode of action of valifenalate in the mouse. These show that liver effects are initiated by activation of receptors CAR, PXR and PPARα. In a review of the mechanistic studies it was concluded that these effects were not likely to occur in humans on a quantitative basis (Annex II). Valifenalate did not induce liver tumours in the rat. There is insufficient evidence to support a classification in category 2 based on the mouse data. In conclusion, the evaluated data show that valifenalate does not meet the classification criteria for carcinogenicity under CLP.

10.9.3 Conclusion on classification and labelling for carcinogenicity

CLP: Not classified (conclusive but not sufficient for classification).

RAC evaluation of carcinogenicity

Summary of the Dossier Submitter's proposal

The CLH-report contains two carcinogenicity studies. That in rats showed no neoplastic findings, while the study in mice showed increased incidence over control and historical control data (HCD) of hepatocellular adenomas in both sexes at 850 and 5000 ppm and increases of hepatocellular carcinoma in males at 5000 ppm. The CLH-report also provides some mechanistic studies for demonstrating that the carcinogenicity in mouse liver is triggered by a mechanism based on a key event consisting in activation of multiple nuclear receptors, followed by a key event consisting in an increase in the DNA replicative synthesis which, in turn, is followed by the last key event, consisting in the formation of the hepatocellular injury. The DS proposed no classification of valifenalate for carcinogenicity based on the lack of relevance for humans of the proposed mechanism of action.

Comments received during consultation

One MSCA questioned the results and conclusions derived from the confidential study number 69 on the basis of: i) inappropriate comparison between strains; ii) a weak induction of peroxisome proliferator-activated receptor (PPAR-α) in the knock-out model; and iii) lack of positive control in this experiment. This same MSCA also questioned the lack of experiments with constitutive androstane receptor (CAR)/pregnane X receptor (PXR) knockout mice in the database in order to clarify the role of these receptors in the hepatocarcinogenesis. Finally, the MSCA also questioned why valifenalate was not able to activate nuclear receptors while positive controls did. Overall, this MSCA considered the receptor activation by valifenalate to be demonstrated but not the lack of relevance for humans because alternative mechanisms of action were not addressed and they therefore supported classification as Carc. 2 H351. The DS replied to these comments as follows:

- Providing an additional historical control data (HCD) from Charles River Laboratories showing that hepatocellular adenoma incidences in males were almost covered and the incidence in females were covered by this new HCD records.
- Highlighting the arguments presented in Annex 2 of the CLH report (and summarised below; see "Supplemental Information") and considering that: i) the "Bradford Hill Considerations" of the WHO International Programme on Chemical Safety support the proposed mechanism of action based on nuclear receptor activation; ii) the lack of relevance for humans, since neither CAR/PXR nor the PPAR-α are regarded as relevant to humans; and iii) evidences that carcinogenicity in liver in this case is not based on alternative mechanisms of action such as genotoxicity, cytotoxicity, aryl hydrocarbon receptor (AhR)- or oestrogen receptor (ER)-mediated mechanism.

One company-manufacturer supported the DS's proposal for no classification.

Assessment and comparison with the classification criteria

A summary of the information contained in the Annex 2 of the CLH-report entitled "*Valifenalate: Mode of Action Analysis using the WHO/IPCS Mode of Action Framework*" is presented in the Background Document.

Table 11 summarises the results of the two carcinogenicity studies found in the CLH-report.

Table 11: *Summary of carcinogenicity studies with valifenalate*.

Method	Results	Reference
2-year combined toxicity and carcinogenicity study OECD TG 453 GLP Rat HsdBrl Han Wistar 50/sex/group: 104 weeks 20/sex/group: 52 weeks Valifenalate (IR5885) Purity: 99.56% 0,15,150, 1000 mg/kg bw/day Continuous dietary administration	**Non-neoplastic findings** See Table 5 for effects at 52 weeks <u>Effects at week 104: 1000 mg/kg bw/day</u> No effects on body weight, haematology and urine analysis Increases of relative liver weight of 9.9% ($p<0.01$) (males) and 7.6% ($p<0.01$) (females) <u>Effects at week 104: 150 mg/kg bw/day</u> Reduction of 8% in male body weight <u>Effects at week 104: 15 mg/kg bw/day</u> No toxicologically significant treatment-related effects **Neoplastic findings** **No treatment-related changes in neoplastic findings at any dose level**	Confidential study number 51
Carcinogenicity study OECD TG 451 Mouse Crl: CD-1™ (ICR) BR 50/sex/group Valifenalate (IR5885) Purity: 99.56%	**Non-neoplastic findings** See Table 6 **Neoplastic findings** <u>Males</u> Dietary concentration (ppm) / 0 / 150 / 850 / 5000 / HCD (%)$ No. Examined: 50 / 50 / 50 / 50 / - Hepatocellular Adenoma (%): 7 (14) / 2 (4) / 14 (28) / 16* (32) / 7.8-21.2	Confidential study number 52

0, 150, 850, 5000 ppm mg/kg bw/day

Continuous dietary administration for 78 weeks

Achieved doses 16.8, 97.2 and 657 mg/kg/day for males and 21.6, 124 and 756 mg/kg/day for females

Hepatocellular carcinoma (%)	2 (4)	4 (8)	4 (8)	10* (20)	1.9-8.0
Combined adenoma + carcinoma** (%)	9 (18)	6 (12)	18 (36)	26 (52)	-

*p ≤ 0.05 compared with control group
**Estimated by RAC, not provided by the DS, no available statistical analysis
$ No contextual information about this HCD was provided

Females

	Dietary concentration (ppm)				
	0	150	850	5000	HCD (%)$
No. Examined	50	50	50	50	
Hepatocellular adenoma	0 (0)	0 (0)	2 (4)	5* (10)	0.0-1.9
Hepatocellular carcinoma	0 (0)	1 (2)	0 (0)	0 (0)	0.0-0.0
Combined adenoma + carcinoma**	0 (0)	1 (2)	2 (4)	5 (10)	

*p ≤ 0.05 compared with control group
**Estimated by RAC, not provided by the DS, no available statistical analysis
$ No contextual information about this HCD was provided

In Han Wistar rats there was no evidence of valifenalate-related carcinogenicity up to and including the limit dose level for carcinogenicity studies of 1000 mg/kg/day (Table 11). In CD-1 mice valifenalate induced hepatocellular adenomas and carcinomas in males. The incidence of these tumours in males and females given 850 or 5000 ppm exceeded the background range in studies performed at this facility (Table 11). For males, at 850 ppm the incidence of adenoma and carcinoma was 28 and 8% respectively, and at 5000 ppm the incidences were 32 and 20%, respectively. The incidences of adenomas exceeded the historical control range at both dose levels. However, the incidence of carcinomas in males at 850 ppm was within the reported historical control incidence. In female mice, valifenalate appeared to be less potent with a smaller, but statistically significant, increase in adenomas only being reported at a dose level of 5000 ppm. The incidence of adenoma was 4 and 10% at 850 and 5000 ppm, respectively. At both dose levels, this incidence was outside the historical control incidence.

Investigative study: Comparison of C57BL/6 mice and CD1 mice to determine if C57BL/6 mice are a suitable strain for a subsequent study in peroxisome proliferator-activated receptor-alpha (PPARα) knock out mice derived from C57BL/6 strain (confidential study number 68)

Two strains of mice (5 males/group) were fed with 7000 ppm valifenalate (purity 99.68%) in diet during days. Several hepatic parameters were determined and compared with controls

of respective strain non-exposed to valifenalate. The results are shown below:

	CD1	**C57Bl/6**
Absolute Liver weight	↑ 19.5%	↑ 13.8%
Relative liver weight	↑ 21%	↑ 16%
PCoA oxidation	↑ 1.6 fold	↑ 1.9 fold
Hepatic pentoxyresorufin-O-dependentylation (PROD)	↑ 2.1 fold	↑ 3.4 fold
Hepatic 12-hydroxylauric acid	↑ 4.9 fold	↑ 7.1 fold

Overall, the DS concluded that the response in both strains was very similar. It was concluded that the C57BL/6 mouse strain is an appropriate background strain for further investigations using the PPARα knockout model

Investigative study: Comparison of response in PPARα knockout mice with wild type controls (confidential study number 69)

C57BL/6 wild type and PPARα knock out CD1 mice (10 males/group) were fed with 7000 ppm valifenalate (purity 99.68%) in diet during 7 and 14 days. Several hepatic parameters were determined and compared with controls of respective strains non-exposed to valifenalate. The results are shown below:

	C57BL/6 wild type		**PPARα knock out CD1**	
	7 days	14 days	7 days	14 days
S-phase	↑ 8.2 fold	↑ 3.5 fold	↑ 5.4 fold	↑ 1.9 fold
Liver pathology:				
↑ minimal to mild centrilobular	10/10	-	2/10	-
hypertrophy	-	10/10	-	-
moderate centrilobular hypertrophy	-	6/10	-	-
increased mitosis				
PCoA oxidation	-	↑ 2.0 fold	-	↑ 1.3 fold
Acox1 mRNA	-	↑ 1.8 fold	-	↑ 1.3 fold
12-hydroxylauric acid levels	-	↑ 7.7 fold	-	↑ 4.0 fold
Cyp2b10 mRNA level	-	↑ 50 fold	-	↑ 50 fold
PROD activity	-	↑ 6.0 fold	-	↑ 7.1 fold
Cyp3a11 mRNA levels	-	↑ 6.3 fold	-	↑ 8.5 fold

Overall, the DS concluded that PPARα pathway is responsible for a portion of the hepatic response, and additional mechanisms mediated by CAR and PXR activation are also involved. RAC also notes that, despite hepatocellular hypertrophy was clearly lower in knock-out mice than in wild mice, there was no significant differences between the wild type and knock out mice in the level of expression of the biomarker of activation of PPAR receptor (Acox1 mRNA level). Moreover, RAC also notes that the level of activation of CAR (Cyp2b10 mRNA level) and PXR (Cyp3a11 mRNA levels) was quite comparable.

Investigative study: Investigate the potential of valifenalate to activate CAR and/or PPARα nuclear hormone receptors and stimulate cell proliferation in isolated hepatocytes (confidential study number 70)

Mouse hepatocytes from CD1 strain were exposed to valifenalate (purity 99.68%), phenobarbital and WY-14.643 as positive controls. Valifenalate 300 µM (a concentration able to reduce the ATP levels by 74%) and also 100 µM (a non-cytotoxic concentration) caused no impact on any of the biochemical marker assessed. However, the positive controls increased DNA synthesis, the mRNA levels of Cyp2b10, Cyp4a10, Cyp4a14c, Cyp4a10, Cyp4a14, Cyp2b10 and Acox1, PCoA oxidation and PROD activity.

Overall, the DS concluded that valifenalate does not activate either mouse CAR or PPARα when assessed *in vitro* as demonstrated by the lack of hypertrophic and hyperplasic responses in the CD-1 mouse hepatocytes.

Investigative study: Investigation of mechanism of possible liver toxicity. Assessments included cell proliferation, CYP enzymes (activity and/or mRNA expression), peroxisomal β-oxidation, catalase histochemistry and oxidative stress (TBARS) (confidential study number 66)

Crl:CD-1 mice (18 males/group) were dosed with 21, 249 and 1050 mg/kg bw/day valifenalate (purity 97.83%) or phenobarbital as positive control during 14 days. Several hepatic parameters were determined and compared with controls of respective strains non-exposed to valifenalate. The results are shown below:

	Dose valifenalate (mg/kg bw/day)		
	21	249	1050
Cyp4a-1 enzyme sub family (Lauric acid 12-hydroxylase)	No effects	↑ 408%	↑ 1106%
Peroxisomal β-oxidation	No effects	↑ 208%	↑ 308%
Relative liver weight	No effects	↑ 13%	↑ 35%
Hepatocellular hypertrophy	No effects	4/6	6/6
Cyp1a1 mRNA level	↓ 0.8 fold	↑ 1.2 fold	↑ 1.2 fold
Cyp1a2 mRNA level	↓ 0.7 fold	↓ 0.8 fold	↓ 0.3 fold
Cyp2b10 mRNA level	↑ 1.6 fold	↑6.2 fold	↑20
Cyp3a11 mRNA level	↑ 1.1 fold	↑ 6.1 fold	↑9.5 fold
Catalase	↑ 6% fold	↑ 12%	↑ 16%

	Dose phenobarbital (mg/kg bw/day)
	130
Cyp 2B10 mRNA level	↑223 fold
Cyp3a11 mRNA level	↑12 fold
Cyp1a1 mRNA level	↑3.6 fold
Cyp1a2 mRNA level	↑2.9 fold
Peroxisomal β-oxidation	No increase
Relative liver weight relative to body weight	↑ 55% by day 3, 37% by day 14
Hepatocellular hypertrophy	↑ 6/6 after 3 and 14 days, severity more marked after 14 days
Catalase	No increase

Overall, the DS concluded that valifenalate appears as moderate and dose dependent liver enzyme inducer of the peroxisomal-proliferator type and that the mode of action as a liver enzyme inducer of the polycyclic aromatic hydrocarbon-, steroid-, or phenobarbital-type can be excluded.

Summary of mechanistic studies on liver effects

The data from these studies have been considered in detail by the DS (see Annex II to the CLH-report) and were summarised below in the section Supplemental information. These mechanistic studies allowed considering a mode of action for the carcinogenic effects of valifenalate with an initiating event based on the co-activation of multiple nuclear receptors, CAR/PXR/PPARα, and as a direct consequence, the associated induction of gene expression and enzyme activity of Cyp2b10, Cyp3a11 and Cyp4a.

The second key event is the increased hepatocellular proliferation and is also initiated in CD-1 mice exposed to valifenalate, on a time scale not dissimilar to the appearance of induction of the hepatic metabolising enzymes.

The final key event is the longer-term formation of carcinomas via the development of altered, hyperplastic, hepatic, foci and the subsequent development of benign and, ultimately, malignant hepatocellular neoplasms.

Comparison with the criteria

Classification in category 1A concerns substances known to have carcinogenic potential for humans and is largely based on human evidence. Since there are no human data it cannot be concluded that valifenalate has known carcinogenic potential for humans; therefore Category 1A is not applicable.

Category 1B is for substances with sufficient evidence of carcinogenic potential for humans. For that, increases incidences of malignant neoplasms or an appropriate combination of benign and malignant neoplasms in (a) two or more species of animals or (b) two or more independent studies in one species carried out at different times or in different laboratories or under different protocols. An increased incidence of tumours in both sexes of a single species in a well-conducted study, ideally conducted under GLP, can also provide sufficient evidence. In the case of valifenalate, the database contains one study showing increment of malignant lesions in a single species and sex and therefore the conditions for category 1B are not met.

Category 2 is reserved for substances with evidences of carcinogenicity not sufficiently convincing to place the substance in Category 1A or 1B and can be set if the evidence of carcinogenicity is restricted to a single experiment, as is the case of valifenalate.

A full range of investigative studies was included in the CLH-dossier to determine the mode of action of valifenalate in the mouse. These experiments show that liver effects are initiated by activation of receptors CAR, PXR and PPARα and it was concluded that these effects were not likely to occur in humans on a quantitative basis.

RAC recognises that the mechanism of action proposed by the DS (nuclear receptor activation → increase of replicative DNA synthesis → hypertrophy → carcinogenesis) is plausible. However, RAC also notes that the database is not robust enough for rule out the relevance of valifenalate-induced hepatocarcinomas in humans. RAC notes the following concerns:

- Weak (up to 3.6 times) increases in the expression of Cyp1a1 and Cyp1a2 were reported after dosing CD-1 mice for 14 days with 850 ppm valifenalate (Table A1 in Annex 2 to the CLH-report); while the level of expression of these Cyp at 7000 ppm (dose at which most of other mechanistic studies were performed) is unknown. It suggests that a potential role of AhR in the mechanism of action cannot be totally

ruled out.

- Inconsistencies detected in the study with PPAR-α mice, where, moreover, lack of positive control was detected
- Lack of data with CAR/PXR knock-out mice
- Lack of data with human hepatocytes
- Fails in the valifenalate to induce *in vitro* changes in biochemistry of hepatocytes without evidences that hepatocytes were not metabolically competent
- Cytoplasmic eosinophilia in hepatocytes in the 1.5-year study in mouse, in the 28-days and 90-days toxicity studies in dogs; hepatocyte and liver macrophage pigmentation in the 1.5-year study in mouse; liver cell necrosis in the 28-day study in dogs and pale cytoplasm in dog hepatocytes in the in the 90-day study and 52-week study suggest cytotoxicity; which could be a carcinogenic mode of action alternative to the proposed PPAR activation.

Overall, there is insufficient evidence to support the non-relevance of the observed liver tumours for humans and therefore RAC supports the **classification of valifenalate as Carc. 2, H351; "Suspected of causing cancer".**

Supplemental information - In depth analyses by RAC

In this section, a summary of the information contained in the Annex 2 of the CLH-report entitled "Valifenalate: Mode of Action Analysis using the WHO/IPCS Mode of Action Framework" is presented:

1 Bradford Hill Considerations for Weight of Evidence Analysis

The Annex 2 contains a very detailed assessment of the Bradford Hill Considerations for Weight of Evidence Analysis of available data/information for Mode of Action Analysis in experimental species. As this regard, the dose response relationships and temporal association can be summarised as follows:

	Time				
Dose ppm (mg/kg bw/day)	**Initiating Event Activation of CAR/PXR/PPARα**	**Key Event 2 Increased replicative DNA synthesis**	**Associated event: Increased hepatocellular hypertrophy**	**Key Event 3 Formation of hepatocellular Carcinoma**	**Reference**
	Measured indirectly from Day 7	*Measured from Day 3*	*Measured from Day 3 to 90*	*Key event: Measured at 78 weeks*	
110 (15.3)			Negative in CD-1 strain of mouse 90 days		*See Annex* *conf. 50.*
150 (20.7)	Negative day 14 in male CD-1 strain of mouse	Negative day 3 and day 14 in CD-1 strain of mouse	Negative in CD-1 strain of mouse at 3 & 14 days		*See Annex conf. 66*
150 (16.8)			Negative in CD-1 strain of mouse at 78 weeks	Negativeweek 78 in CD-1 strain of mouse	*See Annex conf. 52.*

850 (97.2)			Positve in CD-1 strain of mouse at 78 weeks	Negative week 78 in CD-1 strain of mouse	*See Annex conf. 52.*
900 (133.7)			Positive in CD-1 strain of mouse at 90 day		*See Annex conf. 50.*
1750 (249)	Positive day 14 in male CD-1 strain of mouse	Positive day 3 in CD-1 strain of mouse Positive day 14 in CD-1 strain of mouse	Positive in CD-1 strain of mouse at 3 and 14 days		*See Annex conf. 66*
5000 (657)			Positive in CD-1 strain of mouse at 78 weeks	++ week 78 in CD-1 strain of mouse	*See Annex conf. 52.*
7000 (1049.5)		Positive day 3 in CD-1 strain of mouse Positive day 14 in CD-1 strain of mouse	Positive in CD-1 strain of mouse at 3 & 14 days		*See Annex conf. 66*
7000 (995)			Positive in CD-1 strain of mouse at 90 days		*See Annex conf. 50.*
7000 (1050)	Positive day 14 in male CD-1 strain of mouse	Positive day 14 in CD-1 strain of mouse			*See Annex conf 68*
7000 (1324-1636)	Positive day 7 in male C57BL/6 and C57BL/6 (PPARα KO) strains of mouse	Positive day 7 in C57BL/6 strain of mouse + day 7 in C57BL/6 (PPARα KO) strain of mouse	Positive in CD-1 strain of mouse at 7 & 14 days		*See Annex conf. 69*

In this weight of evidence analysis the DS summarised consistency, specificity and biological plausibility of the hypothesised Mode of Action as follows:

	Key Event: Co—activation of CAR/PXR/PPARα	***Key Event 2: Increased hepatocellular proliferation***	***Key Event 3: Formation of Carcinoma***
Consistency & Specificity	Significant evidence in short-term studies for activation of receptors from the 'fingerprint' of the induction of both gene expression and enzyme activities indicating activation of CAR/PXR/PPARα in the species and strain in which	Increased replicative DNA synthesis was dependent on dose and time. Present in short-term studies at dose levels where tumours were reported in long-term studies, and absent in short-term studies at non-carcinogenic dose levels in male CD-1 mice. In PPARα KO mice the extent of replicative DNA synthesis	There have been two guideline carcinogenicity studies with valifenalate: rats and mice. There was no evidence of carcinogenicity in rats.

	hepatocellular adenomas/carcinomas were reported. At dose-levels at which activation is absent in short-term studies, no tumours were reported in long-term studies. These short-term effects were present in another mouse strain (C57BL/6) used in investigative studies.	was reduced relative to the wild type strain. Residual proliferative activity was expected, due to the activation of CAR/PXR, in the absence of PPARα.	
Biological Plausibility	The role of nuclear receptor activation in the formation, in rodents, of hepatocellular adenomas and carcinomas has been much studied and is now a well-accepted mode of action. The relevance of activation of CAR/PXR/PPARα in these rodent findings to potential adverse human health continues to be investigated but is currently considered to be non-relevant for the carcinogenic outcome.	Detailed studies, reported in the scientific literature, on the development of rodent hepatic neoplasia have shown that induction of hepatocyte DNA synthesis is a critical precursor event in the development of hepatocellular adenomas and carcinomas. Such events may not be purely chemical-specific but occur after differing initiating events including cytotoxicity and nuclear receptor activation. Induction of hepatic DNA synthesis is a mode of action of relevance to the production of human hepatic cancer.	Following the induction of hepatic replicative DNA synthesis (2nd key event), the development of altered, hyperplastic, hepatic foci, and the subsequent development of benign and, ultimately, malignant hepatocellular neoplasms is a well-accepted mode of action for the formation of hepatocellular adenomas and carcinomas in rodents with potential relevance to humans.

The third step of the weight of evidence analysis was based on qualitative and quantitative human concordance and was summarised as follows:

Key Event (name)	***(Evidence in Experimental Species)***	***(Evidence in Humans)***	***Quantitative Species Concordance (experimental species and humans)***	***Quantitative Dose Response***	***Confidence / Uncertainty***
Key Event 1 ***Nuclear receptor activation***	Receptor activation (CAR/PXR/PPARα) confirmed in CD-1 mice through quantification of associated	No direct evidence in humans for valifenalate. However, from the study of other chemistries, it	There is no valifenalate-specific data in humans. For other compounds that activate the CAR/PXR/	The dose-response for receptor activation in mice has been ascertained	The correlation between markers for this initiating event in short term studies and

	events. The 'fingerprint' of the induction profile of both Cyp mRNA and enzyme activity is consistent with the above receptors being activated in CD-1 mice treated with valifenalate. This key event was also confirmed in two other strains (C57BL/6 and C57BL/6 KO strains) used in the investigative studies. From the breadth of data generated in mice, this is confirmed as the initiating event of the hypothesised mode of action.	is generally accepted that these nuclear receptors have the potential to be activated in humans. Experimental evidence would suggest that activation of these receptors can induce the hypertrophic but not the replicative hyperplastic response critical to the subsequent development of liver cancer.	PPARα receptors there are clear dose response relationships to receptor activation in animal and human hepatocyte studies *in vitro* whereby threshold doses exist below which nuclear receptor activation will not occur.	in short-term studies. This activation, correlates well with effect and no-effect information from the carcinogenicity study in the same mouse strain. This evidenced-based information for valifenalate is entirely consistent with the initiating event of the hypothesised mode of action.	the final key event, derived from the carcinogenicity study in CD-1 mice, is well-founded. The data generated in short-term studies to confirm receptor activation derives from well-accepted associated events. There is no direct information on valifenalate relating to this key event in humans but it is assumed to be plausible by comparison with other chemicals that activate the three nuclear receptors. Overall, there is a high degree of confidence in this information in mice and an assumption of plausibility in humans.
Key Event 2 Increased hepatocellular proliferation	Increased replicative DNA synthesis has been measured, directly, in short term studies in the CD-1 strain of mice. This key event was also confirmed in two other strains (C57BL/6 and	No direct evidence in humans for valifenalate. For other CAR/PXR and PPARα agonists *in vitro* and *in vivo* evidence has shown the hypertrophic	From experimental animal studies on Wy- 14,643 and phenobarbital (and including human use of phenobarbital) and other chemistries (some in	The dose-response for the induction of replicative DNA synthesis in mice has been ascertained in short-	The correlation between this key event in short and long term studies in CD-1 mice is well-founded based on experimental evidence.

	C57BL/6 (PPARα KO) strains) used in the investigative studies.	but not the hyperplastic consequences of nuclear receptor interaction	hepatocyte cultures), it is generally accepted that this key event is either not activated in humans, or at least activated to a significantly less extent in human cells *in vitro.*	term studies. This activation, (as for key event 1) correlates well with effect and no-effect information from the carcinogenicity study in the same mouse strain (i.e. the third key event). In contrast, it is generally accepted that this key event is either not activated in humans, or activated to a significantly less extent in humans	There is no direct information for valifenalate relating to equivalent events in humans*, in vitro or in vivo*. Indirect evidence, relating to experimental investigations with other chemistries, with a similar mode of action in humans, has been used. Overall, there is a high degree of confidence from evidence-based information in mice. In the absence of direct experimental evidence for valifenalate in humans there is high degree of confidence, based on evidence from other chemistries with a similar mode of action
Key Event 3 Formation of hepatocellular carcinomas	Carcinogenicity studies in rats and mice reported valifenalate to be carcinogenic in mice, particularly males. There was no evidence of carcinogenicity in rats.	No evidence in humans for valifenalate.	There is no valifenalate-specific data in humans. Experimental data with other chemicals that work through activation of these nuclear receptors where the human orthologue of the respective nuclear receptor	The incidence of hepatocellular adenoma and carcinoma in males was dose-related with increased tumour incidences	The guideline carcinogenicity study was well executed and reported. The absence of tumours at the exposure level correlates well with the

			had been inserted into the mouse genome has shown that while the hypertrophic response continues to be seen, the hyperplastic, DNA replicative response, seen with the intact rodent orthologue of the nuclear receptors, is missing from those mice given the human receptors. This data strongly suggests that such a nuclear receptor mode of action (CAR/PXR/PPARα) is unlikely to be relevant to humans	at the top and middle dose-levels. For males exposed to valifenalate at the lower dose-level, there was no increased incidence over control. Female animals had a small but statistically significant increased incidence of adenomas. In contrast, it is generally accepted that such a nuclear receptor mode of action (CAR/PXR/PPARα) is unlikely to be relevant to humans	absence of key events 1 and 2 from short-term studies. Overall, there is a high degree of confidence in this evidence-based information in experimental animals. In the absence of direct experimental evidence for valifenalate in humans there is medium degree of confidence, based on evidence from other chemistries with a similar mode of action

2 The relevance to humans of valifenalate-mediated activation of PPARα and CAR/PXR

The DS reviewed in this Annex the scientific literature as this regard concluding that the evidence indicates that valifenalate acts as a co-activator of CAR/PXR and PPARα and, after taking kinetic and dynamic factors, such as the differential expression of hyperplastic and hypertrophic responses into account, any hepatocellular carcinomas developed through activation of these nuclear receptors by valifenalate in mice, is not likely to occur in humans.

3 Other potential modes of action

The Annex 2 to the CLH-report also assessed other potential modes of action described in the literature for hepatic carcinogenicity. The conclusions of the DS as this regard are summarised below:

3.1 AhR-mediated carcinogenesis:

For valifenalate, an AhR-mediated mode of action for the formation of adenomas and carcinomas in CD-1 mice can be ruled out since neither gene expression nor enzyme activity of hepatic Cyp1a was induced in CD-1 mice exposed to valifenalate at any dose level. Therefore, AhR is not activated at dose levels where valifenalate induces hepatocellular

carcinomas, and does not induce these effects, in male CD-1 mouse.

3.2 Direct reactivity with DNA:

It has been clearly established from a panel of guideline *in vitro* and *in vivo* studies that valifenalate is not genotoxic and hence such a mode of action is not relevant to valifenalate.

3.3 Oestrogen-mediated:

There is no structural similarity between valifenalate and oestrogen that might suggest a similar mode of action of hepatocarcinogenesis and there was no evidence of oestrogenic activity in the guideline two-generation toxicity study in the rat.

3.4 Cytotoxicity-mediated:

Cytotoxicity has been associated with exposure to high levels of valifenalate in (oral, capsule) dog studies, however these effects were not evident after a recovery period. There is no indication of carcinogenic potential in the dog. With respect to cytotoxicity as an alternative mechanism for carcinogenicity in the mouse, all relevant information from rat, mouse and dog studies is entirely consistent with the hypothesised mode of action of carcinogenicity in mice and clearly indicates that cytotoxicity is unlikely to play a role in the carcinogenicity of valifenalate in CD-1 mice.

10.10 Reproductive toxicity

10.10.1 Adverse effects on sexual function and fertility

Table 41: Summary table of animal studies on adverse effects on sexual function and fertility

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
Two generation reproduction (one litter) OECD 416 (2001) GLP Oral (continuous in diet) Rat, HanBrl:WIST 24/sex/group	Valifenalate (IR5885, lot no. T025/02, purity 99.56%) 0, 1250, 4300 or 15000 ppm (reduced to 0, 850, 2900 or 10000 ppm during lactation) Vehicle: laboratory animal diet	***Parental toxicity*** 15000 ppm (10000 ppm) – 986/1150 mg/kg bw/day, males/ females (P generation - pre-pairing) P: ↑ absolute liver weight (males 16%, females 15%); ↑ relative liver weight (males 20%, females 11%); ↑ liver hepatocellular hypertrophy (males 15/24 severity 2.4 cf. 4/24 controls severity 1.3), (females 3/24 severity 2.0 cf. 0/24 controls); ↓ glycogen deposition liver (males 17/24 severity 1.3 cf. 21/24 controls severity 1.6: females 15/24 severity 1.3 cf. 15/24 controls severity 2.3) considered adaptive and not adverse; ↑ severity of renal tubular hyaline change in males (3.4 cf. 2.3 controls) (rat specific effect) F1: 4/24 females with ruffled fur early lactation; ↓ food consumption days 1-7 lactation (19%); ↑ absolute liver weight (males 12%, females 7.5%); ↑ relative liver weight (males 14%, females 10%); ↓ absolute kidney weight (females 7.4%); ↓ relative kidney weight (females 5,6%); ↑ liver hepatocellular hypertrophy (males 21/24 severity 2.2 cf. 2/24 controls severity 2.0), (females 21/24 severity 1.9 cf. 0/24 controls); ↓ glycogen deposition liver (males 19/24 severity 1.5 cf. 23/24 controls severity 2.7: females 2/24 severity 1.0 cf. 13/24 controls severity 1.8) considered adaptive and not adverse; ↑ severity of renal	*See Annex conf. 27.*

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		tubular hyaline change in males (2.3 cf. 1.6 controls) (rat specific effect); ↑ thyroid follicular hypertrophy (males 22/24 severity 2.1 cf. 17/24 controls severity 1.4: females 19/24 severity 1.6 cf. 10/24 controls severity 1.1) 4300 ppm (2900 ppm) – 277/318 mg/kg bw/day, males/ females (P generation - pre-pairing) P: ↑ absolute liver weight (females 6%); ↑ relative liver weight (males 8.5%); ↑ liver hepatocellular hypertrophy (males 7/24 severity 1.3 cf. 4/24 controls severity 1.3); ↓ glycogen deposition liver (males 17/24 severity 1.3 cf. 21/24 controls severity 1.6: females 17/24 severity 1.8 cf. 15/24 controls severity 2.3) considered adaptive and not adverse; ↑ severity of renal tubular hyaline change in males (2.8 cf. 2.3 controls) (rat specific effect) F1: 4/24 females with ruffled fur early lactation; ↓ food consumption days 1-7 lactation (15%); ↑ absolute liver weight (males 6%); ↑ relative liver weight (males 8%); ↑ liver hepatocellular hypertrophy (males 17/24 severity 2.3 cf. 2/24 controls severity 2.0); ↓ glycogen deposition liver (males 23/24 severity 1.9 cf. 23/24 controls severity 2.7: females 7/24 severity 1.4 cf. 13/24 controls severity 1.8) considered adaptive and not adverse; ↑ severity of renal tubular hyaline change in males (2.2 cf. 1.6 controls) (rat specific effect); ↑ thyroid follicular hypertrophy (males 16/24 severity 1.8 cf. 17/24 controls severity 1.4) 1250 ppm (850 ppm) – 80/92 mg/kg bw/day, males/ females (P generation - pre-pairing) P: No treatment related effects F1: No treatment related effects **NOAEL parental toxicity: 80 mg/kg bw/day** ***Reproductive toxicity*** 15000 ppm (10000 ppm) – 986/1150 mg/kg bw/day, males/ females (P generation - pre-pairing) P: No treatment related effects F1: Some differences from control but see text below section 10.10.2 ↑ neonatal pup mortality (15.2% cf. control 7.4%); ↓ viability indices (84.8% cf. control 92.6%); ↑ pup mortality (10 pups/group cf. control 4 pups/group; ↓ weaning indices (93.9% cf. control 97.5%). No treatment related effects 4300 ppm (2900 ppm) – 277/318 mg/kg bw/day, males/ females (P generation - pre-pairing) P: No treatment related effects F1: ↑ neonatal pup mortality (14.8% cf. control 7.4%); ↓ viability indices (85.2% cf. control 92.6%); ↑ pup mortality (9 pups/group cf. control 4 pups/group; ↓ weaning indices (94.2% cf. control 97.5%) 1250 ppm (850 ppm) – 80/92 mg/kg bw/day, males/ females (P generation - pre-pairing) P: No treatment related effects F1: No treatment related effects	

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		NOAEL reproductive toxicity : 986 mg/kg bw/day NB. Study report suggests a NOAEL of 80 mg/kg bw/day based on an effect on post-implantation loss and reduced neonatal viability and weaning index at the mid and high dose levels, F1 only. This is due to the inclusion of 3 litters with total litter loss at the mid dose and 1 litter at the high dose. There is no evidence for the total litter losses being treatment-related. Exclusion of these litters from the calculated mean values confirms the lack of effect on the viability and survival of the offspring. See text below section 10.10.2 **NOAEL reproductive toxicity: 986 mg/kg bw/day** ***Offspring toxicity*** 15000 ppm (10000 ppm) – 986/1150 mg/kg bw/day, males/ females (P generation - pre-pairing) F1a: No treatment related effects F2a: ↓ pup weight gain (8% days 0-21); ↓ absolute spleen weights (males 18%, females 23%), ↓relative spleen weights (males 12%, females 17%) without histological correlate; ↓ glycogen deposition liver (males 18/22 severity 1.5 cf. 20/20 controls severity 2.5: females 14/21 severity 1.3 cf. 20/21 controls severity 1.7) considered not adverse 4300 ppm (2900 ppm) – 277/318 mg/kg bw/day, males/ females (P generation - pre-pairing) F1a: No treatment related effects F2a: ↓ pup weight gain (9% days 0-21); ↓ absolute spleen weights (males 26%, females 25.5%), ↓relative spleen weights (males 20%, females 17%) without histological correlate; ↓ glycogen deposition liver (males 16/19 severity 2.1 cf. 20/20 controls severity 2.5: females 14/18 severity 1.6 cf. 20/21 controls severity 1.7) considered not adverse 1250 ppm (850 ppm) – 80/92 mg/kg bw/day, males/ females (P generation - pre-pairing) F1a: No treatment related effects F2a: No treatment related effects **NOAEL offspring toxicity: 80 mg/kg bw/day**	

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

The reproductive toxicity of valifenalate (IR5885) was investigated in a two generation reproduction toxicity study in rats (*See Annex conf. 27.*). The study was conducted according to the current OECD Test Guideline Number 416 (2001). Systemic toxicity was observed in parents and offspring at the mid and high doses with a NOAEL of approximately 80 mg/kg bw/day.

The NOAEL for reproductive toxicity was 80 mg/kg bw/day based on increased neonatal loss, reduced viability indices and increased pup mortality in the F1 litters in the mid and high dose. However, consideration of the data showed that the apparent effect was attributable to the inclusion of animals with total litter loss in the calculation of the group mean values. The data are presented including and excluding the animals with total litter loss, 3 in the mid dose group and 1 in the high dose group. The data are presented

in Table 42. The evidence is not sufficient however to signal a specific primary toxic effect of valifenalate on the reproduction, the observed signs may be considered as secondary to the maternal effects.

Table 42: Summary table of litter data in F1 animals – selected parameters

F1 Group	Control	Low dose	Mid dose	High dose	HCD$
All dams giving birth	N = 21	N = 23	N = 23	N = 23	21-24
Mean no. implantations	12.7	12.2	12.0	12.6	12.2-13.8
% Post-implantation loss	8.6	11.4	14.2	11.4	@
Mean post implantation loss/female	1.1	1.4	1.7	1.4	0.6-1.7
Mean no. dead pups at 1st check	0.0	0.2	0.8	0.0	0-0.5
Mean no. live pups at 1st check	11.6	10.8	10.3	11.1	10.5-12.6
% Postnatal loss days 0-4	7.4	3.2	14.8	15.2	0-8.5
Mean postnatal loss days 0-4	0.9	0.3	1.5	1.7	0-1.0
% Viability index	92.6	96.8*	85.2**	84.8**	91.5-100
% Weaning index	97.5	99.4	94.2	93.9	88.5-100
All dams weaning young	N = 21	N = 23	N = 19	N = 22	21-24
Mean no. implantations	12.7	12.2	11.5	12.4	12.2-13.8
% Post-implantation loss	8.6	11.4	9.1	12.1	@
Mean post implantation loss/female	1.1	1.4	1.1	1.5	0.6-1.7
Mean no. dead pups at 1st check	0.0	0.2	0.2	0.0	0.0-0.5
Mean no. live pups at 1st check	11.6	10.8	10.5	10.9	10.5-12.6
% Postnatal loss days 0-4	7.4	3.2	5.5	9.6	0-8.5
Mean postnatal loss days 0-4	0.9	0.3	0.6	1.7	0-1.0
% Viability index	92.6	96.8*	94.5	90.4	91.5-100
% Weaning index	97.5	99.4.	98.6	93.9	84.5-100

*/** statistically significant difference from control at 5% /1% level
$ Historical control range for 10 studies initiated from May 2002 to December 2007 (current study initiated November 2002) taken from data provided in Annex III.
@ not available

These data clearly demonstrate that the apparent increase in post-implantation loss / neonatal loss is attributable to the inclusion of the animals with total litter loss. The occurrence of the total litter losses is also considered to be incidental to treatment given the lack of dose response, the absence of pup death amongst surviving litters and the lack of a similar effect in the P litters. In addition, values for post implantation loss and post-natal loss pre-cull in dams weaning young are in line with the historical control data (F1 parents, F2 litters) for 10 studies conducted within a 5 year period in the same laboratory and with the same strain of rat as the valifenalate study. It is therefore concluded that valifenalate has no adverse effect on pup survival in utero or post partum.

In the absence of any effect of valifenalate on oestrus cyclicity, sperm parameters, mating performance, fertility index, gestation duration, the number of implantations, live pup weight at birth together with no clear effect on pup viability, the NOAEL for reproductive toxicity is considered to be 986 mg/kg bw/day, the highest dose tested, and unaffected by the presence of systemic toxicity in the parental generations.

In the classification system, reproductive toxicity is subdivided under two main headings:

(a) Adverse effects on sexual function and fertility

Any effect of substances that has the potential to interfere with sexual function and fertility. This includes, but is not limited to, alterations to the female and male reproductive system, adverse effects on onset of puberty, gamete production and transport, reproductive cycle normality, sexual behaviour, fertility, parturition, pregnancy outcomes, premature reproductive senescence, or modifications in other functions that are dependent on the integrity of the reproductive systems.

(b) Adverse effects on development of the offspring.

Developmental toxicity includes, in its widest sense, any effect which interferes with normal development of the conceptus, either before or after birth, and resulting from exposure of either parent prior to conception, or exposure of the developing offspring during prenatal development, or postnatally, to the time of sexual maturation.

There were no adverse effects on sexual function and fertility or on development of the offspring in the rat, no classification of valifenalate is warranted as a known, presumed or suspected human reproductive toxicant.

10.10.3 Adverse effects on development

Table 43: Summary table of animal studies on adverse effects on development

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
Developmental toxicity OECD 414 (2001) GLP Oral (gavage) Rat, Crl:CD(SD)BR 25 mated females/group	Valifenalate (IR5885, lot no. FCF/T/18000 (ex ZI-068), purity 98.9%) 0, 100, 300 and 1000 mg/kg bw/day Dosing on gestation days 6-19 Vehicle: 0.5% MC	***Maternal toxicity*** 1000 mg/kg bw/day: No treatment related adverse effects Maternal NOAEL 1000 mg/kg bw/day ***Developmental toxicity*** 1000 mg/kg bw/day: No treatment related adverse effects Developmental NOAEL 1000 mg/kg bw/day	*See Annex conf. 9.*
Developmental toxicity OECD 414 (2001) GLP Oral (gavage) Rabbit, NZW (HY/CR) 22 mated females/group	Valifenalate (IR5885, lot no. FCF/T/18000 (ex ZI-068), purity 98.9%) 0, 100, 300 and 1000 mg/kg bw/day Dosing on gestation days 6-28 Vehicle: 0.5% MC	***Maternal toxicity*** 1000 mg/kg bw/day: No treatment related adverse effects Maternal NOAEL 1000 mg/kg bw/day ***Developmental toxicity*** 1000 mg/kg bw/day: No treatment related adverse effects Developmental NOAEL 1000 mg/kg bw/day	*See Annex conf. 10.*

Table 44: Summary table of human data on adverse effects on development

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No human data available on developmental toxicity				

Table 45: Summary table of other studies relevant for developmental toxicity

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No other studies available on developmental toxicity				

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

The developmental toxicity of valifenalate (IR5885) was investigated in two prenatal developmental toxicity studies, one in rats (*See Annex conf. 9.*) and one in rabbits (*See Annex conf. 10.*). Both studies were conducted according to the current OECD Test Guideline Number 414 (2001). In addition, both studies utilised the limit dose of 1000 mg/kg bw/day as the highest dose level. No treatment related adverse maternal effects were observed at any dose level in the rat or the rabbit. Furthermore, no treatment related adverse effects on foetal development were observed and there was no evidence of teratogenicity in either species.

The report of the study in rats (*Annex conf. 9*) provides historical control data relevant to the Charles River Sprague Dawley rat in developmental toxicity studies from the same source and conducted at the same laboratory as the reported study. This comprises 10 studies conducted in the years 1996-2000. However, no treatment-related differences from the concurrent control were identified in any of the reproductive parameters or in the foetal observations. All values were within the historical control range and close to the HC mean. The text table below gives data for key parameters.

Parameter	Dose level (mg/kg bw/day)				HC mean	HC range
	0 (control)	100	300	1000		
Corpora lutea	17.32	17.95	17.77	17.43	18.247	6-30
Implantations	14.63	15.27	14.45	14.67	15.173	0-23
Pre-implantation losses	14.48	14.95	17.48	16.04	16.505	0-100
Post implantation losses	6.81	5.08	4.16	4.64	6.483	0-100
Mean foetal weight (g)	3.97	3.97	4.00	3.96	3.723	1-5.901
Foetuses with external malformations	0/259	0/320	1/305	0/293	0.018	0-1
Foetuses with skeletal malformations	0/130	0/159	0/154	0/147	0.060	0-7
Foetuses with visceral malformations (Wilsons)	0/129	0/161	0/150	0/146	0.018	0-1

The report of the study in rabbits (*Annex conf. 10*) provides historical control data relevant to the New Zealand White rabbit in developmental toxicity studies from the same source and conducted at the same laboratory as the reported study. This comprises studies conducted in the years 1995-2000 with litters from a total of 205 dams. Of these 125 were examined for skeletal malformations and 123 for visceral malformations using the Wilson technique. No treatment-related differences from the concurrent control were identified in any of the reproductive parameters or in the foetal observations. All values were within the

historical control range and close to the HC mean. The text table below gives data for key foetal observations.

Parameter	Dose level (mg/kg bw/day)				HC mean	HC range
	0 (control)	100	300	1000		
Corpora lutea	8.27	9.71	9.81	9.33	10.005	4-17
Implantations	6.67	7.86	7.94	7.07	8.471	3-14
Pre-implantation losses	20.40	18.92	18.94	25.33	15.191	0-70
Post implantation losses	6.45	10.03	4.11	8.43	9.573	0-100
Dead foetuses A	0/100	9/127**	8/135*	5/106*	0.048	0-3.0
Dead foetuses B	0/100	0/110	0/127	5/106*	0.048	0-3.0
Mean foetal weight (g)	48.04	46.38	46.02	46.43	44.621	24.38-58.65
Foetuses with external malformations	0/92	0/98	0/122	1/97	0.015	0-1
Foetuses with skeletal malformations	0/92	0/98	0/122	2/97	0.096	0-1
Foetuses with visceral malformations (Wilsons)	0/32	0/31	0/40	0/31	0.008	0-1

(A) Includes all litters
Includes only viable litters
*/** statistically significant difference from control at 5% /1% level

External malformations comprised one foetus with arthrogryoposis (1000 mg/kg bw/day group) and one foetus with missing testis (100 mg/kg/kg/day group). Skeletal malformations comprised one foetus with scoliosis and one foetus with misshapen sternum (both 1000 mg/kg bw/day). These single incidence findings are considered not to be related to treatment.

A statistically significantly higher frequency per group of dead foetuses was observed in all treated groups without any dose-relationship. Dead foetuses were present in only 1 litter in each group. In the 100 and 300 mg/kg bw/d group all foetuses were dead from litters 33 and 47 respectively which is reflected in the high value for dead foetuses (A). In the 1000 mg/kg bw/d group 5 of 9 foetuses in litter 72 were dead. Only females with live foetuses were included in the calculation of reproductive parameters (B).

10.10.5 Comparison with the CLP criteria

In the classification system, reproductive toxicity is subdivided under two main headings:

(a) Adverse effects on sexual function and fertility

Any effect of substances that has the potential to interfere with sexual function and fertility. This includes, but is not limited to, alterations to the female and male reproductive system, adverse effects on onset of puberty, gamete production and transport, reproductive cycle normality, sexual behaviour, fertility, parturition, pregnancy outcomes, premature reproductive senescence, or modifications in other functions that are dependent on the integrity of the reproductive systems.

(b) Adverse effects on development of the offspring.

Developmental toxicity includes, in its widest sense, any effect which interferes with normal development of the conceptus, either before or after birth, and resulting from exposure of either parent prior to conception, or exposure of the developing offspring during prenatal development, or postnatally, to the time of sexual maturation.

In rat and rabbit prenatal developmental toxicity studies of valifenalate, no treatment related maternal toxicity was demonstrated at the limit dose of 1000 mg/kg bw/day and there was no evidence of developmental toxicity or of teratogenicity in either species. There were no treatment related adverse effects on sexual function and fertility or on development of the offspring in the rat to warrant classification of valifenalate as a known, presumed or suspected human reproductive toxicant.

There were no effects to warrant classification of valifenalate as a developmental toxicant.

10.10.6 Adverse effects on or via lactation

Table 46: Summary table of animal studies on effects on or via lactation

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
Two generation reproduction (one litter) OECD 416 (20010 GLP Oral (continuous in diet) Rat, HanBrl:WIST 24/sex/group	Valifenalate (IR5885, lot no. T025/02, purity 99.56%) 0, 1250, 4300 or 15000 ppm (reduced to 0, 850, 2900 or 10000 ppm during lactation) Vehicle: laboratory animal diet	***Parental toxicity*** 15000 ppm (10000 ppm) – 986/1150 mg/kg bw/day, males/ females (P generation - pre-pairing) P: ↑ absolute liver weight (males 16%, females 15%); ↑ relative liver weight (males 20%, females 11%); ↑ liver hepatocellular hypertrophy (males 15/24 severity 2.4 cf. 4/24 controls severity 1.3), (females 3/24 severity 2.0 cf. 0/24 controls); ↓ glycogen deposition liver (males 17/24 severity 1.3 cf. 21/24 controls severity 1.6: females 15/24 severity 1.3 cf. 15/24 controls severity 2.3) considered adaptive and not adverse; ↑ severity of renal tubular hyaline change in males (3.4 cf. 2.3 controls) (rat specific effect) F1: 4/24 females with ruffled fur early lactation; ↓ food consumption days 1-7 lactation (19%); ↑ absolute liver weight (males 12%, females 7.5%); ↑ relative liver weight (males 14%, females 10%); ↓ absolute kidney weight (females 7.4%); ↓ relative kidney weight (females 5,6%); ↑ liver hepatocellular hypertrophy (males 21/24 severity 2.2 cf. 2/24 controls severity 2.0), (females 21/24 severity 1.9 cf. 0/24 controls); ↓ glycogen deposition liver (males 19/24 severity 1.5 cf. 23/24 controls severity 2.7: females 2/24 severity 1.0 cf. 13/24 controls severity 1.8) considered adaptive and not adverse; ↑ severity of renal tubular hyaline change in males (2.3 cf. 1.6 controls) (rat specific effect); ↑ thyroid follicular hypertrophy (males 22/24 severity 2.1 cf. 17/24 controls severity 1.4: females 19/24 severity 1.6 cf. 10/24 controls severity 1.1) 4300 ppm (2900 ppm) – 277/318 mg/kg bw/day, males/ females (P generation - pre-pairing) P: ↑ absolute liver weight (females 6%); ↑ relative liver weight (males 8.5%); ↑ liver hepatocellular hypertrophy (males 7/24 severity 1.3 cf. 4/24 controls severity 1.3); ↓ glycogen deposition liver (males 17/24 severity 1.3 cf. 21/24 controls severity 1.6: females 17/24 severity 1.8 cf. 15/24 controls severity 2.3) considered adaptive and not adverse; ↑ severity of renal tubular hyaline change in males (2.8 cf. 2.3 controls) (rat specific effect) F1: 4/24 females with ruffled fur early lactation; ↓ food consumption days 1-7 lactation (15%); ↑ absolute liver weight (males 6%); ↑ relative liver weight (males 8%); ↑ liver hepatocellular hypertrophy (males 17/24 severity 2.3 cf. 2/24 controls severity 2.0); ↓ glycogen deposition liver (males 23/24	*See Annex conf. 27.*

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		severity 1.9 cf. 23/24 controls severity 2.7: females 7/24 severity 1.4 cf. 13/24 controls severity 1.8) considered adaptive and not adverse; ↑ severity of renal tubular hyaline change in males (2.2 cf. 1.6 controls) (rat specific effect); ↑ thyroid follicular hypertrophy (males 16/24 severity 1.8 cf. 17/24 controls severity 1.4) 1250 ppm (850 ppm) – 80/92 mg/kg bw/day, males/ females (P generation - pre-pairing) P: No treatment related effects F1: No treatment related effects **NOAEL parental toxicity: 80 mg/kg bw/day** ***Reproductive toxicity*** 15000 ppm (10000 ppm) – 986/1150 mg/kg bw/day, males/ females (P generation - pre-pairing) P: No treatment related effects F1: No treatment related effects **NOAEL reproductive toxicity : 986 mg/kg bw/day** *NB. Study report suggests a NOAEL of 80 mg/kg bw/day based on an effect on post-implantation loss and reduced neonatal viability and weaning index at the mid and high dose levels, F1 only. This is due to the inclusion of 3 litters with total litter loss at the mid dose and 1 litter at the high dose. There is no evidence for the total litter losses being treatment-related. Exclusion of these litters from the calculated mean values confirms the lack of effect on the viability and survival of the offspring. See text below section 10.10.2.* ***Offspring toxicity*** 15000 ppm (10000 ppm) – 986/1150 mg/kg bw/day, males/ females (P generation - pre-pairing) F1a: No treatment related effects F2a: ↓ pup weight gain (8% days 0-21); ↓ absolute spleen weights (males 18%, females 23%), ↓ relative spleen weights (males 12%, females 17%) without histological correlate; ↓ glycogen deposition liver (males 18/22 severity 1.5 cf. 20/20 controls severity 2.5: females 14/21 severity 1.3 cf. 20/21 controls severity 1.7) considered not adverse 4300 ppm (2900 ppm) – 277/318 mg/kg bw/day, males/ females (P generation - pre-pairing) F1a: No treatment related effects F2a: ↓ pup weight gain (9% days 0-21); ↓ absolute spleen weights (males 26%, females 25.5%), ↓ relative spleen weights (males 20%, females 17%) without histological correlate; ↓ glycogen deposition liver (males 16/19 severity 2.1 cf. 20/20 controls severity 2.5: females 14/18 severity 1.6 cf. 20/21 controls severity 1.7) considered not adverse 1250 ppm (850 ppm) – 80/92 mg/kg bw/day, males/ females (P generation - pre-pairing) F1a: No treatment related effects F2a: No treatment related effects	

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		NOAEL offspring toxicity: 80 mg/kg bw/day	

Table 47: Summary table of human data on effects on or via lactation

Type of data/report	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
No human data available on effects on or via lactation				

Table 48: Summary table of other studies relevant for effects on or via lactation

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
No other studies available on effects on or via lactation				

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

The two generation study of valifenalate (IR5885) in rats (*See Annex conf. 27.*). has already been described. The dietary concentrations were lowered for the lactation period in an attempt to maintain the level of test item intake. Nevertheless, mean achieved dose levels were increased above pre-pairing levels (approximately 124, 408 and 1384 mg/kg bw/day in the low, mid and high dose groups respectively cf. 80, 277 and 986 mg/kg bw/day). Parental toxicity was observed at mid and high doses in all generations. Increased neonatal loss, reduced viability indices and increased pup mortality was seen in the F1 litters in the mid and high dose. This is attributable to the inclusion of the animals with total litter loss (see text below section 10.10.2). There were no other treatment related adverse effects on the offspring. The reduction in F1 pup body weight gain was considered to result from direct consumption of the diet and not to be maternally mediated. There was no indication of impaired nursing behaviour during lactation. The results of the study do not indicate any direct, primary adverse effect on the offspring due to transfer of the chemical via the milk or to the quality of the milk.

10.10.8 Comparison with the CLP criteria

The classification is intended to indicate when a substance may cause harm due to its effects on or via lactation and is independent of consideration of the reproductive or developmental toxicity of the substance. There were no effects to warrant classification of valifenalate for effects on or via lactation.

10.10.9 Conclusion on classification and labelling for reproductive toxicity

CLP: Not classified (conclusive but not sufficient for classification).

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

DS proposed no classification of valifenalate for sexual function and fertility, development and lactation based on lack of effects detected in a 2-generation reproduction toxicity study, one developmental toxicity study in rats and one developmental toxicity study in rabbits.

Comments received during consultation

One MSCA supported the proposal of no classification for adverse effect on sexual function and fertility, development and lactation but demanded discussion about the effects on reproductive organs found in some of the repeated dose toxicity studies. The DS provided such discussion and the arguments (supported by RAC) are incorporated into the discussion below.

This same MSCA also requested discussion about the lack of *corpora lutea* and decreased absolute and ovary/brain ratio seen in the F1 parental generation from the high dose of the OECD TG 416 study. The DS replied that the lack of corpora lutea in the parental F1 generation of the 2-generation rat study cannot be confirmed because no difference between the high dose and control group occurred, which indicates a no test item-related effect. Likewise, the mentioned decreased absolute and ovary/brain ratios in the F1 parental generation from the high-dose group cannot be confirmed since the organ/body weight ratios of the ovaries were 0.021, 0.020, 0.022 and 0.020 (ovaries right) and the organ/brain weight ratios 3.177, 2.830, 3.039 and 2.854 (ovaries right) in the order of the ascending doses. They were clearly not affected by the treatment.

A second MSCA also commented that the exclusion of the litter with total loss of pups is not justified. This same MSCA demanded to incorporate into the CLH-report the incidence of the findings "no milk in stomach" as reported in the Annex 1. Finally, this MSCA also raised the opinion that a need for classification regarding developmental toxicity effects or effects on/via lactation because of reduced pup survival. The DS provided the data from litter with total loss (incorporated in the discussion below) and indicated that this data were initially removed because the incidence of dams with total litter loss were not dose-related; which suggests that a relationship with the treatment is very unlikely. Nevertheless, the inclusion of this data (see below) does not alter the main conclusion since no dose-response was observed for all assessed parameters and in most of the cases, the results at the top dose were covered by the HCD. As regard the finding "no milk in stomach" the DS highlighted that no clear dose-response in this parameter was noted with regard to the litter incidence and therefore these findings can be included within the biological variability. Overall, the DS considered that the discussed viability and weaning indices of the F1 generation would be within the HCD and is unlikely that the treatment had an effect on these parameters, especially considering the fact that no effects were detected in the P generation. Thus, the DS maintained the proposal of no classification for reproductive toxicity.

One manufacturer/company agreed with the DS's proposal for no classification.

Assessment and comparison with the classification criteria

Fertility and sexual function

The reproductive toxicity of valifenalate was investigated in a 2-generation reproduction toxicity study in rats. Additionally, some data about effects on sexual organs were reported in several repeated dose toxicity studies.

2-generation reproduction toxicity study in rats (Confidential study number 27)

The study was conducted according current OECD TG 416 and observing GLP. Rats (24/sex/group) were treated with 0, 1250, 4300 or 15000 ppm (reduced to 0, 850, 2900 or 10000 ppm during lactation) valifenalate in laboratory animal diet. Mean achieved test item intakes were as shown below:

		1250 ppm (mg/kg bw/day)	4300 ppm (mg/kg bw/day)	15000 ppm (mg/kg bw/day)
P generation				
Males	Pre-pairing	80.8	277.4	986.3
	After pairing	61.4	216.1	757.9
Females	Pre-pairing	92.7	318.8	1150.3
	Lactation	79.2	273.2	992.8
	Lactation	123.9	408.4	1384.0
F1 generation				
Males	Pre-pairing	83.5	294.2	1024.8
	After pairing	63.8	216.3	763.8
Females	Pre-pairing	93.0	326.1	1145.6
	Gestation	84.1	295.5	1030.8
	Lactation	129.2	429.3	1383.3

The main results and observations in this study are discussed below.

Parental toxicity

See Table 5 above. The main remarkable effects were increases in relative liver weight and liver and thyroid hypertrophy in both P and F1 together with slight clinical signs (ruffled fur) on F1.

Offspring toxicity

No treatment related effects on F1a at any dose were noted.

No treatment related effects at the lowest dose were noted on F2a. The main effects on this F2a at higher doses were:

4300 ppm (2900 ppm)	15000 ppm (10000 ppm)

	M	F	M	F
Pup weight gain (days 0-21)	↓ 9%	↓ 9%	↓ 8%	↓ 8%
Absolute spleen weights (no histological correlate)	↓ 26%	↓ 26%	↓ 18%	↓ 23%
Relative spleen weights (no histological correlate)	↓ 20%	↓ 17%	↓ 12%	↓ 17%
Glycogen deposition liver	16/19 (severity 2.1) vs 20/20 (severity 2.5) controls	14/18 (severity 1.6) vs 20/21 (severity 1.7) controls	18/22 (severity 1.5) vs 20/20 (severity 2.5) controls	14/21 (severity 1.3) vs 20/21 (severity 1.7) controls

RAC noted that glycogen deposition liver was not dose-related and therefore cannot be considered treatment related. No histopathological alterations were noted in spleen and therefore the alterations in spleen weight were not considered relevant.

Reproductive toxicity

No reproductive effects were noted on P generation.

In F1, three dams of the mid dose and one dam of the top dose suffered total litter loss. Next table offers an overview of relevant parameters in regards to pup mortality and survival:

	Dose (ppm)				
Parameter	0	1250/850	4300/2900	15000/10000	HCD[1]
All dams					
Pup loss days 0-4 p.p. (total number)	18	8	35	39	0-23
Pup loss days 0-4 p.p. (% of living pups)	7.4	3.2	14.8	15.2	0-8.5
Mean no. postnatal loss/litter days 0-4 p.p.	0.9	0.3	1.5	1.7	0-1.0
Mean living pups/litter day 4 p.p.	7.7	7.7	6.7	7.1	7.1-8.0
Mean living pups/litter day 21 p.p.	7.5	7.7	6.3	6.7	6.8-8.0
Mean pup loss/litter day 21	0.19	0.04	0.39	0.43	0-1.2
Without dams with total litter loss					
Pup loss days 0-4 p.p. (total number)	18	8	11	25	0-23
Pup loss days 0-4 p.p. (% of living pups)	7.4	3.2	5.5	9.6	0-8.5
Mean no. postnatal loss/litter days 0-4 p.p.	0.9	0.3	0.6	1.7	0-1.0
Mean living pups/litter day 4 p.p.	7.7	7.7	7.8	7.5	7.1-8.0
Mean living pups/litter day 21 p.p.	7.5	7.7	7.7	7.0	6.8-8.0
Mean pup loss/litter day 21	0.19	0.04	0.10	0.43	0-1.2
% Viability index	92.6	96.8*	95.5	90.4	91.5-100
% Weaning index	97.5	99.4	98.6	93.9	84.5-100

[1] Historical control data from 10 studies conducted from May 2002 to December 2007 (current study started November 2002)

RAC noted that no dose-response was observed in the effect total litter loss (no incidence at the lowest dose, 3 dams at the mid dose and 1 dam at the top dose). It suggests that

this effect can be incidental and not treatment related.

When all dams were considered, the total number of pup loss on days 0-4, percentage of living pups on days 0-4 and mean number of post-natal loss on days 0-4 in the mid and top dose was higher than HCD. These parameters were higher than HCD only at the top dose when dams with total litter loss were removed. Nevertheless, RAC noted that dose-response was not observed in these parameters since the increment of dose of 3.4 times between mid and high dose barely has effect on incidence. By the other hand, no negative effects on survival is evident since the records for mean living pups/litter day on days 4 and 21 and mean pup loss/litter day 21 were (in both cases with all dams and without dams with total litter loss) were covered by the HCD.

Effects on sexual organs in the repeated dose toxicity studies

Repeated dose toxicity studies in dogs showed certain effects on sexual organs (Table 7). These effects were mainly immaturity in prostate gland and reductions in weights of testis, epididymis and ovaries.

The findings on prostate glands are relatively common in short-term studies in dogs. Reductions in prostate gland weights were reported in all three studies in dogs. However, these reductions were noted in some cases also in control group or even in all animals of all groups. These findings, together with the small group size (3-4 animals/group) that bias the assessment of dose-response and the lack of alteration with histopathological correlation in the 52-week study suggest that prostate gland alterations cannot be addressed to valifenalate effects.

Reductions in testis, ovary and epididymis weights were also reported in these studies in dogs. However, these reductions were not correlated with histopathological changes and therefore are not considered by RAC as toxicologically relevant, especially considering that these effects were not reported in mice and rats.

Development

Table 12 summarises the available developmental toxicity studies with valifenalate.

Table 12: *Summary for animal studies on developmental toxicity with valifenalate*.

Method	Results	Reference
Developmental toxicity OECD TG 414 (2001) GLP Oral (gavage) Rat Crl:CD(SD)BR 25 mated females/group Valifenalate (IR5885)	Maternal toxicity 1000 mg/kg bw/day: No treatment related adverse effects at any dose Developmental toxicity **No treatment related adverse effects.** Incidences of corpora lutea, implantations, pre-implantation losses, post implantation losses, mean foetal weight, foetuses with external malformations, foetuses with skeletal malformations and foetuses with visceral malformations in all cases not statistically different from concurrent controls and within HCD	Confidential study number 9

Purity: 98.9% 0, 100, 300 and 1000 mg/kg bw/day Dosing on gestation days 6-19 Vehicle: 0.5% MC		
Developmental toxicity OECD 414 (2001) GLP Oral (gavage) Rabbit NZW (HY/CR) 22 mated females/group Valifenalate (IR5885) Purity: 98.9% 0, 100, 300 and 1000 mg/kg bw/day Dosing on gestation days 6-28	<u>Maternal toxicity</u> 1000 mg/kg bw/day: No treatment related adverse effects <u>Developmental toxicity</u> **No treatment related adverse effects.** Incidences of corpora lutea, implantations, pre-implantation losses, post implantation losses, dead foetuses, mean foetal weight, foetuses with external malformations, foetuses with skeletal malformations, and foetuses with visceral malformations in all cases not-statistically different from concurrent controls and within HCD	Confidential study number 10

Lactation

The two-generation study of valifenalate in rats has already been described. The dietary concentrations were lowered for the lactation period as an attempt to maintain the level of test item intake. Nevertheless, mean achieved dose levels were increased above pre-pairing levels (approximately 124, 408 and 1384 mg/kg bw/day in the low, mid and high dose groups respectively cf. 80, 277 and 986 mg/kg bw/day). Parental toxicity was observed at mid and high doses in all generations. Increased neonatal loss, reduced viability indices and increased pup mortality was seen in the F1 litters in the mid and high dose. There were no other treatment related adverse effects on the offspring.

The incidence of the finding 'no milk in stomach' was increased in the mid dose and high dose groups, but with regard to the litter incidences 1/21, 1/23, 6/23 and 4/23 in ascending order of doses. No clear relationship with doses could be established and this was most likely due to variability. Such findings, including cannibalism are background findings, which often occur in reproductive toxicity studies as non-treatment-related phenomenon. It is consistent with the fact that this observation was also made in the control group in this study and it occurred mainly in the litters with the mentioned losses, where the possibility of milk uptake by pups was apparently limited. There is no evidence of treatment-related impairment of the nursing behaviour of the dams.

The discussed viability and weaning indices of the F1 generation would be within the HCD if the dams with total litter loss were taken out of the evaluation, as can be seen in the table above. Therefore, the treatment has unlikely had an effect on these parameters, which is further supported by the fact that no effects on these parameters occurred in the P generation.

Comparison with the criteria

Sexual function and fertility

No effects on reproductive performance parameters and reproductive performance could be attributed to valifenalate. Therefore, RAC supports the DS's proposal for **no classification of valifenalate for adverse effects on sexual function and fertility.**

Development

In rat and rabbit prenatal developmental toxicity studies of valifenalate, no treatment related maternal toxicity was demonstrated at the limit dose of 1000 mg/kg bw/day and there was no evidence of developmental toxicity or of teratogenicity in either species. There were no treatment related effects on development of the offspring in the 2-generation toxicity study in rats rat to warrant classification of valifenalate as a known, presumed or suspected human reproductive toxicant, especially considering that the effects on pup loss days 0-4 are not considered by RAC robust enough because they were not noted in P litters. Therefore, RAC supports the DS's proposal for **no classification of valifenalate for development.**

Adverse effects on or via lactation

There was no indication of impaired nursing behaviour during lactation. The results of the study do not indicate any direct, primary adverse effect on the offspring due to transfer of the chemical via the milk or to the quality of the milk. Thus, RAC supports the DS's proposal for **no classification of valifenalate for adverse effects on or via lactation.**

10.11 Specific target organ toxicity-single exposure

Table 49: Summary table of animal studies on STOT SE

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

Acute neurotoxicity OECD 424 GLP Rat (Crl:CD(SD) 10/sex/group	Valifenalate technical Purity 98.9% Oral (gavage) 0, 500, 1000, 2000 mg/kg bw. Vehicle: 0.5% w/v methylcellulose in water. Single dose followed by 14 day observation period.	**2000 mg/kg bw:** No mortalities and no treatment related clinical or neurological signs on day 0, 7 or 14. *Signs of neurotoxicity at time of peak effect (2 hours):* No differences from control. *Signs of neurotoxicity after 7 days:* No differences from control. *Signs of neurotoxicity after 14 days:* No differences from control. *Pathology:*Slight increased incidence of axonal degeneration in multiple nerves but no clear dose response. ***1000 mg/kg bw:*** No effects. ***5000 mg/kg bw:*** No effects. **NOAEL for acute neurotoxicity: 2000 mg/kg bw**	*See Annex conf. 67*

Table 50: Summary table of human data on STOT SE

Type of data/report	Test substance	Route of exposure Relevant information about the study (as applicable)	Observations	Reference
No human data available on target organ toxicity-single exposure				

Table 51: Summary table of other studies relevant for STOT SE

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No other studies available on target organ toxicity-single exposure				

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

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.

In an acute neurotoxicity study in rats (*See Annex conf.67*), oral doses of up to 2000 mg/kg bw in Sprague Dawley rats did not cause any signs of neurotoxicity. There were no changes in FOB and motor activity evaluations. Histological changes were limited to a slight increase in the incidence of axonal degeneration in multiple nerves when considered in combination (with particular emphasis on the lumbar spinal nerve (females), lumbar dorsal root fibres (males) and sciatic nerve) in animals dosed with 2000 mg/kg valifenalate, but there was no clear dose-response in the number of animals affected. The NOAEL for neurotoxicity following a single dose in rats was determined as 2000 mg/kg. The results of this study revealed no indication of acute neurotoxicity.

10.11.2 Comparison with the CLP 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 exposure to valifenalate were indicative of nonspecific, general acute toxicity. No adverse effects were observed after acute dermal and inhalation exposure. As there was no clear evidence of specific target 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 warranted.

10.11.3 Conclusion on classification and labelling for STOT SE

CLP: Not classified (conclusive but not sufficient for classification).

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

Summary of the Dossier Submitter's proposal

The DS proposed no classification of valifenalate based on the absence of specific effects reported in the acute toxicity tests (see Table 1) and the absence of neurotoxicity in one acute neurotoxicity study using doses up to 2000 mg/kg bw.

Comments received during consultation

One manufacturer/company agreed with the DS's proposal for no classification.

Assessment and comparison with the classification criteria

Comparison with the criteria

RAC notes the absence of organ specific effects in the acute studies via oral, dermal and inhalation routes (Table 1). The CLH-report presents also an acute neurotoxicity study performed in rats conducted following OECD TG 424 and observing GLP. On this study, 10 rats/sex/group were treated with single doses of 500, 1000 and 2000 mg/kg bw of valifenalate (purity 98.9%) in 0.5% w/v methylcellulose in water. Animals were further observed for 14 days. Doses lower than 2000 mg/kg bw caused no effects on rats. The top dose (2000 mg/kg bw) caused a slight incidence of axonal degeneration in multiple nerves but without observing a clear dose-response.

Overall, none of the single-dose animal studies contained in the CLH-report provided evidence of organ-specific toxicity; which prevents for classification as STOT SE Cat 1 or 2. Moreover, no narcotic effects or respiratory tract irritation were found in such studies; which prevents for classification as STOT SE Cat 3. Therefore, RAC supports the DS's proposal for **no classification of valifenalate as STOT SE.**

10.12 Specific target organ toxicity-repeated exposure

The specific target-organ toxicity of valifenalate upon repeated exposure has been investigated in 28-day and 90-day studies in rats, in mice and dogs and a one-year study in dogs. Additional information is provided by

carcinogeneticity studies in rats and mice and the parental information for a 2-generation study in rats, which are reported in sections 10.9 and 10.10.

Table 52: Summary table of animal studies on STOT RE

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results	Reference
Studies in rats			
28-Day oral toxicity study Based on OECD 407 (1995) but no compliance claimed. Preliminary study for a 90 day. Non GLP Oral (continuous in diet) Rat, Han Wistar 5/sex/group	Valifenalate (IR5885, batch no. FCF/T/180-00 (ex ZI068), purity 98.9%) 0, 120, 600, 3000 and 15000 ppm Vehicle: laboratory animal diet	No treatment-related deaths in any dose group **15000 ppm (1518/1537 mg/kg bw/day males/females)** ↓ body weight gain weeks 0-4 (25% males); ↓ food consumption weeks 0-4 (12% males, 10% females); ↓ food conversion efficiency weeks 1-4 (14.5 % males); ↓ haematocrit (5% males, 4% females); ↓ haemoglobin (5% males, 4% females); ↓ total lymphocyte count (22% males, 34% females); ↑ activated partial thromboplastin time (23.1% males); ↑ aspartate aminotransferase activity (24% females); ↓ calcium (3% males, 5% females); ↓ phosphorous (21% females); ↓ total protein (3% males, 7% females); ↑ A/G ratio (7% females); ↓ absolute thymus weight (32% males, 14% females); ↑ thymic lymphocytosis (2/5 males cf. 0/5 controls, 4/5 females cf. 2/5 controls) **3000 ppm (311/314 mg/kg bw/day males/females)** ↓ haematocrit (10% males); ↓ haemoglobin (7% males); ↓ total lymphocyte count (10.5% males, 33% females); ↓ calcium (3.5% males, 5% females); ↓ phosphorous (19% females); ↓ total protein (3% males, 9% females); ↑ A/G ratio (13% females); ↓ absolute thymus weight (14% females); ↑ thymic lymphocytosis (4/5 males cf. 0/5 controls) **600 ppm (63/64 mg/kg bw/day males/females)** ↓ haematocrit (5% males), haemoglobin (4% males); ↓ calcium (4% males, 4.5% females); ↓ phosphorous (15% females); ↓ total protein (3% males, 6% females); ↑ A/G ratio (9% females); ↑ thymic lymphocytosis (3/5 males cf. 0/5 controls) **120 ppm (13 mg/kg bw/day males & females)** No adverse effects **NOAEL males 3000 ppm (311 mg/kg bw/day)** **NOAEL females 3000 ppm (314 mg/kg bw/day)**	*See Annex conf. 48.*
90-Day oral toxicity study 4 week recovery period OECD 408 (1998) GLP Oral (continuous in diet) Rat, Han Wistar 10/sex/group 5/sex/control & high dose groups for recovery phase	Valifenalate (IR5885, batch no. FCF/T/180-00 (ex ZI068), purity 98.9%) 0, 7, 150, 1000 mg/kg bw/day Vehicle: laboratory animal diet	**There were no deaths or overt signs of toxicity in any dose group.** **1000 mg/kg bw/day** ↓ haematocrit (5% males); ↓ haemoglobin (4% males); ↓ red blood cell count (2% males); ↓ white blood cell count (13% males); ↓ monocyte count (28% males); ↑ platelet count (7% males); ↓ prothrombin time (10% males); ↓ neutrophil count (31% females); ↓ triglycerides (36% males); ↑ chloride (2% males); ↑ calcium (3% females); ↑ urine volume (60% males, 68% females); ↓ specific gravity (1039 g/L females cf. 1050 g/L controls)); ↑ pH (7.3 males cf. 6.9 controls, 6.4 females cf. 5.9 controls); ↑ relative liver weight (15% males, 13% females); ↑ distended caecum (7/10 males, 1/10 females, no occurrence in controls) **150 mg/kg bw/day** ↓ haematocrit (2% males); ↓ haemoglobin (3% males); ↓ white	*See Annex conf. 49.*

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results**	**Reference**
		blood cell count (24% males); ↓ monocyte count (24% males); ↑ platelet count (11% males); ↓ prothrombin time (8% males); ↓ triglycerides (34% males); ↑ chloride (1% males); ↑ urine pH (7.3 males cf. 6.9 controls, 6.4 females cf. 5.9 controls) **7 mg/kg bw/day** ↑ urine pH (7.4 males cf. 6.9 controls) Recovery from all treatment-related effects occurred in the 4 week recovery period. **NOAEL 150 mg/kg bw/day**	
52-Week chronic toxicity (from 2 year study) OECD 453 (1981) GLP Oral (continuous in diet) Rat, Han Wistar 20/sex/group	Valifenalate (IR5885, batch T025/02, purity 99.56%) 0, 15, 150, 1000 mg/kg bw/day Vehicle: laboratory animal diet	**1000 mg/kg bw/day**: ↓ body weight 9% in males carcinogenicity phase weeks 0-104. No effect in females. ↓ haemoglobin (2.5–3.8% males weeks 13. 26 and 52); ↓ red cell count and mean cell haemoglobin concentration (1.4-3.5% males weeks 13 and 26); ↑ platelet count (9-16% males, approximately 10% females); ↑ APTT time (19-28% males). ↑ urine volume (75-210% females); ↓ specific gravity (1035-1041 g/L females cf. 1047-1066 g/L controls); ↑ relative liver weights (19% males, 12% females); ↑ relative kidney weights (7.6% males); ↑ thyroid follicular cell hypertrophy 11/20 males week 52 (3/20 controls). **150 mg/kg bw/day**: ↓ mean cell haemoglobin concentration (1.7% week 13, 1.4% week 26 males); *Pathology*: no treatment-related changes **15 mg/kg bw/day**: No toxicologically significant treatment-related effects. **NOAEL 150 mg/kg/day for males and 1000 mg/kg/day in females**	*See Annex conf. 51.*
28-Day dermal toxicity study OECD 410 (1981) GLP Dermal (6 hours/day) Rat, Han Wistar 10/sex/group	Valifenalate (IR5885 technical, batch no. T025/02, purity 99.6%) 0, 1000 mg/kg bw/day Vehicle: sterile water	**1000 mg/kg bw/day** No treatment-related effects **NOEL 1000 mg/kg bw/day**	*See Annex conf. 23.*
Two generation reproduction (one litter) OECD 416 (2001) GLP Oral (continuous in diet) Rat, HanBrl:WIST	Valifenalate (IR5885, lot no. T025/02, purity 99.56%) 0, 1250, 4300 or 15000 ppm (reduced to 0, 850, 2900 or 10000 ppm during lactation) Vehicle:	***Parental toxicity*** 15000 ppm (10000 ppm) – 986/1150 mg/kg bw/day, males/ females (P generation - pre-pairing) P: ↑ absolute liver weight (males 16%, females 15%); ↑ relative liver weight (males 20%, females 11%); ↑ liver hepatocellular hypertrophy (males 15/24 severity 2.4 cf. 4/24 controls severity 1.3), (females 3/24 severity 2.0 cf. 0/24 controls); ↓ glycogen deposition liver (males 17/24 severity 1.3 cf. 21/24 controls severity 1.6: females 15/24 severity 1.3 cf. 15/24 controls severity 2.3) considered adaptive and not adverse; ↑ severity of renal tubular hyaline change in males (3.4 cf. 2.3 controls) (rat	*See Annex conf. 27.*

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results	Reference
24/sex/group	laboratory animal diet	specific effect) F1: 4/24 females with ruffled fur early lactation; ↓ food consumption days 1-7 lactation (19%); ↑ absolute liver weight (males 12%, females 7.5%); ↑ relative liver weight (males 14%, females 10%); ↓ absolute kidney weight (females 7.4%); ↓ relative kidney weight (females 5,6%); ↑ liver hepatocellular hypertrophy (males 21/24 severity 2.2 cf. 2/24 controls severity 2.0), (females 21/24 severity 1.9 cf. 0/24 controls); ↓ glycogen deposition liver (males 19/24 severity 1.5 cf. 23/24 controls severity 2.7: females 2/24 severity 1.0 cf. 13/24 controls severity 1.8) considered adaptive and not adverse; ↑ severity of renal tubular hyaline change in males (2.3 cf. 1.6 controls) (rat specific effect); ↑ thyroid follicular hypertrophy (males 22/24 severity 2.1 cf. 17/24 controls severity 1.4: females 19/24 severity 1.6 cf. 10/24 controls severity 1.1) 4300 ppm (2900 ppm) – 277/318 mg/kg bw/day, males/ females (P generation - pre-pairing) P: ↑ absolute liver weight (females 6%); ↑ relative liver weight (males 8.5%); ↑ liver hepatocellular hypertrophy (males 7/24 severity 1.3 cf. 4/24 controls severity 1.3); ↓ glycogen deposition liver (males 17/24 severity 1.3 cf. 21/24 controls severity 1.6: females 17/24 severity 1.8 cf. 15/24 controls severity 2.3) considered adaptive and not adverse; ↑ severity of renal tubular hyaline change in males (2.8 cf. 2.3 controls) (rat specific effect) F1: 4/24 females with ruffled fur early lactation; ↓ food consumption days 1-7 lactation (15%); ↑ absolute liver weight (males 6%); ↑ relative liver weight (males 8%); ↑ liver hepatocellular hypertrophy (males 17/24 severity 2.3 cf. 2/24 controls severity 2.0); ↓ glycogen deposition liver (males 23/24 severity 1.9 cf. 23/24 controls severity 2.7: females 7/24 severity 1.4 cf. 13/24 controls severity 1.8) considered adaptive and not adverse; ↑ severity of renal tubular hyaline change in males (2.2 cf. 1.6 controls) (rat specific effect); ↑ thyroid follicular hypertrophy (males 16/24 severity 1.8 cf. 17/24 controls severity 1.4) 1250 ppm (850 ppm) – 80/92 mg/kg bw/day, males/ females (P generation - pre-pairing) P: No treatment related effects F1: No treatment related effects **NOAEL parental toxicity: 80 mg/kg bw/day**	
Studies in mice			
28-Day oral toxicity study Based on OECD 407 (1995) but no compliance claimed. Preliminary study for a 90 day.	Valifenalate (IR5885, batch no. FCF/T/180-00 (ex ZI068), purity 98.9%) 0, 110, 440, 1750 and 7000 ppm Vehicle: laboratory animal	**7000 ppm (1105/1536 mg/kg bw/day males/females)** ↓ body weight gain weeks 0-4 (37.5% males, not significant); ↓ food conversion efficiency weeks 1-4 (31% males); ↓ haematocrit (10% males), haemoglobin (11% males), red blood cell count (10% males); ↑ glucose (39% males, 31% females); ↓ triglycerides (71% females); ↑ cholesterol (31% males); ↑ potassium (15% males, 19% females); ↓ sodium (2% females); ↓ chloride (3% females); ↓ total protein (10% females); ↓ albumin (7% females); ↑ A/G ratio (4% females); ↑ relative liver weight	*See Annex conf. 48.*

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results**	**Reference**
Non GLP Oral (continuous in diet) Mouse, CD-1 6/sex/group	diet	(52% males, 40.5% females); ↑ relative adrenal weights (45% males); ↑ centrilobular hepatocytic hypertrophy (6/6 males cf. 0/6 controls; 5/6 females cf. 1/6 controls) **1750 ppm (266/402 mg/kg bw/day males/females)** ↓ body weight gain weeks 0-4 (19% males, not significant); ↓ haematocrit (4% males), haemoglobin (6% males), red blood cell count (5% males); ↑ glucose (38% males, 32% females); ↓ triglycerides (44% females); ↓ sodium (2% females); ↓ chloride (3.5% females); ↓ total protein (4% females); ↓albumin (3% females); ↑ A/G ratio (2% females); ↑ relative liver weight (30.5% males, 14% females); ↑centrilobular hepatocytic hypertrophy (6/6 males cf. 0/6 controls; 2/6 females cf. 1/6 controls) **440 ppm (68/96 mg/kg bw/day males/females)** ↑ liver weight (10% females); ↑ centrilobular hepatocytic hypertrophy (6/6 males cf. 0/6 controls) **110 ppm (18/27 mg/kg bw/day males/females)** No treatment-related effects **NOAEL males 440 ppm (68 mg/kg bw/day)** **NOAEL females 7000 ppm (1536 mg/kg bw/day)**	
90-Day oral toxicity study Based on OECD 408 (1998) but no compliance claimed. Prelim carcinogenicity study. GLP Oral (continuous in diet) Mouse, CD-1 10/sex/group	Valifenalate (IR5885, batch no. FCF/T/180-00 (ex ZI068), purity 98.9%) 0, 110, 900 and 7000 ppm Vehicle: laboratory animal diet	**7000 ppm (995/1144 mg/kg bw/day males/females)** ↓ body weight gain weeks 0-13 (26% males); ↓ food conversion efficiency weeks 1-13 (22% males); ↓ haematocrit (4% males, 5% females); ↓ haemoglobin (4% males, 3% females); ↓ mean cell haemoglobin (5% males, 3% females); ↓ mean cell volume (5% males, 4% females); ↑ relative liver weight (51% males, 35% females); ↑ centrilobular hepatocellular vacuolation (8/10 males cf. 2/10 controls); ↑ periportal hepatocellular vacuolation (3/10 males cf. 0/10 controls) due to increased fat storage **900 ppm (133/147 mg/kg bw/day males/females)** ↓ body weight gain weeks 0-13 (15% males, not significant); ↓ food conversion efficiency weeks 1-13 (22% males); ↑ relative liver weight (12% males) **110 ppm (15/16 mg/kg bw/day males/females)** No treatment-related effects **NOAEL males 900 ppm (133.7 mg/kg bw/day)** **NOAEL females 900 ppm (147.5 mg/kg bw/day)**	*See Annex conf. 50.*
Carcinogenicity study: OECD 451 Mouse (Crl: CD-1™ (ICR) BR) 50/sex/group	Valifenalate (IR5885) Lot T025/02, purity 99.56% 0, 150, 850, 5000 ppm mg/kg bw/day Continuous dietary	**Non-neoplastic findings** **5000ppm**: *Body weight*: ↓ 22 % in males (weeks 0 to 78) *Liver weight:* ↑ 97.0 % and 23.1% relative weight in males and females. *Kidney weight*: ↑ 11.9 % relative weight in females *Liver pathology*: ↑ Centrilobular hepatocyte hypertrophy: 25/50 females (8/50 controls), Generalised hepatocyte hypertrophy:	*See Annex conf. 52.*

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results	Reference
	administration for 78 weeks Achieved doses 16.8, 97.2 and 657 mg/kg/day for males and 21.6, 124 and 756 mg/kg/day for females.	29/50 males (3/50 controls), Centrilobular hepatocyte vacuolation 32/50 males (11/50 controls), Cytoplasmic eosinophilia 29/50 males (0/50 controls); Pigment in hepatocytes 18/50 males, 13/50 females (0-1/50 controls), Pigment in macrophages 12/50 males, 31/50 females (control 1/50 males and 12/50 females) *Gall bladder pathology*: ↑ Choleliths 8/45 females (1/47 controls). **850ppm**: *Clinical findings:* No adverse effects. *Liver weight*: ↑ 28.6% relative weight males *Pathology*: ↑ Liver findings Centrilobular hepatocyte vacuolation 33/50 males (11/50 controls) **150ppm**: *Clinical findings:* No adverse effects. *Organ weights*: Increased liver weights (males) . *Pathology*: Centrilobular (34/50) and/or generalised liver hypertrophy (6/50) in males (21/50 and 3/50 controls). **NOAEL for toxicity** : 150 ppm equivalent to 16.8 mg/kg bw/day in males and 21.6 mg/kg bw/day in females	
Studies in dogs			
28-Day oral toxicity study OECD 409 (1998) GLP Oral (capsule) Dog, Beagle 3/sex/group	Valifenalate (IR5885, batch no. FCF/T/180-00 (ex ZI068), purity 98.9%) 0, 250, 500 and 1000 mg/kg bw/day Vehicle: gelatine capsule	**1000 mg/kg bw/day** ↑ pale faeces (3/3 males, 2/3 females, cf. no occurrence in controls); ↓ cholesterol (60% males, 67% females); ↓ phospholipid (53% males, 61% females); ↑ alkaline phosphatase activity (203% males); ↑ gamma glutamyl-transferase (80% males); ↑ total protein (13% males, 18% females); ↓ albumin (20% males, 23% females); ↓ calcium (8% males, 11% females); ↓ magnesium (10% males); ↑ phosphorous (18% males); ↑ absolute liver weight (66% males, 32.5% females); ↓ hepatocellular glycogen content (0/3 males cf. 2/3 controls severity 2.5; 1/3 females severity 1.0 cf. 3/3 controls severity 3.0); ↑ hepatocellular hypertrophy 3/3 males severity 4.0 cf. 1/3 controls severity 1.0; 3/3 females severity 3.3 cf. 0/3 controls); ↑ liver eosinophilic cytoplasmic inclusions (3/3 males severity 2.3, cf. 0/3 controls; 2/3 females severity 3.0 cf. 0/3 controls); ↑ liver single cell necrosis 3/3 males, 1/3 females, 0/3 per sex, controls); ↑increased apoptosis (1/3 males, 0/3 females, 0/3 per sex, controls) **500 mg/kg bw/day** ↑ pale faeces (3/3 males cf. no occurrence in controls); ↓ cholesterol (41% males, 52% females); ↓ phospholipid (38% males, 44% females); ↑ total protein (9% males, 14% females); ↓ albumin (18% males, 21% females); ↓ calcium (10% females); ↑ absolute liver weight (49% males, 42% females); ↓ hepatocellular glycogen content (3/3 males severity 2.0 cf. 2/3 controls severity 2.5; 3/3 females severity 2.0 cf. 3/3 controls severity 3.0); ↑ hepatocellular hypertrophy 3/3 males severity 3.0 cf. 1/3 controls severity 1.0; 3/3 females severity 2.7 cf. 0/3 controls); ↑ liver eosinophilic cytoplasmic inclusions (3/3 males severity 2.0, cf.	*See Annes conf.7.*

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results	Reference
		0/3 controls; 2/3 females severity 1.5 cf. 0/3 controls) **250 mg/kg bw/day** No adverse effects ↓ cholesterol (42% males, 19% females); ↓ phospholipid (39.5% males); ↑ total protein (8% males); ↓ albumin (23% males); ↑ absolute liver weight (34% males, 19% females); ↓ hepatocellular glycogen content (3/3 males severity 2.7 cf. 2/3 controls severity 2.5; 3/3 females severity 1.3 cf. 3/3 controls severity 3.0); ↑ hepatocellular hypertrophy 3/3 males severity 1.3 cf. 1/3 controls severity 1.0; 3/3 females severity 2.0 cf. 0/3 controls); ↑ liver eosinophilic cytoplasmic inclusions (2/3 males severity 1.5, cf. 0/3 controls; 1/3 females severity 1.0 cf. 0/3 controls) Neither of these observations are considered to be related to administration of the test item. **NOAEL 500 mg/kg bw/day** (liver findings were considered adaptive and blood findings minimal or within historical range)	
90-Day oral toxicity study OECD 409 (1998) GLP Oral (capsule) Dog, Beagle 4/sex/group	Valifenalate (IR5885, batch no. T025/02 purity 98.56%) 0, 50, 250 and 750 mg/kg bw/day Vehicle: gelatine capsule	**750 mg/kg bw/day** 1 female taken off-dose after 7 weeks due to weight loss adverse laboratory results and retained until the end of the study; ↑ white discoloured faeces or white/yellow powder in faeces from day 3, 7/8 dogs cf. none in controls; ↓ body weight gain (48% males, 33% females weeks 0-13); ↓ food consumption (12% males & females); ↑ platelets (week 6, 20% males, 74% females: week 13, 33% males, 42% females); ↓ RBC (week 6, 8% males, week 13, 8% males and 9% females); ↑ MCH (males 9% week 6, 10% week 13); ↑ MCV (males 3% week 6, 6.5% week 13); ↓ reticulocytes (week 6, 49.5% males, 60% females: week 13, 43% females); ↑ ALP (week 6, 337% males, 370% females: week 13, 517% males, 446% females); ↑ ALT (week 6, 109% males, 303% females: week 13, 272% males, 58% females); ↑ GGT (week 6, 67% males, 133% females: week 13, 133% males, 100% females); ↓ cholesterol (week 6, 53% males, 65% females: week 13, 57.5% males, 69% females); ↓ total protein (week 6, 15% males, 17% females: week 13, 18% males, 17% females); ↓ albumin (week 6, 21% males, 28% females: week 13, 23% males, 27% females); ↓ A/G ratio (week 6, males 1.01 cf. 1.15 controls, females 0.95 cf. 1.26 controls: week 13, males 0.96 cf. 1.15 controls, females 0.94 cf. 1.28 controls); ↑ AST (week 13, 28% males, 23.5% females); ↑ glucose (week 13, 11.5% males, 22% females); ↑ relative liver weight (60% males, 70% females); ↑ relative thyroid/parathyroid weights (64% males); ↓ prostate weight (64%); ↓ testis weight (28%); ↑ epididymis weight (14%); ↑ hepatocyte hypertrophy (4/4 males, 3/3 females: none in controls); ↑ hepatocytes pale cytoplasm, peripheral clumping (4/4 males, 3/3 females: none in controls); ↑ eosinophilic intracytoplasmic inclusions in hepatocytes (4/4 males, 3/3 females: none in controls); ↑ thyroid follicular hypertrophy (2/4 males, 2/3 females: none in controls) **250 mg/kg bw/day** ↑ white discoloured faeces or white/yellow powder in faeces from day 10, 5/8 dogs cf. none in controls; ↓ body weight gain (21%	*See Annex conf. 12.*

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results	Reference
		males weeks 0-13); ↑ platelets (week 6, 14% males: week 13, 42% males); ↓ reticulocytes (week 6, 31% males, 39% females); ↑ ALP (week 6, 255% males, 91% females: week 13, 430% males, 194% females); ↑ ALT (week 13, 42% females); ↑ GGT (week 13, 33% males, 33% females); ↓ cholesterol (week 6, 47% males, 36% females, week 13, 40% male , 31% females); ↓ total protein (week 6, 11% males, 11.5% females: week 13, 12.5% males, 9% females); ↓ albumin (week 6, 14% males, 14% females: week 13, 20% males, 13% females); ↓ A/G ratio (week 6, males 1.01 cf. 1.15 controls: week 13, males 1.00 cf. 1.15 controls); ↑ AST(week 13, 29% females); ↑ relative liver weight (44% males, 34% females); ↑ relative thyroid/parathyroid weights (61% males); ↑ hepatocyte hypertrophy (4/4 males, 4/4 females: none in controls); ↑ hepatocytes pale cytoplasm, peripheral clumping (4/4 males, 4/4 females: none in controls); ↑ eosinophilic intracytoplasmic inclusions in hepatocytes (4/4 males, 4/4 females: none in controls); ↑ thyroid follicular hypertrophy (1/4 males, 2/4 females: none in controls) **<u>50 mg/kg bw/day</u>** ↑ ALP (week 6, 12% males, 73% females: week 13, 142% males, 134% females); ↑ relative liver weight (33% females); ↑ hepatocyte hypertrophy (4/4 males, 4/4 females: none in controls); ↑ thyroid follicular hypertrophy (1/4 females: none in controls) **NOAEL 250 mg/kg bw/day** (findings not of toxicological importance).	
52-Week chronic toxicity Additionally 13 weeks subchronic toxicity with 8 week recovery. OECD 452 (1981) GLP Oral (capsule) Dog, Beagle 4/sex/group	Valifenalate (IR5885, batch no. T025/02 purity 99.56%) 0, 1, 7, 50 and 250 mg/kg bw/day Vehicle: gelatine capsule	**<u>250 mg/kg bw/day</u>** ↑ platelets (weeks 26-52, 43-74% males; ↑ ALP (550% males, 340% females week 13, 1281-1360% males, 565-746% females week 26-52); ↓ cholesterol (week 13, 28% males, 25% females); ↓ total protein (weeks 13-52, 9-13% males, 7-10% females), ↓ albumin (weeks 13-52, 13-19% males, 13-16% females); ↓ A/G ratio (weeks 26-52, males 0.99-1.01 cf. 1.18-1.19 controls: weeks 26 and 39 females 1.11 and 1.08 cf. 1.28 and 1.33 controls); ↑ triglycerides (91% males week 39), ↓ calcium ions (5-8% males weeks 13-52) ↑ relative liver weight (61% males, 36% females); ↑ relative thyroid/parathyroid (31% males); ↓ relative prostate weight 29%; ↓ relative ovary weights 57% ↑ hepatocyte hypertrophy (4/4 males, 4/4 females: none in controls), cytoplasmatic inclusion; ↑ hepatocytes with pale cytoplasm and peripheral clumping hypertrophy (4/4 males, 3/4 females: none in controls) **<u>50 mg/kg bw/day</u>** ↑ ALP (131-217% males weeks 13 -52, 179-398% females weeks 13-52) ↓ relative ovary weights 48% ↑ hepatocyte hypertrophy (4/4 males, 4/4 females: none in controls), cytoplasmatic inclusion; ↑ hepatocytes with pale cytoplasm and peripheral clumping hypertrophy (1/4 females: none in controls)	*See Annex conf. 65*

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results	Reference
		7 mg/kg bw/day ↑ ALP (165 and 150% females weeks 26 and 39) ↑ hepatocyte hypertrophy (1/4 males, 2/4 females: none in controls) **1 mg/kg bw/day** ↑ ALP (55% females week 39) **NOAEL 50 mg/kg bw/day**	

Table 53: Summary table of human data on STOT RE

Type of data/report	Test substance	Route of exposure Relevant information about the study (as applicable)	Observations	Reference
There are no relevant human data				

Table 54: Summary table of other studies relevant for STOT RE

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
There are no additional studies				

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

Oral dosing with Valifenalate was well tolerated. In 28 day, 90 day and 1 year dietary toxicity studies in rats toxicologically significant findings were observed at doses of 1000 mg/kg bw/day and above (*See Annex conf. 48., 49., 51., 52.*). These comprised effects on body weight and food consumption, changes in haematology and clinical chemistry, increased liver and thyroid weights and histopathological changes in the liver (centrilobular hepatocyte hypertrophy).

Although changes in haematology parameters were consistent, treatment differences from control were minimal, even at the limit dose of 1000 mg/kg bw/day, as can be seen when compared with historical control data. Mean values were all within the 5-95% confidence intervals taken from the historical control data for the same strain and in the same laboratory (Annex III). The tables below show a comparison of the data obtained with the historical control data (HCD). The haematology HCD for 1999-2009 are split into 2 periods 1999-2004 and 2004-2009, as those are the periods the data were provided by the laboratory. It is evident that the mean values and confidence intervals for the relevant data are very consistent for the 2 periods. The differences from control cannot be considered adverse as they are within the range of “normal” (HCD) values.

Summary table haematology data 90 day study – selected parameters

MALES							
Parameter	**Control**	**Low dose**	**Mid dose**	**High dose**	**HCD Means**	**HCD 5% CI**	**HCD 95% CI**
Hct (L/L)	0.446	0.443	0.436	0.425**	0.448 0.451	0.414 0.415	0.485 0.487
Hb (g/dL)	15.8	15.6	15.3**	15.1**	15.7 15.6	14.6 14.4	16.9 17.0
WBC (x10^9/L)	8.5	7.24	6.48*	7.40*	7.55 7.49	4.39 4.54	11.51 11.38
Lymphocytes (x10^9/L)	6.91	5.91	4.99*	5.96*	5.89 5.84	3.33 3.42	9.26 9.06
Monocytes (x10^9/L)	0.25	0.21	0.19*	0.18*	0.20 0.17	0.07 0.07	0.39 0.34
Platelets (x10^9/L)	833	786	928**	895**	848 842	698 682	1022 1014
PT (sec)	15.7	15.0	14.5**	14.2**	14.8 15.1	13.1 13.6	16.6 16.6

Statistically significant when compared with Control: * - p<0.05; ** - p<0.01
HCD data are presented as two separate values from data for 1999-2004 and 2004-2009

Summary table haematology data up to 52 weeks 2 year rat study – selected parameters and time points

MALES							
Parameter	**Control**	**Low dose**	**Mid dose**	**High dose**	**HCD Means**	**HCD 5% CI**	**HCD 95% CI**
Hct week 13 (L/L)	0.460	0.460	0.47*	0.453	0.448 0.451	0.414 0.415	0.485 0.487
Hb week 13 (g/dL)	16.2	16.0	16.3	15.7**	15.7 15.6	14.6 14.4	16.9 17.0
Hb week 26 (g/dL)	15.6	15.5	15.4	15.0**	15.8 15.5	14.9 14.4	16.7 16.4
Hb week 52 (g/dL)	15.7	15.4	15.6	15.3**	15.7 15.4	14.9 13.5	16.7 16.5
RBC wk 13 (x10^{12}/L)	8.90	8.82	8.85	8.59**	8.54 8.63	7.82 7.88	9.24 9.40
RBC wk 26 (x10^{12}/L)	8.55	8.54	8.47	8.30*	8.63 8.48	7.98 7.78	9.28 9.09
WBC wk 13 (x10^9/L)	9.80	9.59	9.61	8.96	7.55 7.49	4.39 4.54	11.51 11.38
Plat wk 13 (x10^9/L)	882	891	917	970**	848 842	698 682	1022 1014
APTT wk 13 (sec)	21.9	20.3	22.0	26.1**	19.4 18.1	15.1 13.9	24.9 23.0
APTT wk 26 (sec)	19.7	19.2	20.4	25.2**	17.4 18.6	11.9 12.0	22.5 23.5

APTT wk 52 (sec)	17.5	17.9	17.9	19.2**	18.3 18.2	13.1 11.6	22.6 25.0
FEMALES							
Hct wk 13 (L/L)	0.423	0.423	0.421	0.413*	0.421 0.427	0.385 0.393	0.451 0.465
Hb wk 13 (g/dL)	15.1	15.1	15.1	14.8	14.8 14.8	13.5 13.8	15.9 15.9
RBC wk 13 (x10^{12}/L)	7.77	7.77	7.67	7.68	7.63 7.79	6.88 7.15	8.42 8.48
WBC wk 13 (x10^{9}/L)	7.00	5.77	6.23	6.42	5.48 5.61	2.76 3.16	9.12 9.41
Platelets wk13 (x10^{9}/L)	906	942	951	996*	867 890	690 690	1068 1152
APTT wk 13 (sec)	18.1	16.5	14.9**	14.8**	18.2 16.9	12.0 11.7	23.5 21.7
APTT wk 26 (sec)	20.0	17.7	19.1	19.5	18.2 18.2	12.7 13.6	23.2 22.5

Statistically significant when compared with Control: * - p<0.05; ** - p<0.01
HCD data are presented as two separate values from data for 1999-2004 and 2004-2009

Changes in blood biochemistry parameters were minimal, even at the limit dose of 1000 mg/kg bw/day, as can be seen when compared with historical control data. Mean values were generally close to the mean and within the 5-95% confidence intervals taken from the historical control data for the same strain and in the same laboratory (Annex III). The tables below show a comparison of the data obtained with the historical control data (HCD). The blood chemistry HCD for 1999-2009 are split into 2 periods 1999-2004 and 2004-2009, as those are the periods the data were provided by the laboratory. It is evident that the mean values and confidence intervals for the relevant data are very consistent for the 2 periods.

Summary table clinical chemistry data up to 52 weeks 90 day and 2 year rat studies – selected parameters and time points

MALES	**90 DAY**	**STUDY**					
Parameter	**Control**	**Low dose**	**Mid dose**	**High dose**	**HCD Means**	**HCD 5% CI**	**HCD 95% CI**
Trig male (mmol/L)	1.36	1.20	0.90**	0.87**	0.86 0.80	0.35 0.35	1.61 1.48
Cl male (mmol/L)	106	106	107*	108**	104 102	100 99	107 104
MALES	**2 YEAR**	**STUDY**					
Urea w26 (mmol/L)	6.05	5.76	6.31	5.42*	5.90 5.90	4.22 4.22	7.53 7.83
Urea w 52 (mmol/L)	4.99	5.10	6.08*	5.49*	5.10 5.23	3.78 3.77	6.61 6.89
Gluc w 52 (mmol/L)	8.67	8.00	7.43*	7.84*	8.44 8.33	6.84 7.11	11.23 10.13
FEMALES	**2 YEAR**	**STUDY**					
ALP w 26 (u/L)	28	27	23	21**	61 63	40 46	90 92

Gluc w 26 (mmol/L)	6.29	7.14**	7.00**	7.40**	6.52 6.32	4.56 4.84	8.64 8.21
Gluc w 52 (mmol/L)	6.06	6.74*	6.76*	7.24**	7.04 7.22	5.44 5.27	9.27 9.67
Creat w 52 (µmol/L)	55	60*	60*	59*	55 50	47 37	62 62

In 28 and 90 day dietary studies in the mouse (*See Annex conf. 48., 50.*) the effects were consistent with those described in the rat i.e. on body weight and food consumption, changes in haematology and clinical chemistry, increased liver and thyroid weights and histopathological changes in the liver (centrilobular hepatocyte hypertrophy). These occurred at doses of 995 mg/kg bw/day and above.

In the dog 90 day capsule dosing study (*See Annex conf. 12.*) one female dog dosed at 750 mg/kg bw/day was taken off-dose after 7 weeks due to weight loss and adverse clinical and laboratory results. In the remaining dogs at this dose level changes in body weight and food consumption, haematology and clinical chemistry parameters were seen. Pathology findings comprised pale cytoplasm and peripheral clumping in hepatoctyes, eosinophilic intracytoplasmic inclusions in hepatocytes and thyroid follicular hypertrophy. In the 52 weeks dog study, effects were seen at the highest dose of 250 mg/kg bw/day (*See Annex conf. 65*).

In a 28 day dermal toxicity study in the rat (*See Annex conf. 23.*) there was no evidence of systemic toxicity at the highest doses tested.

Overall, the repeat dose studies indicate an absence of significant target organ toxicity. Although haematology changes are described in three species, the magnitude of these changes is small and insufficient to be classed as significant. In addition there was no accompanying organ damage at necropsy or at microscopic examination in the spleen, kidney or liver.

The only evidence of significant target organ toxicity was at a very high dose (750 mg/kg bw/day) in the dog in 1 of 8 animals where the severity of the body weight loss and clinical results resulted in the discontinuation of dosing after 7 weeks. The remaining 7 animals in this group survived without signs of severe organ toxicity.

The target organs identified in all three species were the liver and thyroid. In the liver pathology findings were centrilobular hepatocyte hypertrophy and pale cytoplasm and peripheral clumping in hepatoctyes. The liver pathology, relative liver weights increases and clinical biochemistry changes, the most marked of which was increased ALP activity, are all considered to reflect adaptive changes i.e. the normal response of the target tissue to substances. In the thyroid there was evidence of increased weight and follicular cell hyperplasia. Although thyroid hormones were investigated in the 52 week study (*See Annex conf.65*), the results were variable and there was no conclusive evidence of an effect. However it is established (ECHA CLP guidance, 2015) that test substances that cause induction of liver enzymes, interfere with the regulation of thyroid hormones and that rodents are highly sensitive to a reduction in thyroid hormone levels (T4), resulting in thyroid toxicity (e.g. hypertrophy, hyperplasia) after repeated stimulation exposure of this organ. Thus, 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.

10.12.2 Assessment and comparison with the CLP criteria

STOT-RE is assigned on the basis of a substance demonstrating evidence of significant or severe toxicity, generally at or below the oral guidance value of 100 mg/kg/d (for a classification in category 2) obtained in a 90-day rat study. The oral guidance value for a classification in category 1 is ≤ 10 mg/kg/d. The equivalent guidance values for a 28-day study are ≤ 300 mg/kg/d and ≤ 30 mg/kg/d, respectively; for a one-year study, they are ≤ 25 mg/kg/d and 2.5 mg/kg/d, respectively, and for a two-year study, ≤ 12.5 mg/kg/d and 1.25 mg/kg/d. For dermal exposure, the 90-day guidance value is ≤ 200 mg/kg/d in rats or rabbits

Table 55: Extrapolation of equivalent effective dose for toxicity studies of greater or lesser duration than 90 days

Study	Adjusted guidance value category 1/ 2 (mg kg bw/d)	Effects at doses below guidance cut-off values
28 day rat study	30/300	Category 1: Small changes in haematology and clinical chemistry parameters at 63/64 mg/kg bw/day male /female Category 2: Changes in haematology and clinical chemistry parameters No observed adverse effect level 311/314 mg/kg bw/day male /female
28 day mouse study	30/300	Category 1: No adverse effects at 18/27 mg/kg bw/ day in males and females Category 2: 68 mg/kg bw/day increased relative liver weight and centrilobular hepatocyte hypertrophy in males. No observed adverse effect level 68/1536 mg/kg bw/day) male/female
28 day dog study	30/300	Category 1: Lowest dose = 250 mg/kg bw/day Category 2 : No adverse effects at 250 mg/kg bw/day
90 day rat study	10/100	Category 1: No adverse effects at lowest dose 7 mg/kg bw/day Category 2: 150 mg/kg bw/day Small changes in haematology (HCt and Hb ≤ 3%) and clinical chemistry parameters. No observed adverse effect level :150 mg/kg bw/day
90 day mouse study	10/100	Category 1: Lowest dose = 15/16 mg/kg bw/day in males and females Category 2: 15/16 mg/kg bw/day in males and females no treatment related effects No observed adverse effect level: 133.7/147.75 mg/kg bw/day in males/females
90 day dog study	10/100	Category 1: Lowest dose = 50 mg/kg bw/day Category 2: 50 mg/kg bw/day changes in blood chemistry and hepatocyte hypertrophy
Multigeneration study	10/100* [* underestimate exposure at least 16 weeks]	Category 2: – 80/92 mg/kg bw/day, males/ females (P generation - pre-pairing: No treatment related effects. No observed adverse effect level parental toxicity: 80 mg/kg bw/day
1 year dog study	2.5/25	Category1: 1 mg/kg bw/day increase in ALP in both sexes Category 2: 7 mg/kg bw/day increase in ALP and hepatocyte hypertrophy.
2 year rat study	2.5/25 (one year interim kill) 1.25/12.5 (two year)	Category 1: Lowest dose = 15 mg/kg bw/day Category 2: 15 mg/kg bw/day no adverse effects; No observed adverse effect level: 150 mg/kg bw/day

Study	Adjusted guidance value category 1/ 2 (mg kg bw/d)	Effects at doses below guidance cut-off values
78 week mouse study	1.7/17	Category 1: Lowest dose = 16.8/21.6 mg/kg bw/day in males and females Category 2: 16.8/21.6 mg/kg bw/day in males/ females increased liver weights in males. No observed adverse effect level: 16.8/21.6 mg/kg bw/day in males/ females

Comparison with CLH criteria. The effects noted in this study comprise small changes in blood chemistry and clinical chemistry parameters and hepatocyte hypertrophy in rats, increased relative liver weight and centrilobular hepatocyte hypertrophy in mice and an increase in ALP and hepatocyte hypertrophy in the dog. These effects were generally seen at doses above the guidance cut-off values and were considered by the authors of the reports as non-adverse adaptations to administration of the test material. For example, the decrease in haemoglobin and related parameters in the 90 day rat study were ≤ 3% and even after administration of a limit dose of 1000 mg/kg bw/day for 52 weeks in the 2 year carcinogenicity study the values were < 4% below control. Other changes seen are considered adaptive changes in response to administration of a xenobiotic substance. These were centrilobular hypertrophy and associated increases in liver weight and in the activity of ALP. Hence the treatment-related changes seen in all available toxicity studies are consistent with points b), c) and/or d) of the CLP Guidance (Guidance on the Application of the CLP Criteria Version 5 – July 2017).

"Annex I: 3.9.2.8. Effects considered not to support classification for specific target organ toxicity following repeated exposure Annex I: 3.9.2.8.1. It is recognised that effects may be seen in humans and/or animals that do not justify classification. Such effects include, but are not limited to:

(a) Clinical observations or small changes in bodyweight gain, food consumption or water intake that have toxicological importance but that do not, by themselves, indicate "significant" toxicity.

(b) Small changes in clinical biochemistry, haematology or urinalysis parameters and/or transient effects, when such changes or effects are of doubtful or minimal toxicological importance

(c) Changes in organ weights with no evidence of organ dysfunction.

(d) Adaptive responses that are not considered toxicologically relevant.

(e) Substance-induced species-specific mechanisms of toxicity, i.e. demonstrated with reasonable certainty to be not relevant for human health, shall not justify classification."

Effects corresponding to the classification in STOT RE 2

In rats, no adverse effects below the guidance cut-off for category 2 occurred in 28-day, 90-day and 2-year studies.

In dogs, effects were reported in the dose range-finding and 1 year studies and in the 90 day study at doses below the threshold for classification in Category 2.

In the 1 year study, at 1 or 7 mg/kg/day the only treatment related findings were confident to the liver and were considered to be adaptive in nature and thus not of toxological importance within the context of this study. In addition, there was no dosage relationship with regard to the incidence of the liver findings noted at either 1 or 7 mg/kg/day.

In the 90-day study, the treatment-related effects on liver and thyroids seen at 50 and 250 mg/kg/day were considered not to be indicative of toxicity. Clear evidence of toxicity was observed at 750 mg/kg bw/day, therefore, it was considered that longer term dosing at a level approaching 750 mg/kg bw/day may result in toxic changes in the liver that may not be tolerated and thus lead to the early termination of the animals. . This is clearly in excess of the relevant cut-off level ≤ 100 mg/kg required for classification as Cat 2 for STOT RE (ECHA CLP Guidance, 2015).

In mice, there were no adverse effects at 90-day study below the threshold reference value.

In 28-day study the slightly high liver weights were associated with centrilobular hepatocyte hypetrtrophy which was observed for males and females which received 1750ppm or 7000 ppm and in males which received 440 ppm. This is a common response to the administration of xenobiotics in rodents and relates to metabolic adaptation rather than a toxic effect of treatment.

In 18-month study, compared to Controls, high absolute and bodyweight-relative liver weights in males and females receiving 850 and 5000 ppm and in males receiving 150 ppm. Males were affected to a greater extent than females. Absolute and bodyweight-relative kidney weights were also marginally higher than those of the Control in females at the highest dosage.
All other inter-group differences attaining statistical significance were present in one sex only, lacked dosage-relationship and were therefore attributed to normal biological variation.

The assessment for STOT RE includes data by the oral and dermal routes. No repeat dose inhalation studies have been conducted, therefore no comparison with the STOT RE criteria is possible. However, the acute inhalation study showed no evidence of impairment of the respiratory system up to the limit dose.

For valifenalate no toxicologically significant effects were seen in rats, mice and dogs and no classification is required.

10.12.3 Conclusion on classification and labelling for STOT RE

CLP: Not classified (conclusive but not sufficient for classification).

RAC evaluation of specific target organ toxicity – repeated exposure (STOT RE)

Summary of the Dossier Submitter's proposal

The repeated dose toxicity studies with animals showed that valifenalate is able to cause small changes in blood and clinical chemistry parameters as well as hepatocyte hypertrophy in rats, increased relative liver weight and centrilobular hepatocyte hypertrophy in mice, and an increase in alkaline phosphatase (ALP) and hepatocyte hypertrophy in dogs. The DS noted that these effects were generally seen at doses above the guidance cut-off values and were of low severity (i.e. the alterations in blood and clinical chemistry). Other changes (centrilobular hypertrophy and associated increases in liver weight and in the activity of ALP) were considered adaptive in response to administration of valifenalate. The DS proposed no classification of the substance for STOT RE.

Comments received during consultation

One manufacturer/company agreed with the DS's proposal for no classification.

Assessment and comparison with the classification criteria

Tables 5, 6 and 7 summarise the results of the repeated dose toxicity studies in rats, mice and dogs; respectively.

Table 5: *Summary of repeated dose toxicity studies in rats with valifenalate*. In all cases the effects were statistically different from controls for at least $p<0.05$. ND = No statistical differences with control.

Method	Results	Reference
28-day oral toxicity study Based on OECD TG 407 (1995) but no GLP compliance claimed Preliminary study for a 90 day Non GLP Oral (continuous in diet) Rat Han Wistar 5/sex/group Valifenalate (IR5885) Purity: 98.9% 0, 120, 600, 3000 and 15000 ppm Vehicle: laboratory animal diet	No treatment-related deaths in any dose group <u>15000 ppm (1518/1537 mg/kg bw/day males/females)</u> ↓ Body weight gain weeks 0-4: 25% males; ND- females ↓ Food consumption weeks 0-4: 12% males; 10% females ↓ Haematocrit: 5% males; 4% females ↓ Haemoglobin: 5% males; 4% females ↓ Total lymphocyte count: 22% males; 34% females ↑ Activated partial thromboplastin time: 23% males; ND females ↑ Aspartate aminotransferase activity: ND males; 24% females ↓ Calcium: 3% males; 5% females ↓ Phosphorous: - males; 21% females ↓ Total protein: 3% males; 7% females ↑ A/G ratio: - males; 7% females ↓ Absolute thymus weight: 32% males; 14% females Thymic lymphocytosis (always slight grade): 2/5 vs 0/5 controls males; 4/5 vs 2/5 controls females <u>3000 ppm (311/314 mg/kg bw/day males/females)</u> ↓ Haematocrit: 10% males; ND females ↓ Total lymphocyte count: 11% males; 33% females ↓ Calcium: 4% males; 5% females ↓ Phosphorous: - males; 19% females ↓ Total protein: 3% males; 9% females ↑ A/G ratio: ND males; 13% females ↓ Absolute thymus weight: ND males; 14% females Thymic lymphocytosis (always slight grade): 4/5 vs 0/5 controls males; ND females <u>600 ppm (63/64 mg/kg bw/day males/females)</u> ↓ Haematocrit: ND males; 5% females ↓ Haemoglobin: 4% males; ND females ↓ Calcium: 4% males; 5% females ↓ Phosphorous: ND males; 15% females ↓ Total protein: 3% males; 6% females ↑ A/G ratio: ND males; 9% females Thymic lymphocytosis (always slight grade): 3/5 vs 0/5 controls males; ND females <u>120 ppm (13 mg/kg bw/day males & females)</u> No adverse effects. **Conclusion:** **NOAEL: 311 mg/kg bw/day** **LOAEL: 1518 mg/kg bw/day**	Confidential study number 48

90-day oral toxicity study

4 week recovery period

OECD GT 408 (1998)

GLP

Oral (continuous in diet)

Rat

Han Wistar

10/sex/group

5/sex/control & high dose groups for recovery phase

Valifenalate (IR5885)

Purity: 98.9%

0, 7, 150, 1000 mg/kg bw/day

Vehicle: laboratory animal diet

There were no deaths or overt signs of toxicity in any dose group.

Confidential study number 49

<u>1000 mg/kg bw/day</u>

	males	females
↓ Haematocrit	5%	ND
↓ Haemoglobin	4%	ND
↓ Red blood cell	2%	ND
↓ White blood cell	13%	ND
↓ Monocyte count	28%	ND
↑ Platelet count	7%	ND
↓ Prothrombin time	10%	ND
↓ Neutrophil count	ND	31%
↓ Triglycerides	36%	ND
↑ Chloride	2%	ND
↑ Calcium	ND	3%
↑ Urine volume	60%	68%
↓ Specific gravity	ND	1039 g/l vs 1050 g/l control
↑ pH	7.3 vs 6.9 controls	6.4 vs 5.9 controls
↑ Relative liver weight	15%	13%
Distended caecum	7/10 vs 0/10 controls	1/10 vs 0/10 controls

<u>150 mg/kg bw/day</u>

	males	females
↓ Haematocrit	2%	ND
↓ Haemoglobin	3%	ND
↓ White blood cell	24%	ND
↓ Monocyte count	24%	ND
↑ Platelet count	11%	ND
↓ Prothrombin time	8%	ND
↓ Triglycerides	34%	ND
↑ Chloride	1%	ND
↑ pH	7.3 vs 6.9 controls	6.4 vs 5.9 controls

<u>7 mg/kg bw/day</u>

	males	females
↑ pH	7.3 vs 6.9 controls	ND

Recovery from all treatment-related effects occurred in the 4 weeks recovery period.

Conclusion:
NOAEL: 150 mg/kg bw/day
LOAEL: 1000 mg/kg bw/day

52-week chronic toxicity (from 2 year study)

OECD TG 453 (1981)

<u>1000 mg/kg bw/day</u>

Confidential study number 51

	males	females
↓ Body weight	9%	ND
↓ Haemoglobin	2.5-3.8%	ND
↓ Red cell count and mean cell haemoglobin	1.4-3.5%	ND

GLP Oral (continuous in diet) Rat Han Wistar 20/sex/group Valifenalate (IR5885) Purity: 99.56% 0, 15, 150, 1000 mg/kg bw/day Vehicle: laboratory animal diet	concentration			
	↑ Platelet count	9-16%	10%	
	↑ APTT time	19-28%	ND	
	↑ Urine volume	ND	75-210%	
	↓ Specific gravity	1035-1041 g/l vs 1047-1066 g/l controls	ND	
	↑ Relative liver weights	19%	12%	
	↑ Relative kidney weights	8%	ND	
	Thyroid follicular cell hypertrophy	10 slight + 1 moderate vs 3 slight controls	ND	
	<u>150 mg/kg bw/day</u>			
		males	females	
	↓ Mean cell haemoglobin concentration	1.7%	ND	
	Thyroid follicular cell hypertrophy	5 slight vs 3 slight controls	ND	
	<u>15 mg/kg bw/day</u>			
		males	females	
	Thyroid follicular cell hypertrophy	2 slight vs 3 slight controls	ND	
	Conclusion: **NOAEL: 150 mg/kg/day** **LOAEL: 1000 mg/kg/day**			
28-day dermal toxicity study OECD TG 410 (1981) GLP Dermal (6 hours/day) Rat Han Wistar 10/sex/group Valifenalate (IR5885) Purity: 99.6% 0, 1000 mg/kg bw/day Vehicle: sterile water	No treatment-related effects **Conclusion:** **NOEL: 1000 mg/kg bw/day**			Confidential study number 23

Two generation reproduction (one litter)

OECD TG 416 (2001)

GLP

Oral (continuous in diet)

Rat

HanBrl:WIST

Valifenalate (IR5885)

Purity: 99.56%

0, 1250, 4300 or 15000 ppm (reduced to 0, 850, 2900 or 10000 ppm during lactation)

Vehicle: laboratory animal diet

Parental toxicity

Confidential study number 27

<u>15000 ppm (10000 ppm) – 986/1150 mg/kg bw/day, males/ females (P generation - pre-pairing)</u>

	P		F1	
	male	female	male	female
↑ Absolute liver weight	16%	15%	12%	8%
↑ Relative liver weight	20%	11%	14%	10%
Hepatocellular hypertrophy	15/24 (severity 2.4) vs 4/24 (severity 1.3) controls	3/24 (severity 2.0) vs 0/24 controls	21/24 (severity 2.2) vs 2/24 (severity 2.0) controls	21/24 (severity 1.9) vs 0/24 controls
Glycogen deposition liver	17/24 (severity 1.3) vs 21/24 (severity 1.6) controls	15/24 (severity 1.3) vs 15/24 (severity 2.3) controls	19/24 (severity 1.5) vs 23/24 (severity 2.7) controls	2/24 (severity 1.0) vs 13/24 (severity 1.8) controls
Ruffled fur early lactation	ND	ND	ND	4/24
↓ Absolute kidney weight	ND	ND	ND	7%
↓ Relative kidney weight	ND	ND	ND	6%
Thyroid follicular hypertrophy	ND	ND	22/24 (severity 2.1) vs 17/24 (severity 1.4) controls	19/24 (severity 1.6) vs 10/24 (severity 1.1) controls

<u>4300 ppm (2900 ppm) – 277/318 mg/kg bw/day, males/ females (P generation - pre-pairing)</u>

	P		F1	
	male	female	male	female
↑ Absolute liver weight	6%	ND	6%	ND
↑ Relative liver weight	9%	ND	8%	ND
Hepatocellular hypertrophy	7/24 (severity 1.3) vs 4/24 (severity 1.3) controls	ND	17/24 (severity 2.3) vs 2/24 (severity 2.0) controls	ND
Glycogen deposition liver	17/24 (severity 1.3) vs 21/24 (severity 1.6) controls	17/24 (severity 1.8) vs 15/24 (severity 2.3) controls	23/24 (severity 1.9) vs 23/24 (severity 2.7) controls	7/24 (severity 1.4) vs 13/24 (severity 1.8) controls

Ruffled fur early lactation	ND	ND	ND	4/24
Thyroid follicular hypertrophy	ND	ND	16/24 (severity 1.8) vs 4/24 (severity 1.3) controls	16/24 (severity 1.8) vs 17/24 (severity 1.7) controls

1250 ppm (850 ppm) – 80/92 mg/kg bw/day, males/ females (P generation - pre-pairing)

No treatment related effects in both P and F1 generations

Conclusion:
NOAEL parental toxicity: 80 mg/kg bw/day
LOAEL parental toxicity: 318 mg/kg bw/day

The 28-days, 90-days and 53-weeks repeated dose toxicity studies in rats showed that valifenalate was able to induce minor changes in blood and clinical chemistry (Table 5). Although these changes were consistent among different studies, the severity is relatively low. The CLH-report provides historical control data (HCD) showing that the minor differences between treated and control animals were of no toxicological relevance because the records of the altered parameters were within the HCD. Therefore, RAC notes that the changes in blood and clinical chemistry found in the repeated dose toxicity studies in rat do not support a classification as STOT RE.

The repeated dose toxicity studies in rat suggest that thymus is a potential target organ of valifenalate. Indeed, decreases in absolute thymus weight and increases in thymic lymphocytosis were used for setting the LOAEL of the 28-days repeated dose toxicity study (Table 5).

Thyroid follicular cell hypertrophy was reported in the 52-weeks repeated toxicity study and in the F1 generation of the 2-generation reproduction toxicity study (Table 5), although in the latter the meaning of this effect is unclear because no clear dose response was found and high background incidence was noted. Overall, RAC notes that these thyroid effects could support a potential classification as STOT RE.

The incidence of distended caecum was also clearly increased in males versus controls in the 90-days repeated dose toxicity study (Table 5). The toxicological significance of this effect is still unclear but RAC notes that it could support a potential classification as STOT RE.

The repeated dose toxicity studies in rat suggest that also liver is a target organ of valifenalate. Increases in liver weight were reported in the 90-days, 52-weeks and 2-generation oral toxicity studies (Table 5). RAC notes that these increases in liver weight were moderate and can be an adaptive response to valifenalate administration and therefore cannot be considered for setting classification as STOT RE. A dose-dependent hepatocellular hypertrophy was reported in both P and F1 generations in the 2-generation study (Table 5). However, liver hypertrophy is cited in the Guidance on the Application of the CLP Criteria as an adaptive (compensatory) response that is generally reversible with no adverse consequences on cessation of exposure. Thus, the observed liver hypertrophy does not warrant classification as STOT RE.

Glycogen deposition in liver was reported in the 2-generation toxicity study (Table 5).

However, RAC notes that no clear dose-response was observed and there was also a high incidence in control groups. Thus, the observed glycogen deposition in liver does not warrant a potential classification as STOT RE.

***Table 6*:** *Summary of repeated dose toxicity studies in mice with valifenalate*. In all cases the effects were statistically different from controls for at least p<0.05. ND = No statistical differences with control.,

Method	Results	Reference
28-day oral toxicity study Based on OECD TG 407 (1995) but no compliance claimed Preliminary study for a 90 day Valifenalate (IR5885, batch no. FCF/T/180-00 (ex ZI068) Purity: 98.9% 0, 110, 440, 1750 and 7000 ppm Vehicle: laboratory animal	7000 ppm (1105/1536 mg/kg bw/day males/females)	Confidential study number 48

	males	females
↓ Haematocrit	10%	ND
↓ Haemoglobin	11%	ND
↓ Red blood cell	10%	ND
↑ Glucose	39%	31%
↓ Triglycerides	ND	71%
↑ Cholesterol	31%	ND
↑ Potassium	15%	19%
↓ Sodium	ND	2%
↓ Chloride	ND	3%
↓ Total protein	ND	10%
↓ Albumin	ND	7%
↑ A/G ratio	ND	4%
↑ Relative liver weight	52%	41%
↑ Relative adrenal weights	45%	ND
Centrilobular hepatocytic hypertrophy	4 slight + 2 moderate vs 0/6 controls	5 (slight) vs 1 slight control

1750 ppm (266/402 mg/kg bw/day males/females)

	males	females
↓ Haematocrit	4%	ND
↓ Haemoglobin	6%	ND
↓ Red blood cell	5%	ND
↑ Glucose	38%	32%
↓ Triglycerides	ND	44%
↑ Potassium	ND	2%
↓ Chloride	ND	3.5%
↓ Total protein	ND	4%
↓ Albumin	ND	3%
↑ A/G ratio	ND	2%
↑ Relative liver weight	31%	14%
Centrilobular hepatocytic hypertrophy	6 slight vs 0/6 controls	2 moderate vs 1 slight control

440 ppm (68/96 mg/kg bw/day males/females)

	males	females
↑ Relative liver weight	ND	10%
Centrilobular hepatocytic hypertrophy	6 slight vs 0/6 controls	ND

110 ppm (18/27 mg/kg bw/day males/females)

No treatment-related effects

Conclusion:
NOAEL: 68 mg/kg bw/day
LOAEL: 266 mg/kg bw/day

90-day oral toxicity study

Based on OECD TG 408 (1998) but no compliance claimed

Prelim carcinogenicity study

GLP

Oral (continuous in diet)

Mouse

CD-1

10/sex/group

Valifenalate (IR5885, batch no. FCF/T/180-00 (ex ZI068)

Purity: 98.9%

0, 110, 900 and 7000 ppm

Vehicle: laboratory animal diet

Confidential study number 50

7000 ppm (995/1144 mg/kg bw/day males/females)

	males	females
↓ Body weight gain weeks 0-13	26%	ND
↓ Haematocrit	4%	5%
↓ Haemoglobin	4%	3%
↓ Mean cell haemoglobin	5%	3%
↓ Mean cell volume	5%	4%
↑ Relative liver weight	51%	35%
Centrilobular hepatocellular vacuolation	4 slight + 4 moderate vs 1 minimal + 1 slight controls	ND
Periportal hepatocellular vacuolation	1 minimal + 1 slight + 1 moderate vs 0/10	ND

900 ppm (133/147 mg/kg bw/day males/females)

	males	females
Relative liver weight	12%	ND

110 ppm (15/16 mg/kg bw/day males/females)

No treatment-related effects

Conclusion:
NOAEL: 133 mg/kg bw/day
LOAEL: 995 mg/kg bw/day

Carcinogenicity (1.5-year) study

OECD TG 451

Mouse

Crl: CD-1™ (ICR) BR

50/sex/group

Valifenalate (IR5885)

Purity: 99.56%

0, 150, 850,

Confidential study number 52

Non-neoplastic findings

5000 ppm (657/756 mg/kg bw/day)

	males	females
↓ Body weight	22%	ND
↑ Relative liver weight	97%	23%
↑ Relative kidney weight	ND	12%
↑ Centrilobular hepatocyte hypertrophy	ND	22 slight + 3 moderate vs 5 slight + 2 moderate + 1 marked controls
Generalised hepatocyte hypertrophy	18 slight + 11 moderate vs 3 slight controls	ND

5000 ppm

Continuous dietary administration for 78 weeks

Achieved doses 16.8, 97.2 and 657 mg/kg/day for males and 21.6, 124 and 756 mg/kg/day for females

Centrilobular hepatocyte vacuolation	11 slight + 20 moderate + 1 marked vs 3 minimal + 7 slight + 1 moderate controls	ND
Cytoplasmic eosinophilia in hepatocytes	29/50 vs 0/50 controls	ND
Pigment in hepatocytes	18/50 vs 0/50 controls	13/50 vs 0/50 controls
Pigment in hepatocyte macrophages	12/50 vs 1/50 controls	31/50 vs 12/50 controls
Gall bladder choleliths	ND	8/45 vs 1/47

850 ppm (97.2/124 mg/kg bw/day)

	males	females
↑ Relative liver weight	29%	ND
Centrilobular hepatocyte vacuolation	2 minimal + 11 slight + 22 moderate vs 3 minimal + 7 slight + 1 moderate controls	ND

150 ppm (17/22 mg/kg bw/day)

	males	females
Centrilobular hepatocyte vacuolation	21 slight + 13 moderate vs 3 minimal + 7 slight + 1 moderate controls	ND

Conclusion:
NOAEL: 17 mg/kg bw/day
LOAEL: 97 mg/kg bw/day

The effects reported in mice (Table 6) were consistent with the effects reported in rats (Table 5). Moderate alterations of blood and clinical values were reported in the 28-days and 90-days repeated toxicity studies (Table 6). The incidence of these alterations were relatively moderate and, in concordance with changes reported in rats, RAC does not consider these effects enough robust for supporting a STOT RE classification.

The studies in mice also highlight liver as target organ of valifenalate. Moderate increases in relative liver weight (up to 50%) were noted in the 28-days and 90-days repeated dose toxicity studies (Table 6). This increase was more notable (around 100%) in the carcinogenicity study (Table 6). Histopathological alterations in liver were noted in several studies in mice. These alterations include mainly hepatocyte hypertrophy and vacuolation, cytoplasmic eosinophilia and hepatocyte and macrophage pigmentation (Table 6). RAC notes that all these changes in liver are indeed adaptive responses by the same reason outlined in the case of rat studies and therefore should be considered for setting classification as STOT RE.

Other effects were also described in these repeated dose toxicity studies in mice as 45% increase in adrenal weight, 12% increase in relative kidney weight and increases in incidences of gall bladder choleliths (Table 6). However, RAC notes that these effects were not consistently reported among different studies in mice and were not noted in rat and dog studies and therefore RAC does not consider these effects for classification as STOT RE.

Table 7**: Summary of repeated dose toxicity studies in dogs with valifenalate.** In all cases the effects were statistically different from controls for at least $p<0.05$. ND = No statistical differences with control.,

<table>
<tr><th>Method</th><th>Results</th><th>Reference</th></tr>
<tr><td>28-day oral toxicity study

OECD TG 409 (1998)

GLP

Oral (capsule)

Dog

Beagle

3/sex/group

Valifenalate (IR5885)

Purity: 98.9

0, 250, 500 and 1000 mg/kg bw/day

Vehicle: gelatine capsule</td><td><u>1000 mg/kg bw/day</u>

<table><tr><th></th><th>males</th><th>females</th></tr><tr><td>↑ Pale faeces</td><td>3/3</td><td>2/3</td></tr><tr><td>↓ Cholesterol</td><td>60%</td><td>67%</td></tr><tr><td>↓ Phospholipid</td><td>53%</td><td>61%</td></tr><tr><td>↑ Alkaline phosphatase</td><td>203%</td><td>ND</td></tr><tr><td>↑ Gamma glutamyl-transferase</td><td>80%</td><td>ND</td></tr><tr><td>↑ Total protein</td><td>13%</td><td>18%</td></tr><tr><td>↓ Albumin</td><td>20%</td><td>23%</td></tr><tr><td>↓ Calcium</td><td>8%</td><td>11%</td></tr><tr><td>↓ Magnesium</td><td>10%</td><td>ND</td></tr><tr><td>↑ Phosphorous</td><td>18%</td><td>ND</td></tr><tr><td>↑ Absolute liver weight</td><td>66%</td><td>33%</td></tr><tr><td>Hepatocellular glycogen content</td><td>0/3 vs 2/5 (severity 2.5) controls</td><td>1/3 (severity 1.0) vs 3/3 (severity 3.0) controls</td></tr><tr><td>Hepatocellular hypertrophy</td><td>3/3 (severity 4.0) vs 1/3 (severity 1.0) controls</td><td>3/3 (severity 3.3) vs 0/3 controls</td></tr><tr><td>Liver eosinophilic cytoplasmic inclusions</td><td>3/3 (severity 2.3) vs 0/3 controls</td><td>2/3 (severity 3.0) vs 0/3 controls</td></tr><tr><td>Liver single cell necrosis</td><td>3/3 (severity 1.0) vs 0/3 controls</td><td>1/3 (severity 0.33) vs 0/3 controls</td></tr><tr><td>Liver apoptosis</td><td>1/3 (severity 0.6) vs 0/3 controls</td><td>ND</td></tr></table>
<u>500 mg/kg bw/day</u>

<table><tr><th></th><th>males</th><th>females</th></tr><tr><td>↑ Pale faeces</td><td>3/3</td><td>ND</td></tr><tr><td>↓ Cholesterol</td><td>41%</td><td>52%</td></tr></table></td><td>Confidential study number 7</td></tr>
</table>

	↓ Phospholipid	38%	44%
	↑ Total protein	9%	14%
	↓ Albumin	18%	21%
	↓ Calcium	ND	10%
	↑ Absolute liver weight	49%	42%
	Hepatocellular glycogen content	3/3 (severity 2.0) vs 2/3 (severity 2.5) controls	3/3 (severity 2.0) vs 3/3 (severity 3.0) controls
	Hepatocellular hypertrophy	3/3 (severity 3.0) vs 1/3 (severity 1.0) controls	3/3 (severity 2.7) vs 0/3 controls
	Liver eosinophilic cytoplasmic inclusions	3/3 (severity 2.0) vs 0/3 controls	2/3 (severity 1.5) vs 0/3 controls

250 mg/kg bw/day

	males	females
↓ Cholesterol	42%	19%
↓ Phospholipid	40%	ND
↑ Total protein	8%	ND
↓ Albumin	23%	ND
Hepatocellular glycogen content	3/3 (severity 2.7) vs 2/3 (severity 2.5) controls	3/3 (severity 1.3) vs 3/3 (severity 2.0) controls
Liver eosinophilic cytoplasmic inclusions	2/3 (severity 1.5) vs 0/3 controls	1/3 (severity 1.0) vs 0/3 controls

Conclusion:
NOAEL: 500 mg/kg bw/day
LOAEL: 1000 mg/kg bw/day

90-day oral toxicity study

OECD TG 409 (1998)

GLP

Oral (capsule)

Dog

Beagle

4/sex/group

Valifenalate (IR5885)

750 mg/kg bw/day

1 female taken off-dose after 7 weeks due to weight loss adverse laboratory results and retained until the end of the study

White discoloured faeces or white/yellow powder in faeces from day 3, 7/8 dogs

	males	females
↓ Body weight gain	48%	33%
↓ Food consumption	12%	12%
↑ Platelets	Up to 33%	Up to 74%
↓ RBC	8%	9%
↑ MCH	9%	10%
↑ MCV	7%	ND
↓ Reticulocytes	50%	Up to 60%

Confidential study number 12

Purity: 98.56%

0, 50, 250 and 750 mg/kg bw/day

Vehicle: gelatine capsule

↑ ALP	Up to 517%	Up to 446%
↑ ALT	Up to 109%	Up to 303%
↑ GGT	Up to 133%	Up to 133%
↓ Cholesterol	Up to 60%	Up to 69%
↓ Total protein	Up to 18%	Up to 17%
↓ Albumin	Up to 23%	Up to 28%
↑ AST	28%	24%
↑ Glucose	12%	22%
↑ Relative liver weight	60%	70%
↑ Relative thyroid/parathyroid weights	64%	ND
↓ Prostate weight	64%	ND
↓ Testis weight	28%	ND
↑ Epididymis weight	14%	ND
Hepatocyte hypertrophy	4 moderate vs 0/4 controls	3 moderate vs 0/4 controls
Hepatocytes pale cytoplasm, peripheral clumping	2 slight + 2 moderate vs 0/4 controls	3 moderate vs 0/4 controls
Eosinophilic intracytoplasmic inclusions in hepatocytes	2 slight + 2 moderate vs 0/4 controls	1 slight + 2 moderate vs 0/4 controls
Thyroid follicular hypertrophy	1 minimal + 1 slight vs 0/4 controls	2 minimal vs 0/4 controls

<u>250 mg/kg bw/day</u>

↑ white discoloured faeces or white/yellow powder in faeces from day 10, 5/8 dogs

	males	females
↓ Body weight gain	21%	ND
↑ Platelets	Up to 42%	ND
↓ Reticulocytes	31%	39%
↑ ALP	Up to 430%	Up to 194%
↑ ALT	ND	42%
↑ GGT	33%	33%
↓ Cholesterol	Up to 47%	Up to 36%
↓ Total protein	Up to 13%	Up to 11%
↓ Albumin	Up to 20%	Up to 13%
↑ AST	ND	29%
↑ Relative liver weight	44%	34%
↑ Relative thyroid/parathyroid weights	61%	ND
Hepatocyte hypertrophy	2 slight + 2 moderate vs 0/4 controls	1 minimal + 1 slight + 2 moderate vs 0/4 controls
Hepatocytes pale	2 slight + 2	1 minimal + 1

	cytoplasm, peripheral clumping	moderate vs 0/4 controls	slight + 2 moderate vs 0/4 controls
	Eosinophilic intracytoplasmic inclusions in hepatocytes	2 slight + 2 moderate vs 0/4 controls	3 minimal + 1 slight vs 0/4 controls
	Thyroid follicular hypertrophy	1 minimal vs 0/4 controls	2 slight vs 0/4 controls

50 mg/kg bw/day

	males	females
↑ ALP	Up to 142%	Up to 134%
↑ Relative liver weight	-	33%
Hepatocyte hypertrophy	3 minimal + 1 slight vs 0/4 controls	2 minimal + 2 slight vs 0/4 controls
Thyroid follicular hypertrophy	ND	1 slight vs 0/4 controls

Conclusion:
NOAEL: 250 mg/kg bw/day
LOAEL: 750 mg/kg bw/day

52-week chronic toxicity

Additionally 13 weeks sub-chronic toxicity with 8 week recovery

OECD TG 452 (1981)

GLP

Oral (capsule)

Dog

Beagle

4/sex/group

Valifenalate (IR5885)

Purity: 99.56%

0, 1, 7, 50 and 250 mg/kg

250 mg/kg bw/day

	males	females
↑ Platelets	Up to 74%	ND
↑ ALP	Up to 1360%	Up to 746%
↓ Cholesterol	28%	25%
↓ Total protein	Up to 13%	Up to 10%
↓ Albumin	Up to 19%	Up to 16%
↑ Triglycerides	91%	ND
↓ Calcium ions	Up to 8%	ND
↑ Relative liver weight	61%	36%
↑ Relative thyroid/parathyroid	31%	ND
↓ Relative prostate weight	29%	ND
↓ Relative ovary weights	ND	57%
Hepatocyte hypertrophy	3 slight + 1 moderate vs 0/4 controls	3 slight + 1 moderate vs 0/4 controls
Hepatocytes with pale cytoplasm and peripheral clumping hypertrophy	4 minimal vs 0/4 controls	3 minimal vs 0/4 controls

50 mg/kg bw/day

	males	females
↑ ALP	Up to 217%	Up to 398%
↓ Relative ovary weights	-	48%

Confidential study number 65

bw/day

Vehicle: gelatine capsule

Hepatocyte hypertrophy	2 minimal + 2 slight vs 0/4 controls	3 minimal + 1 slight vs 0/4 controls
Hepatocytes with pale cytoplasm and peripheral clumping hypertrophy	ND	1 minimal vs 0/4 controls

7 mg/kg bw/day

	males	females
↑ ALP	165%	150%
Hepatocyte hypertrophy	1 minimal vs 0/4 controls	1 minimal + 1 slight vs 0/4 controls

1 mg/kg bw/day

	males	females
↑ ALP	ND	55%

Conclusion:
NOAEL: 50 mg/kg bw/day
LOAEL: 250 mg/kg bw/day

The database with dogs shows a scenario consistent with information obtained with rats and mice. Alterations in clinical and blood chemistry were noted in the three available studies. However, most of these changes were of low magnitude; the largest changes reported were the high increase of transaminase activities (ALP and ALT) (Table 7). RAC notes that the changes in transaminases are secondary to liver response and therefore should not be considered as supporting for classification as STOT RE.

The assessment of the dog studies shows again the liver as target organ of valifenalate. Indeed, increases in relative liver weight, hepatocellular hypertrophy and liver eosinophilic cytoplasmic inclusions were consistently reported through the whole database. Again, as in the case of rats and mice, RAC noted that at exposure levels below the guidance values, these changes are adaptive responses rather than adverse effects and therefore cannot be used as basis for supporting a classification. However, RAC notes certain incidences of liver single cell necrosis in the 28-day study. On the opposite to hypertrophy, necrosis is a non-reversible event that might notably alter the performance of liver and therefore should be taken into consideration for classification as STOT RE.

Some changes were noted in reproductive organs (reductions in prostate, testis and ovary weight and increases in epididymis weight) (Table 7). However, these alterations will be assessed within the reproductive toxicity hazard class and not for STOT RE. The thyroid, in the mice studies, exhibited certain alterations after valifenalate exposure. These changes were mainly reduction in relative thyroid/parathyroid and thyroid follicular hypertrophy (Table 7). However, RAC noted that these effects were not reported in all studies and no dose-response was observed in the case of thyroid follicular hypertrophy (Table 7). Overall, RAC does not consider the effects in thyroid robust enough for supporting a potential classification as STOT RE.

Comparison with the criteria

Table 8 summarises all findings of Tables 5, 6 and 7 on adverse effects relevant for STOT-RE classification that were consistently observed in available repeated toxicity studies.

Table 8: *Adverse effects of valifenalate relevant for STOT-RE classification.* **Bolded text** refers to those effects that appear at doses relevant for classification as STOT RE.

Effect	Study	Lowest reported dose (mg/kg bw/day)	Guidance value for STOT-RE classification Cat 1/Cat 2 (mg/kg bw/day)
↓ Absolute thymus weight, thymic lymphocytosis, distended caecum	28-day study (rats)	1518	30/300
Thyroid follicular cell hypertrophy	52-week (rats)	1000	2.5/25
Thyroid follicular cell hypertrophy	2-generation reproduction (rats)	277	8.9/89 (assuming 112 days of exposure)
Liver single cell necrosis	28-days study (dogs)	1000	30/300

Table 8 shows as none of the effects considered for supporting a classification as STOT RE appear at concentrations within the corresponding guidance values. Therefore, RAC supports **no classification of valifenalate for STOT RE** based on the observed effects.

10.13 Aspiration hazard

Not relevant for solid substances.

Table 56: Summary table of evidence for aspiration hazard

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

10.13.1 Short summary and overall relevance of the provided information on aspiration hazard

Data lacking

10.13.2 Comparison with the CLP criteria

Because of the lack of data, a definitive conclusion on aspiration cannot be made.

10.13.3 Conclusion on classification and labelling for aspiration hazard

CLP: Data lacking

RAC evaluation of aspiration toxicity

Summary of the Dossier Submitter's proposal

DS proposed no classification of valifenalate for aspiration toxicity based on data lacking.

Comments received during consultation

No comments were received during consultation.

Assessment and comparison with the classification criteria

RAC notes that the hazard class aspiration toxicity is not relevant for solids and therefore **supports no classification for valifenalate**.

11 EVALUATION OF ENVIRONMENTAL HAZARDS

11.1 Rapid degradability of organic substances

Table 57: Summary of relevant information on rapid degradability

Method	Results	Remarks	Reference
Effects on the activity of sludge micro-organisms OECD 209 GLP	Respiration rate EC_{50} >100 mg/L (higher than water solubility)	Test material: valifenalate technical Purity: 97.97 w/w % Reference item: 3,5-dichlorophenol (EC_{50} 21.9 mg/L)	*See Annex conf. 40.*
Ready biodegradability EEC method C.4-D (1992) Manometric Respirometry Test; OECD 301 F GLP	Not readily biodegradable	Test material: valifenalate Purity: 99.56 w/w % Reference item: aniline (43 % biodegradation within 14 d and 50 % biodegradation after 28 d incubation, based on $ThOD_{NH4}$.)	*See Annex conf. 15.*

The were no adverse effects of valifenalate technical on the respiration rate of activated sludge (*See Annex conf. 40.*). In a biodegradability test performed with manometric respirometry (*See Annex conf. 15.*), valifenalate was reported to be not readily biodegradable.

Study 1: Effects on the activity of sludge micro-organisms (*See Annex conf. 40.*)

The purpose of this study was to determine potential effects of the test item on the activity of micro-organisms of activated sludge from a sewage treatment plant.

Based on the results of a non-GLP range-finding test and agreed with the sponsor/study monitor one test item treatment (5 replicates), one control treatment (two replicates) and one solvent control (two replicates) were tested. The test concentration of the test item has been chosen on the basis of the range finding test and the water solubility of the test item (24.1 mg/L at room temperature).

The method is based on the measurement of the respiration rate of micro-organisms (measured as oxygen consumption) after a contact time of three hours with the test item. The respiration rate is measured over a period of ten minutes.

No adverse effects of the test item valifenalate technical on the activity of the micro-organisms of activated sludge were observed at the tested concentration of 100 mg/L (limit test) compared to the solvent control. The organic solvent (methanol) did not show significant inhibition of the activity of the micro-organisms (measured as O_2 consumption).

Therefore it is concluded that the EC_{50} is higher than 100 mg/L (i.e. higher than the water solubility 24.1 mg/L).

Study 2: Ready biodegradability (*See Annex conf. 15.*)

Ready biodegradability of valifenalate was investigated in a biodegradability test performed with manometric respirometry. The test item was exposed to activated sludge from the aeration tank of a domestic waste water treatment plant for 28 days. The biodegradation was followed by the oxygen uptake of the micro-organisms during exposure. As a reference item aniline was tested simultaneously under the same conditions as the test item, and functioned as a procedure control. This study is recognised by the OECD and EEC guidelines and should provide a basis to assess the ready biodegradation properties of the test item when incubated with activated sludge. Under the test conditions the percentage biodegradation of valifenalate reached 3 % after 28 days of incubation, based on $ThOD_{NH4}$. If the calculation is based on $ThOD_{NO3}$, a mean of 2% biodegradation was found after 28 days of incubation. Valifenalate can therefore be considered to be not readily biodegradable. In the toxicity control containing both the test item and the reference item Aniline, 43% biodegradation was noted within 14 days and 50% biodegradation was determined after 28 days of incubation, based on $ThOD_{NH4}$.

11.1.1 BOD_5/COD

No data available.

11.1.2 Hydrolysis

Table 58: Summary of relevant information on hydrolysis

Method	Results	Remarks	Reference
Hydrolysis rate at pH 4, 7 and 9 under sterile conditions in the absence of light OECD 111 GLP	pH 4: no significant degradation pH 7: DT50 = 2.09 d (65ºC) DT50 = 5.21 d (55ºC) DT50 = 7.62 d (50ºC) DT50 = 90.94 d (25ºC) (estimated using Arrhenius plot) pH 9: DT50 = 0.33 d (50ºC) DT50 = 4.15 d (25ºC)	Pseudo first order kinetics; two main compounds found: valifenalate and IR5839. Chemical purity of the test material: > 99 w/w % Radiochemical purity of the test material: >97% Specific activity: 5.089 MBq/mg	*See Annex conf. 35.*

Study 1: Hydrolysis as pH 4, 7 and 9 (*See Annex conf. 35.*)

The hydrolysis rate of valifenalate was determined in three buffered aqueous solutions (pH 4, 7 and 9) at a concentration of 1 µg/mL. The study was carried out in the absence of light, under sterile conditions. Study results showed that no significant degradation of ^{14}C-valifenalate occurred in buffered solution at pH 4, while at pH 7 and pH 9 a pseudo-first order kinetic hydrolysis reaction was observed. The values of DT_{50} (Disappearance Time for 50% of the starting concentraton)and DT_{90} were determined for pH 7 and 9 at different temperatures (See Table 55 above).

Two main compounds found were the unchanged parent substance valifenalate and IR5839 (3-(4-chlorophenyl)-3-({(2S)-2-[(isopropoxycarbonyl) amino]-3-methylbutanoyl}amino) propanoic acid, also referred to as IR5885 acid). For both of the compounds the diasteroisomeric ratio (S,R/S,S) was approximately 1:1.

In conclusion, the parent compound was hydrolytically stable at pH 4 (50°C). The hydrolytic degradation of valifenalate increased with higher pH values. The major hydrolytic product in this study was IR5839.

Photochemical degradation in water is not expected to be significant since the molar absorption coefficient (ε) is <10 $M^{-1} \times cm^{-1}$ at λ >290 nm.

11.1.3 Other convincing scientific evidence

In a water-sediment study carried out using radiolabelled valifenalate, the DT_{50} value of valifenalate in the water-sediment system were 4.51-4.7 days, while the two main degradation products were IR5839 and PCBA. No photochemical degradation study has been performed with valifenalate.

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

No other relevant data available.

11.1.3.2 Inherent and enhanced ready biodegradability tests

No data available.

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

Table 59: Summary of relevant information on water-sediment and soil degradation data

Method	Results	Remarks	Reference
Degradation in - water/sediment OECD Guideline 308 GLP	DT_{50} whole system: 4.5 d (Pond) and 4.71 d (River) DT_{90} whole system: 14.9 d (Pond) and 15.64 d (River)	Chemical purity of the test material: > 99 w/w % Radiochemical purity of the test material: > 98% Surface water - The radioactivity: 40.84% AR (Pond) and 43.74% AR (River). Sediment - The radioactivity increased: 50.64% AR (Pond) and 45.51% AR (River). In both aquatic systems - valifenalate degraded after 22 d: 5.92% AR (Pond) and 5.51% AR (River). The main degradation products out of eight found in the water/sediment degradation study were S2 (IR5839) and S3 (PCBA). IR5839: 52.80% AR (Pond) and 56.34% AR (River). PCBA: 13.77% AR (Pond) and 8.16% AR (River).	*See Annex conf. 38.*

NOTE: Since some of the results of the original study were found to be unreliable, data featured in the 'Remarks' column are data from the original study report, while data featured in the 'Results' column are re-calculated results from the RMS review procedure.

Study 1: Water/sediment study (*See Annex conf. 38.*)

In a water/sediment study, the degradation of ^{14}C-valifenalate was assessed in two aquatic systems, named "Pond" and "River" systems. The study was conducted in compliance with OECD and SETAC guidelines.

Samples of each aquatic system were dispensed into glass cylinders to obtain incubation units containing a 2.5 cm soil layer flooded with associated water to a depth of 10 cm. The incubation units were gently agitated on an orbital shaker. Moistened carbon dioxide-free air was drawn over the water surface and the units were maintained in the dark at 20 ± 2 °C for 32 days to allow the samples to reach the stage of equilibrium.

Following the acclimation period, ^{14}C-valifenalate was applied to each unit at the maximum recommended field application (240 g a.i./ha). Each unit was connected to a glass Dreschel containing KOH solution to trap evolved carbon dioxide.

Duplicate incubation units were collected and analysed 0, 1, 2, 4, 6, 8, 14, and 22 days after the application for both systems. The surface water and the corresponding sediment were analyzed separately. The surface water was separated from the soil by pipette and the radioactivity content was determined by Liquid Scintillation Counting (LSC). Suitable aliquots of water were concentrated and analysed by Thin Layer Chromatography (TLC) and, for representative samples, also by High Performance Liquid Chromatography (HPLC). Sediments were extracted with different solvent mixtures and the extractable radioactivity was determined by LSC. Suitable aliquots of soil extracts were combined, concentrated, and analyzed by TLC and, for representative samples, also by HPLC. The radioactivity content in KOH solution was determined by LSC. The non-extractable radioactivity was determined by LSC after oxidation by means of a biological oxidizer.

The radioactivity in the surface water decreased during all the study and it was 40.84% and 43.74% of applied radioactivity (AR) at the end of incubation period in the Pond and River systems, respectively. The radioactivity in the sediment increased throughout the study reaching 50.64% AR and 45.51% AR at the end of incubation period in the Pond and River systems, respectively.

Valifenalate degraded in both aquatic systems: after 22 days it accounted for 5.92% AR and 5.51% AR in the Pond and River systems, respectively. The DT_{50}/DT_{90} surface water values were considered to be not reliable during the RMS review and they were re-calculated with these DT_{50} lab and DT_{90} lab values, in days, being listed in Table 56 above. In the whole system the DT_{50} values were 4.5 days (Pond) and 4.71 days (River) and DT_{90} values, 14.9 days (Pond) and 15.64 days (River).

Six compounds were found in the surface water and in the sediment extracts. The main degradation products were S2 and S3: S2 reached 52.80% AR and 56.34% AR in Pond and River systems, respectively. S2 was identified as 3-(4-chlorophenyl)-3-({(2S)-2-[(isopropoxycarbonyl) amino]-3-methylbutanoyl}amino) propanoic acid (also referred to as IR5839 or IR5885 acid). The compound S3, that increased up to a maximum of 13.77% AR and 8.16% AR (in the Pond and River systems, respectively), was identified as 4-chlorobenzoic acid (also referred to as PCBA). The fraction S6 slowly increased reaching 8.93% AR and 8.04% AR. It was represented by a pool of 4 compounds and none of these reached values higher than 3.13% AR. None of the other compounds, S4 and S5, ever reached levels higher than 5% AR. The non-extractable radioactivity (bound residue) increased to 8.99% and 16.24% AR in Pond and River systems, respectively.

The radioactivity in the ^{14}C-CO_2 traps was always lower than the detection limit in both the systems except at the last three sampling times when it reached values ranging between 0.77% AR and 1.24% AR. The ^{14}C-Mass Balance was always higher than 90% AR and ranged from 90.61% to 104.12% AR for Pond system and from 90.49% to 107.96% AR for River system.

It is concluded that valifenalate is neither readily biodegradable nor rapidly degradable in the environment.

11.1.3.4 Photochemical degradation

Since both valifenalate and its metabiltes have effectively no absorption above wavelengths greater than 290 nm (*See Annex conf. 5.*) no photochemical degradation study has been performed.

11.2 Environmental transformation of metals or inorganic metals compounds

Not applicable.

11.2.1 Summary of data/information on environmental transformation

Not applicable.

11.3 Environmental fate and other relevant information

Based on a soil adsorption/desorption study, valifenalate is a moderately mobile compound. It's physical properties (vapour pressure, water solubility) suggest no volatilisation.

11.3.1.1 Adsorption/Desorption

Table 60: Summary of relevant information on soil adsorption / desorption

Method	Results	Remarks	Reference
Soil adsorption / desorption OECD 106 GLP	Arithmetic mean/median K_{oc} = 753(mL/g) K_{Fads} = 23.2 (mL/g) K_{Foc} = 859 (mL/g) 1/n = 1.038 (-)	Chemical purity of the test material: > 99 w/w % Radiochemical purity of the test material: 99.21%	*See Annex conf. 39.*

Sudy 1: Soil adsorption/desorption (*See Annex conf. 39.*)

Batch soil adsorption / desorption studies were performed with valifenalate in five soils. The study was carried out with the following five different, characterized, fresh and sterilised soils: AR-1 – loamy sand; Stirone – clay; Cal – clay; G-2 – loam; SP-2.1 – sand.

This study was divided into three tiers, with preliminary and screening tests followed by the definitive determination of adsorption and desorption isotherms with all the five soils. The following were determined: parameters of the Freundlich equations for adsorption and desorption isotherms to study the influence of concentration on the extent of adsorption and desorption from soils and the distribution coefficient at desorption equilibrium (K_{des}, also referred to as apparent desorption coefficient).

The adsorption-desorption study was conducted under sterile conditions. All the glassware and the materials necessary for the study were sterilized at 121°C for 20 minutes by autoclaving. Handling of sterile materials and sample preparation were performed by using a bacteriological hood equipped with a UV lamp.

Table 61: K_d and K_{oc} values of valifenalate

Soil	**K_d** (mL/g)	**Organic carbon content of soil** (w/w %)	**K_{oc}** (mL/g)	**K_{Fads}** (mL/g)	**K_{Foc}** (mL/g)	**1/n** (-)
AR-1	54	14.42	375	73	506	0.998
Stirone	15	0,89	1686	19	2134	1.169
Cal	9	1.8	472	9	500	0.955
G-2	9	2.13	400	8	375	1.038
SP-2.1	8	0.9	834	7	777	1.031

Arithmetic mean/median	753	23.2	**859**	1.038

Based on the arithmetic mean values derived above, valifenalate could be categorised as a moderately mobile compound.

11.3.1.2 Volatilisation

Pure valifenalate has a vapour pressure of 9.6×10^{-8} Pa at 20°C (*See Annex conf. 42.*) and a water solubility of 24.1 mg/L at 20°C (*See Annex conf. 5.*) resulting in a calculated Henry's Law constant of 1.6×10^{-6} Pa m^3/mol (at 20°C and pH 5.4 ± 0.5). This combination of properties suggests no volatilisation and thus no significant amounts of valifenalate are to be expected in air. The Atkinson calculated oxidative photochemical degradation half life is 7.5 hours assuming a hydroxyl radical concentration of 5×10^5 molecules/cm^3 (*Fisk, 2003*).

11.4 Bioaccumulation

Table 62: Summary of relevant information on bioaccumulation

Method	Results	Remarks	Reference
Partition coefficient n-octanol/water Calculation based on solubility in water and n-octanol	Results determined at 20 °C applying the HPLC method(OECD 117). pH = 4 I° 3.07 ± 0.03 Log(P) II° 3.04 ± 0.02 pH= 7 I° 3.11 ± 0.07 Log(P) II° 3.05 ± 0.03 pH= 9 I° 3.08 ± 0.02 Log(P) II° 3.06 ± 0.03	Log P_{ow} is not pH dependent A preliminary measurement of Log P with 60% CH_3OH confirmed the obtained values higher than 3.00 (3.07) for the I° component and 3.19 for the II° component.	D'Olimpio, P. (2001)
Experimentel aqutic BCF OECD 305 GLP	BCF < 4	*Oncorhynchus mykiss* Flowthrough Chemical purity of the test material: 99.63 w/w % Radiochemical purity of the test material: 97.1%	*See Annex conf. 21.*

11.4.1 Estimated bioaccumulation

As relevant experimental data are available, estimations are not included.

11.4.2 Measured partition coefficient and bioaccumulation test data

The partition coefficient n-octanol/water was determined according to HPLC method (OECD 117). The log P_{OW} of valifenalate is 3.05 – 3.11 at pH 7 and 20 °C.

The bioconcentration and depuration of valifenalate technical in rainbow trout (*Oncorhynchus mykiss*) was investigated in edible and non-edible tissues in a dynamic flow through system. Based on the results bioconcentration factor for the whole fish was calculated.

The fish were continuously exposed to ^{14}C-valifenalate at an average high dose concentration of 893.5 µg-eq/L and an average low dose concentration of 93.5 µg-eq/L for 14 days at a temperature ranging from 13.3

to 14.8°C, a pH ranging from 8.0 to 8.2 and an oxygen concentration ranging from 8.2 to 9.5 mg/L. Thereafter, the fish were transferred to flowing untreated water and the depuration of radioactivity was monitored for 14 days.

Due to the extremely low accumulation of valifenalate in fish at both dose levels, no relevant plateau levels and consequently no half-lives or accumulation/depuration kinetics could be determined.

At the high dose level, the residual radioactivity found in fish during the whole exposure period amounted to 1160 ± 355, 2685 ± 325 and 1940 ± 367 µg-eq/kg for edibles, non-edibles and whole fish, respectively being about 2 fold higher than the high dose exposure concentration. Thereafter, radioactivity was depurated from fish during 14 days. At the end of the depuration period, concentrations ranged from 428 to 474 µg-eq/kg.

At the low dose level, the residual radioactivity found in fish during the whole exposure period amounted to 142 ± 36, 283 ± 53 and 215 ± 46 µg-eq/kg for edibles, non-edibles and whole fish, respectively being about two-fold higher than the low dose exposure concentration. Thereafter, radioactivity was depurated from fish during 14 days. At the end of the depuration period, concentrations ranged from 48 to 60 µg-eq/kg.

Based on the total radioactivity concentration in the exposure water and the residual radioactivity found in fish parts, ratios between fish and water (BCF) amounted to 1.3, 3.0 and 2.3 for edibles, non-edibles and whole fish, respectively, indicating lack of bioconcentration at both dose levels.

Analyses of radioactivity of the test water showed mainly the presence of the parent compound at both dose levels throughout the entire exposure period. Besides the constant levels of parent compound ranging on average from 96.2 to 98.0% of the radioactivity recovered, three unknown radioactive fractions W0, W2 and W3/4 were found in minor amounts (< 3% of the radioactivity recovered).

In conclusion, valifenalate technical did not bioconcentrate (BCF < 4) in rainbow trout during the exposure period.

11.5 Acute aquatic hazard

Table 63: Summary of relevant information on acute aquatic toxicity

Test material	Species	Method	Results[1]	Remarks	Reference
Acute toxicity to fish					
Valifenalate Purity 99.56 w/w %	*Oncorhynchus mykiss* (rainbow trout)	OECD 203	96 hr LC_{50} > 100 mg/L	Static Nominal concentrations	*See Annex conf. 16.* *See Annex conf. 59.*
Valifenalate Purity 99.56 w/w %	*Brachydanio rerio* (zebrafish)	OECD 203	96 hr LC_{50} >100 mg/L	Static Nominal concentrations	*See Annex conf. 19.* *See Annex conf. 25.*
Valifenalate Purity 99.63 w/w %	*Cyprinodon variegatus* (sheepshead minnow)	US EPA OPPTS 850.1075	96 hr LC_{50} >15 mg/L	Static Mean measured concentrations	*See Annex conf. 29.*
Valifenalate technical Purity 98.9 w/w %	*Lepomis macrochirus* (bluegill sunfish)	US EPA OPPTS 850.1075	96 hr LC_{50} >40 mg/L	Static Nominal concentrations	*See Annex conf.*
Acute toxicity to Aquatic invertebrates					

Valifenalate Purity 99.56 w/w %	*Daphnia magna* (water flea)	OECD 202	48 hr EC_{50} >100 mg/L Immobilization	Static Nominal concentrations	*See Annex conf. 13.* *See Annex conf. 24.*
Valifenalate Purity 99.63 w/w %	*Americamysis bahia* (mysid shrimp)	US EPA OPPTS 850.1035	96 hr LC_{50} 2.8 mg/L NOEC 1.9 mg/L Mortality	Static Mean measured concentrations	*See Annex conf. 31.*
Valifenalate Purity 99.63 w/w %	*Crassostrea virginica* (eastern oyster)	US EPA OPPTS 850.1025	96 hr EC_{50} 3.1 mg/L NOEC 1.5 mg/L Shell deposition	Static Mean measured concentrations	*See Annex conf. 32.*
Valifenalate technical Purity 98.9 w/w %	*Leptocheirus plumulosus* (marine amphipod)	US EPA OCSPP 850.1740	10 d LC_{50} >109 mg a.i./kg dry sediment 10 d NOEC: 109 mg/kg Mortality	Nominal concentrations. Dry sediment	*Aufderheide, 2015a*
Toxicity to Algae and aquatic plants					
Valifenalate Purity 99.56 w/w %	*Scenedesmus subspicatus* (green algae)	OECD 201	NOEC >100 (mg/L) 72 hr E_bC_{50} >100 mg/L 72 hr E_rC_{50} >100 mg/L	Nominal concentrations	*See Annex conf. 14.* *See Annex conf. 26.*
Valifenalate technical Purity 98.9 w/w %	*Skeletonema costatum* (marine diatom)	US EPA OCSPP 850.4500	**NOEC: 0.106 (mg/L)** 96 hr I_bC_{50} > 9.48 mg/L 96 hr I_rC_{50} > 9.48 mg/L 96 hr I_yC_{50} > 9.48 mg/L	Geometric mean measured concentrations	*Hicks, 2015b*
Valifenalate technical Purity 98.9 w/w %	*Navicula pelliculosa* (freshwater diatom)	US EPA OCSPP 850.4500	NOEC: 5.45 (mg/L) 96 hr I_bC_{50} > 5.45 mg/L 96 hr I_rC_{50} > 5.45 mg/L 96 hr I_yC_{50} > 5.45 mg/L	Geometric mean measured concentrations	*Bergfield, 2015a*
Valifenalate technical Purity 98.9 w/w %	*Anabaena flos-aquae* (green algae)	US EPA OCSPP 850.4550	NOEC:2.15(mg/L) 96 hr I_bC_{50} > 4.13 mg/L 96 hr I_rC_{50} > 4.13 mg/L 96 hr I_yC_{50} > 4.13 mg/L	Geometric mean measured concentrations	*Aufderheide, 2015b*

Valifenalate technical Purity 98.9 w/w %	*Lemna gibba* (duckweed)	US EPA OCSPP 850.4400	NOEC:5.02 (mg/L) 7 d EC_{50} >5.02 mg/L	Static-renewal Geometric mean measured concentrations	*Bergfield, 2015b*
Acute toxicity to other aquatic organisms					
Valifenalate technical Purity 98.9 w/w %	*Chironomus dilutus* (freshwater midge)	US EPA OCSPP 850.1735	10 d NOEC: 108 mg/kg Mortality 10 d NOEC: 14.1 mg/kg Growth	Static Mean measured concentrations	*Aufderheide, 2015c*

11.5.1 Acute (short-term) toxicity to fish

Study 1: Acute toxicity to rainbow trout (*See Annex conf. 16.*)

Groups of 7 young Rainbow trout (*Oncorhynchus mykiss* – length: 4.78 ± 0.48 cm; wet weight: 1.01 ± 0.23 g) were exposed in a static test to aqueous test media containing valifenalate. After a range finding test and a pre-experiment have been carried out to find out the range of concentrations to be tested in the definitive test and the solubility of valifenalate in the test medium, the limit concentration of 100 mg/L of the test item suspended in methyl cellulose and two controls containing water and water with methyl cellulose, respectively were tested in order to determine the mortality and symptoms of intoxication over periods of 2.5, 24, 48, 72, 96 hours after start of test. pH, dissolved oxygen concentration and water temperature were recorded daily in each experimental group as well as the other environmental parameters such as light intensity, light regime. Duplicate samples from the freshly prepared test media of the only test concentration and the vehicle control were taken at the start of the test in order to verify the concentration of the test item in the test media. For the determination of the stability of the test item under the test conditions and the maintenance of the test item concentrations during the test period, samples of the test media and vehicle control were taken in duplicates on Day 2 and Day 4 of exposure.

The environmental parameters (pH, dissolved oxygen concentration and water temperature) were in the acceptable range.

The analytically determined concentration of valifenalate was 95 % of the nominal concentration, on average. The active ingredient concentration was judged to be sufficiently stable during the test period of 96 hours. Thus, all results were related to the nominal concentration of the test item.

In the control and at the test concentration of 100 mg/L no mortality and no symptoms of intoxication were observed. The NOEC of valifenalate in rainbow trout resulted to be 100 mg/L, while the LC_{50} and the LOEC were higher than 100 mg/L.

Study 2: Acute toxicity to zebra fish (*See Annex conf. 19.*)

Groups of 7 juvenile zebrafish (*Brachydanio rerio)* were exposed in a static test to aqueous test media containing valifenalate. Based on preliminary tests, the range of concentrations to be tested in the definitive test and the solubility of valifenalate in the test medium, , one concentration (100 mg/L) of the test item suspended in methyl cellulose and two controls containing water and water with methyl cellulose, were tested in order to determine the mortality and symptoms of intoxication over periods of 2.5, 24, 48, 72, 96 hours after start of test. pH, dissolved oxygen concentration and water temperature were recorded daily in the test media of 100 mg/L and in the controls, as well as other environmental parameters such as light intensity, light regime. For the analytical dose verification of valifenalate duplicate samples from the freshly prepared test media of the test concentration and the vehicle control were taken at the start of the test. For the determination of the stability of the test item under the test conditions, the maintenance of the test item

concentrations during the test period, samples of the test media and vehicle control were taken in duplicate on Day 4 of exposure.

The environmental parameters (pH, dissolved oxygen concentration and water temperature) were in the acceptable range.

The analytically determined concentration of valifenalate in the test medium analysed varied between 90% to 95% of the nominal concentration. The active ingredient was judged to be sufficiently stable during the test period of 96 hours. Thus, all results were related to the nominal concentration of the test item.

In the control and at the test concentration of 100 mg/L no mortality and no signs of intoxication were observed. The NOEC of valifenalate in zebrafish resulted to be at least 100 mg/L, while the LC_{50} and the LOEC were higher than 100 mg/L.

Study 3: Acute toxicity to sheepshead minnow (*See Annex conf. 29.*)

The objective of this study was to determine the toxicity of valifenalate to sheepshead minnow, *Cyprinodon variegatus*, during a 96-hour exposure period under static test conditions.

Sheepshead minnows (mean total length: 2.7 cm; mean wet weight: 0.3 g) were exposed to a geometric series of five test concentrations, a negative control (filtered saltwater), and a solvent control (dimethyl formamide). Test chambers were 25-L stainless steel aquaria containing 20 L of test solution. Two replicate test chambers were maintained in each treatment and control group, with 10 sheepshead minnows in each test chamber, for a total of 20 fish per test concentration. Nominal test concentrations selected were 1.9, 3.8, 7.5, 15 and 30 mg valifenalate/L. All organisms were observed periodically to determine the number of mortalities in each treatment group. The numbers of individuals exhibiting signs of toxicity or abnormal behaviour were also reported. Observations were made approximately 5, 24, 48, 72 and 96 hours after test initiation.

The 96-hour LC_{50} and the LOEC for the sheepshead minnows were >15 mg valifenalate/L based on mean measured concentrations. The no observed effect concentration (NOEC) after 96 h was 15 mg a.s./L, the highest concentration tested at below which there was no toxicant related mortality or behavioural abnormalities.

Study 4: Acute toxicity to bluegill sunfish (*See Annex conf. 65*)

In a 96-hour acute toxicity study, bluegill sunfish (*Lepomis macrochirus*) were exposed to valifenalate technical at nominal concentrations 0 (control), 0 (vehicle control), and 40 mg a.i./L under static conditions in accordance with the OPPTS 850.1075 guideline. The control treatment met the acceptability criteria for survival set by the study protocol.

The 24, 48, 72 and 96-hour LC_{50} values, based on nominal concentrations, were estimated to be >40 mg a.i./L, the highest concentration tested. The 96 hour NOEC was 40 mg a.i./L, based on less than 10% mortality and a lack of observed sublethal effects at the highest nominal test substance concentration.

Table 64: Summary of acute toxicity tests with fish

Method	Species	Test material (purity)	Results	Reference
OECD 203	*Oncorhynchus mykiss* (rainbow trout)	Valifenalate (99.56 w/w %)	96 hr LC_{50} >100 mg/L	*See Annex conf. 16.*
	Brachydanio rerio (zebrafish)		96 hr LC_{50} >100 mg/L	*See Annex conf. 19.*
US EPA OPPTS 850.1075	*Cyprinodon variegatus* (sheepshead minnow)	Valifenalate (99.63 w/w %)	96 hr LC_{50} > 15 mg/L	*See Annex conf. 29.*

	Lepomis macrochirus (bluegill sunfish)	Valifenalate technical (98.9 w/w %)	96 hr LC_{50} > 40 mg/L	*See annex conf. 65*

11.5.2 Acute (short-term) toxicity to aquatic invertebrates

Study 1: Acute toxicity to *Daphnia magna* (*See Annex conf. 13.*)

A range-finding test was carried out to determine the range of concentrations to be tested in the definitive test. Groups of 20 young *Daphnia* were exposed, in static conditions, to the test item valifenalate suspended in methylcellulose for a period of 48 hours. The concentrations tested were: 4.6, 10, 21, 46 and 100 mg/L. Two control groups were tested in parallel; one containing water and the other containing water with 100 mg methyl cellulose/L. A parallel test (analytical test) was carried out to verify that the concentration of test item was above 80 % of initial concentration throughout the test period. Regarding the analytical phase, under test conditions the active ingredient was sufficiently stable during the test period (48 h) with mean of recoveries of 93% from the test samples. Concerning the biological results no significant immobility or mortality up to the highest concentration tested was observed. Thus the EC_{50} and the NOEC were determined to be higher than 100 mg/L and 100 mg/L, respectively.

Study 2: Acute toxicity to mysid shrimp (*See Annex conf. 31.*)

The objective of this study was to determine the toxicity of valifenalate to the saltwater mysid, *Americamysis bahia* (< 24 hours old), during a 96-hour exposure period under static test conditions. Saltwater mysids were exposed to a geometric series of five test concentrations, a negative control (filtered saltwater), and a solvent control (dimethyl formamide). Test chambers were 2-L glass beakers containing approximately 1.5 L of test solution. Two replicate test chambers were maintained in each treatment and control group, with 10 mysids in each test chamber, for a total of 20 mysids per test concentration. Nominal test concentrations selected were 0.50, 1.0, 2.0, 4.0 and 8.0 mg valifenalate/L.

Observations of mortality and other signs of toxicity were made approximately 5, 24, 48, 72 and 96 hours after test initiation.

Based on the test results, the 96-hour LC_{50} for *Americamysis bahia* for valifenalate with a purity of 99.56 w/w % was 2.8 mg/L, with a 95% confidence interval of 1.9 to 3.6 mg/L based on mean measured concentrations. The no observed effect concentration (NOEC) after 96 h was estimated to be 1.9 mg/L, the highest concentration tested at and below which there were no toxicant related mortality and signs of toxicity.

Study 3: Acute toxicity to *Crassostrea virginica* (*See Annex conf. 32.*)

The objective of this study was to determine the effects of valifenalate on the shell deposition of the eastern oyster, *Crassostrea virginica*, during a 96-hour exposure period under flow-through test conditions. Eastern oysters were exposed to a geometric series of five test concentrations, a negative control (filtered saltwater), and a solvent control (dimethyl formamide). Test chambers were 54-L glass aquaria filled with approximately 27 L of test water. One test chamber was maintained in each treatment and control group with 20 eastern oysters in each test chamber. Nominal test concentrations selected were 0.38, 0.75, 1.5, 3.0 and 6.0 mg valifenalate/L.

Observations of mortality and other clinical signs were made approximately 6, 24, 48, 72 and 96 hours after test initiation. Measurements of shell deposition for each oyster were made at 96 hours, and were used to determine the EC_{50} value and the no-observed-effect-concentration (NOEC). The EC_{50} is the concentration of test substance in water that is calculated to induce a 50% reduction in shell deposition, relative to the control.

Based on inhibition of shell deposition, the 96-hour EC_{50} for *Crassostrea virginica* for valifenalate was 3.1 mg/L, with a 95% confidence interval of 1.8 to 3.4 mg/L. Results are based on mean measured concentrations. The no observed effect concentration (NOEC) after 96 h was estimated to be 1.5 mg/L, based on the statistically significant inhibition of shell growth observed at 3.0 and 4.3 mg/L.

Study 4: 10-day acute toxicity to *Leptocheirus plumulosus* (*Aufderheide, 2015a*)

In a 10-day acute toxicity study the marine amphipod, *Leptocheirus plumulosus*, was exposed to valifenalate technical at nominal concentrations of 0 (control) and 201 mg a.i./kg dry sediment in accordance with the US EPA OCSPP 850.1740 guideline. The NOEC and LOEC values based on mean measured concentrations in sediment were ≥109 and >109 mg a.i./kg for survival. The LC_{50} value was therefore >109 mg a.i./kg. There were no abnormalities noted in any of the test substance treatments during the 10 day test. This toxicity study is classified as acceptable and satisfies the guideline requirements for the marine amphipod, *Leptocheirus plumulosus* acute toxicity study.

Table 65: Summary of acute toxicity tests with aquatic invertebrates

Method	Species	Test material (purity)	Results	Reference
OECD 202	*Daphnia magna* (water flea)	Valifenalate (99.56 w/w %)	48 hr EC_{50} >100 mg/L Immobilization	*See Annex conf. 13.*
US EPA OPPTS 850.1025	Crassostrea virginica (*eastern oyster*)	Valifenalate (99.63 w/w %)	96 hr EC_{50}: 3.1 mg/L Shell deposition	*See Annex conf. 32.*
US EPA OPPTS 850.1035	*Americamysis bahia* (mysid shrimp)	Valifenalate (99.63 w/w %)	96 hour hr LC_{50}: 2.8 mg/L	*See Annex conf. 31.*
US EPA OCSPP 850.1740	*Leptocheirus plumulosus* (marine amphipod)	Valifenalate technical (98.9 w/w %)	10 d LC_{50}>109 mg/kg dry sediment	*Aufderheide, 2015a*

11.5.3 Acute (short-term) toxicity to algae or other aquatic plants

Study 1: Growth Inhibition Test on *Scenedesmus subspicatus* (*See Annex conf. 14.*)

The 72 hour E_bC_{50} and E_rC_{50} values of valifenalate were determined, by a limit test, on the unicellular green algae *Scenedesmus subspicatus*. Exponentially growing cultures of this green algal species were exposed in a static test to aqueous test media containing valifenalate suspended in methyl cellulose at a concentration of 100 mg/L under defined conditions. Two controls groups containing water and water with methyl cellulose, were tested in parallel. Prior to the definitive test, a range-finding experiment was carried out to determine the range of concentrations to be tested in the definitive test. The solubility of the test item in the test water was also determined by another pre-experiment. Furthermore, analytical monitoring was carried out to verify that the concentration of test item was above 80 % of initial concentration throughout the test period.

The inhibition of growth in relation to control cultures, measured as growth rate and biomass, was determined over test periods of 24, 48 and 72 hours and thus over several algal generations. At the end of test neither the biomass nor the growth rate were significantly different from the control parameters. Thus the NOEC and the EC_{50} values were determined to be at least 100 mg/L and higher than 100 mg/L, respectively.

Study 2: Growth Inhibition Test on *Skeletonema costatum* (*Hicks, 2015b*)

In a 96-hour acute toxicity study, cultures of marine diatom, *Skeletonema costatum* were exposed to valifenalate technical at nominal concentrations of 0 (control), 0 (vehicle control; 50 µL DMF/L), 0.040, 0.12, 0.37, 1.1, 3.3, and 10 mg a.i./L under static conditions in accordance with the OCSPP 850.4500 guideline. The NOEC values based on area under the growth curve, growth rate, and mean yield were all 0.106 mg a.i./L, respectively. The 96-hour IC_{10}, IC_{20}, and IC_{50} values based on geometric mean measured concentrations for area under the growth curve were 0.183, 1.47, and >9.48 mg a.i./L, respectively. The percent area under the growth curve inhibition in the treated algal culture as compared to the control ranged from -1 to 32%. The 96-hour IC_{10}, IC_{20}, and IC_{50} values based on growth rate were >9.48 mg a.i./L. The percent growth rate inhibition in the treated algal culture as compared to the control ranged from 0 to 3%. The 96-hour IC_{20} and IC_{50} values based on mean yield were both >9.48 mg a.i./L. The 96-hour yield data

did allow for calculation of the IC_{10}; therefore, the value was estimated to be 0.976 mg a.i./L based on the treatment mean percent inhibition. The percent yield inhibition in the treated algal culture as compared to the control ranged from -2 to 12%. There were no abnormalities observed in any of the test substance treatments during the 96-hour test.

Study 3: Growth Inhibition Test on *Navicula pelliculosa* (*Bergfield, 2015a*)

In a 96-hour acute toxicity study, cultures of freshwater diatom, *Navicula pelliculosa* were exposed to valifenalate technical at nominal concentrations of 0 (control), 0 (vehicle control; DMF 50 µL/L), 0.38, 0.75, 1.5, 3.0, and 6.0 mg a.i./L under static conditions in accordance with the OCSPP 850.4500 guideline. The NOEC values based on geometric mean measured concentration for area under the growth curve, growth rate, and mean yield were all 5.45 mg a.i./L. The 96-hour IC_{10}, IC_{20}, and IC_{50} values based on geometric mean measured concentration from area under the growth curve and were >5.45 mg a.i./L. The 96-hour percent area under the growth curve inhibition in the treated algal culture as compared to the control ranged from -4 to 4%. The 96-hour IC_{10}, IC_{20}, and IC_{50} values based on geometric mean measured concentration for growth rate were >5.45 mg a.i./L. The 96-hour percent growth rate inhibition in the treated algal culture as compared to the control was 0%. The 96-hour IC_{10}, IC_{20} and IC_{50} values based on geometric mean measured concentration mean yield were >5.45 mg a.i./L. The 96-hour percent yield inhibition in the treated algal culture as compared to the control ranged from -1 to 3%. There were no abnormalities observed in any of the test substance treatments during the 96-hour test.

Study 4: Growth Inhibition Test on *Anabaena flos-aquae* (*Aufderheide, 2015b*)

In a 96-hour acute toxicity study, cultures of freshwater algae, *Anabaena flos-aquae* were exposed to valifenalate technical at nominal concentrations of 0 (control), 0 (vehicle control; DMF 50 µL/L), 0.38, 0.75, 1.5, 3.0, and 6.0 mg a.i./L under static conditions in accordance with the OCSPP 850.4550 guideline. The functional solubility of valifenalate technical in test medium, as determined as part of this study, was approximately 6 mg/L. The NOEC values based on area under the growth curve, growth rate, and mean yield were 2.15, 4.13, and 4.13 mg a.i./L geometric mean measured concentration, respectively. The 96-hour IC_{10}, IC_{20}, and IC_{50} values based on area under the growth curve and geometric mean measured concentration were >4.13 mg a.i./L, respectively. The percent area under the growth curve inhibition in the treated algal culture as compared to the control ranged from -9 to 9%. The 96-hour IC_{10}, IC_{20}, and IC_{50} values based on growth rate and geometric mean measured concentration were >4.13 mg a.i./L. The percent growth rate inhibition in the treated algal culture as compared to the control ranged from -4 to 1%. The 96-hour IC_{10}, IC_{20} and IC_{50} values based on mean yield and geometric mean measured concentration were >4.13 mg a.i./L. The percent yield inhibition in the treated algal culture as compared to the control ranged from -16 to 5%. There were no abnormalities observed in any of the test substance treatments during the 96 hour test.

Study 5: Growth Inhibition Test on *Lemna gibba* (*Bergfield, 2015b*)

In a 7-day acute toxicity study, the cultures of the freshwater aquatic plant duckweed, *Lemna gibba* were exposed to valifenalate technical at nominal concentrations of 0 (control), 0 (vehicle control; DMF 50 µL/L), 0.38, 0.75, 1.5, 3.0, and 6.0 mg a.i./L under static-renewal conditions in accordance with the OCSPP 850.4400 guideline (*See Bergfield, 2015a*). The NOEC values based on geometric mean measured concentration for frond average specific growth rate, frond yield, biomass yield as dry weight, and biomass average specific growth rate as dry weight were all 5.02 mg a.i./L. The 7-day EC_{10}, EC_{20}, and EC_{50} values based on geometric mean measured concentration for frond average specific growth rate were >5.02 mg a.i./L. The percent average specific growth rate inhibition in the treated duckweed culture as compared to the control ranged from 0 to 1%. The 7 day EC_{10}, EC_{20}, and EC_{50} values based on geometric mean measured concentration for frond yield were >5.02 mg a.i./L. The percent frond yield inhibition in the treated duckweed culture as compared to the control ranged from -1 to 4%. The 7-day EC_{10}, EC_{20}, and EC_{50} values based on biomass as dry weight and geometric mean measured concentration were >5.02 mg a.i./L. The percent biomass as dry weight inhibition in the treated duckweed culture as compared to the control ranged from -13 to -1%. The 7-day EC_{10}, EC_{20}, and EC_{50} values based on geometric mean measured concentration for biomass average specific growth rate were >5.02 mg a.i./L. The percent biomass average specific growth rate inhibition in the treated duckweed culture as compared to the control ranged from -4 to -1%.

Table 66: Summary of acute toxicity tests with algae and other aquatic plants

Method	Species	Test material (purity)	Results	Reference
OECD 201	*Scenedesmus subspicatus* (green algae)	Valifenalate (99.56 w/w %)	72 hr E_rC_{50} >100 mg/L	*See Annex conf. 14.*
US EPA OCSPP 850.4500	*Skeletonema costatum* (marine diatom)	Valifenalate technical (98.9 w/w %)	96 hr I_rC_{50} > 9.48 mg/L	*Hicks 2015b*
	Navicula pelliculosa (freshwater diatom)		96 hr I_rC_{50} > 5.45 mg/L	*Bergfield, 2015a*
US EPA OCSPP 850.4550	*Anabaena flos-aquae* (green algae)		96 hr I_rC_{50} > 4.13 mg/L	*Aufderheide, 2015b*
US EPA OCSPP 850.4400	*Lemna gibba* (duckweed)		7 d I_rC_{50} > 5.02 mg/L	*Bergfield, 2015b*

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

Study 1: Acute toxicity to the freshwater midge, *Chironomus dilutus* (*Aufderheide, 2015c*)

In a 10-day acute toxicity study, the freshwater midge *Chironomus dilutus* was exposed to valifenalate technical at nominal concentrations of 0 (control), 13, 25, 50, 100 and 200 mg a.i./ kg dry sediment in accordance with the OCSPP 850.1735 guideline. The NOEC values based on mean calculated concentrations in sediment were 108 mg/kg for survival and 14.1 mg/kg for growth (ash-free dry weights). The LOEC values based on mean calculated concentrations in sediment were >108 mg/kg for survival and 37.7 mg/kg for growth (ash-free dry weights). The LC_{50} value based on mean calculated concentration in sediment was >108 mg/kg (ash-free dry weights) There were no abnormalities observed in any of the test substance treatments during the 10-day test. This toxicity study is classified as acceptable and satisfies the guideline requirements of the *Chironomus dilutus* acute toxicity study.

Table 67: Summary of acute toxicity tests with other aquatic organisms

Method	Species	Test material (purity)	Results	Reference
US EPA OCSPP 850.1735	*Chironomus dilutus* (freshwater midge)	Valifenalate technical (98.9 w/w %)	10 d NOEC: 108 mg/kg dry sediment Mortality 10 d NOEC: 14.1 mg/kg dry sediment Growth	*Aufderheide, 2015c*

11.6 Long-term aquatic hazard

Table 68: Summary of relevant information on chronic aquatic toxicity

Method	Species	Test material (purity)	Results	Remarks	Reference
Chronic toxicity to fish					
OECD 215	*Oncorhynchus mykiss* (rainbow trout)	Valifenalate (99.56 w/w %)	28 d NOEC ≥ 100 mg/L Growth	Semi-static Nominal concentrations	*See Annex conf. 17.* *See Annex conf. 61.*
EPA OPPTS 850.1400	*Pimephales promelas* (fathead minnow)	Valifenalate (99.63 w/w %)	33 d NOEC: 12 mg/L Growth	Flow-through Nominal concentrations	*See Annex conf. 30.*
Chronic toxicity to aquatic invertebrates					
OECD 211	*Daphnia magna* (water flea)	Valifenalate (99.56 w/w %)	22 d NOEC: 3.2 mg/L Growth 22 d NOEC: 10 mg/L Mortality	Semi-static Nominal concentrations	*See Annex conf. 18.* *See Annex conf. 60.*
Chronic toxicity to algae or other aquatic plants					
OECD 201	*Scenedesmus subspicatus* (green algae)	Valifenalate (99.56 w/w %)	72 hr NOEC: ≥ 100 mg/L Growth	Static Nominal concentrations	*See Annex conf. 14.* *See Annex conf. 26.*
OCSPP 850.4500	*Skeletonema costatum* (marine diatom)	Valifenalate technical (98.9 w/w %)	96 hr NOEC: 0.106 m/L Growth	Static Geometric mean measured concentrations	*Hicks, 2015b*
	Navicula pelliculosa (freshwater diatom)		96 hr NOEC: 5.45 mg /L Growth	Static Geometric mean measured concentrations	*Bergfield, 2015a*
US EPA OCSPP 850.4550	*Anabaena flos-aquae* (cyanobacteria)		96 hr NOEC: 2.15 mg/L Growth	Static Geometric mean measured concentrations	*Aufderheide, 2015a*
US EPA OCSPP 850.4400	*Lemna gibba* (duckweed)		7 d NOEC: 5.02 mg/L Growth	Static-renewal Geometric mean measured concentrations	*Bergfield, 2015b*

11.6.1 Chronic toxicity to fish

Two chronic fish studies were submitted all according to GLP and considered acceptable. One was an early life stage studie with EPA OPPTS 850.1400 and one was a test on juvenile fish with OECD 215.

The 28 day NOEC (growth) in rainbow trout (*O. mykiss*) was 100 mg/L (*See Annex conf. 17.*) and the corresponding 33 day endpoint in the fathead minnow (*Pimephales promelas*) was 11.0 mg/L (*See Annex conf. 30.*).

Study 1: Chronic prolonged toxicity test on juvenile rainbow trout, *Oncorhynchus mykiss* (*See Annex conf. 17.*)

Juvenile rainbow trout were exposed in a semi-static test system to aqueous test media containing the test item for 28 days. Since in the acute toxicity test with rainbow trout no effect was determined up to 100 mg test item/L, in this prolonged study 100 mg test item/L, a control and a solvent control (50 mg methyl cellulose/L) were tested. Mortality and symptoms of intoxication were recorded throughout the study and bodyweight of surviving fish were recorded at the start and the end of the test.

During the test period test item concentrations were in the range 82-129% of the nominal value and the mean measured test concentration in the test media was 98%. Under the test conditions the test item was sufficiently stable during the test medium renewal period of 48 and 72 hours. Therefore all the results are related to the nominal concentration of the test item.

No mortality or symptoms of intoxication were observed during the test at the nominal test concentration of 100 mg test item/L.

No significant difference was determined comparing the pseudo specific growth rates of the test concentration with the one of control and the solvent control. The 28-day NOEC was at least 100 mg test item/L; the 28-day LOEC and the 28-day Lowest Lethal Concentration (LLC) were higher than 100 mg test item/L.

Study 2: Fish early life stage toxicity test with fathead minnow *Pimephales promelas* (*See Annex conf. 30.*)

The objective of this study was to determine the effects of valifenalate on the time to hatch, hatching success, survival and growth of fathead minnow (*Pimephales promelas)*, during early life-stage development. Fathead minnows embryos (< 24 hours old) were exposed to a geometric series of five test concentrations, a negative control (dilution water) and a solvent control (dimethyl formamide) under flow-through conditions. The test chambers were 9-L glass aquaria filled with approximately 7 L of test solution. The depth of the test water in a representative test chamber was approximately 15 cm. Nominal test concentrations were 0.75, 1.5, 3.0, 6.0 and 12.0 mg valifenalate/L. The exposure period included a 5-day embryo hatching period and a 28-day post-hatch juvenile growth period. Larvae were fed live brine shrimp nauplii (*Artemia sp.*)

During the first day of exposure, embryos were checked twice for mortality and eggs were checked for fungus. Thereafter, until hatching was complete, observations of embryo mortality and the removal of dead embryos were performed once a day. During the 28 day post-hatch exposure period, the larvae were observed daily to evaluate the number of mortalities and the number of individuals exhibiting clinical signs of toxicity or abnormal behaviour.

There were no statistically significant treatment-related effects on hatching success, survival, or growth at any concentration tested. Consequently, the NOEC was 11 mg/L, the highest concentration tested, the LOEC was >11 mg/L, and the MATC was 11 mg/L. The RMS commented that measured concentrations of 12 mg/L treatment group ranged between 90 – 95 %, and remained > 80 % by the end of the test, therefore nominal concentrations are considered appropriate to express toxicity. Thus the NOEC is concluded to be 12 mg/L, the highest concentration tested and the LOEC is >12 mg/L.

Table 69: Summary of chronic toxicity tests with fish

Method	Species	Test material (purity)	Results	Reference
OECD 215	*Oncorhynchus mykiss* (rainbow trout)	Valifenalate (99.56% w/w)	28 d NOEC ≥ 100 mg/L Growth	*See Annex conf. 17.* *See Annex conf. 61.*
EPA OPPTS 850.1400	*Pimephales promelas* (fathead minnow)	Valifenalate (99.63 w/w %)	33 d NOEC: 12 mg/L Growth	*See Annex conf. 30.*

11.6.2 Chronic toxicity to aquatic invertebrates

Study 1: Reproduction of *Daphnia magna* (*See Annex conf. 18.*)

Groups of 10 young *Daphnia* (7.5 – 22.5 hours old) for each control and test concentration were exposed in semi-static conditions to the test item valifenalate for a period of 22 days. The concentrations tested (0.32, 1.0, 3.2, 10, 32 and 100 mg/L) were based on results of the previous acute toxicity test on *Daphnia.* The two control groups tested contained reconstituted water and water with methylcellulose. The test media of all test concentrations and of the control were renewed on days 2, 5, 7, 9, 12, 14, 16 and 19 of the exposure period. At these times the animals were transferred from the old test vessels into the freshly prepared test media of the corresponding concentrations. Observations of adult survival and number of young were carried out daily while pH, dissolved oxygen concentration and water temperature were measured at the start and at the end of each treatment period in the control and in all test concentrations.

In order to verify the stability of the test item under the test conditions, a sufficient volume of the freshly prepared test media of the control and of all concentrations were incubated under the same conditions as the test, but without animals or food for 48 or 72 hours. Samples were collected on days 0-2, 12-14 and 16-19. During the test period the mean measured test item concentrations of nominal 1.0 to 100 mg/L were determined in the range from 81 to 92% of the nominal values. The lowest test concentration of nominal 0.32 mg/L was below the limit of quantification.

At the end of the test period (22 days), the NOEC and the LOEC for reproduction based on nominal test concentrations were 3.2 mg/L and 10 mg/L respectively. The EC_{50} reproduction rate resulted to be 5.9 mg/L. The NOEC and LOEC for survival were 10 and 32 mg/L, respectively.

Table 70: Summary of chronic toxicity tests with aquatic invertebrates

Method	Species	Test material (purity)	Results	Reference
OECD 211	*Daphnia magna* (water flea)	Valifenalate (99.56% w/w)	22 d NOEC: 3.2 mg/L Growth 22 d NOEC: 10 mg/L Mortality	*See Annex conf. 18.* *See Annex conf. 60.*

11.6.3 Chronic toxicity to algae or other aquatic plants

No additional data other than that reported in section 11.5.3.

Table 71: Summary of chronic toxicity tests with algae and other aquatic plants

Method	Species	Test material (purity)	Results	Reference
OECD 201	*Scenedesmus subspicatus* (green algae)	Valifenalate (99.56 w/w %)	72 hr NOEC: ≥ 100 mg/L Growth	*See Annex conf. 14. See Annex conf. 26.*
OCSPP 850.4500	*Skeletonema costatum* (marine diatom)	Valifenalate technical (98.9 w/w %)	96 hr NOEC: 0.106 m/L Growth	*Hicks, 2015b*
	Navicula pelliculosa (freshwater diatom)		96 hr NOEC: 5.45 mg /L Growth	*Bergfield, 2015a*
US EPA OCSPP 850.4550	*Anabaena flos-aquae* (cyanobacteria)		96 hr NOEC: 2.15 mg/L Growth	*Aufderheide, 2015a*
US EPA OCSPP 850.4400	*Lemna gibba* (duckweed)		7 d NOEC: 5.02 mg/L Growth	*Bergfield, 2015b*

11.6.4 Chronic toxicity to other aquatic organisms

No chronic toxcitiy test with valifenalate to other aquatic organisms were performed.

11.7 Comparison with the CLP criteria

11.7.1 Acute aquatic hazard

Acute aquatic toxicity data on valifenalate are available for fish, invertebrates, algae and aquatic plants. Invertebrates are the most acutely sensitive trophic group. The lowest reliable acute value is the 96-hour EC50 of 2.8 mg valifenalate/L for *Americamysis bahia*, this is >1 mg/L and therefore no acute hazard classification is warranted.

Table 72: Summary of relevant information on acute aquatic toxicity

Taxonomic group	Species	Lowest representative $L(E)C_{50}$	Endpoint	Reference
Fish	*Cyprinodon variegatus*	>15 mg/L	LC_{50}	*See Annex conf. 29.*
Aquatic invertebrates	*Americamysis bahia*	2.8 mg/L	LC_{50}	*See Annex conf. 31.*
Aquatic plants	*Anabaena flos-aquae*	>4.13 mg/L	IC_{50}	*Aufderheide, 2015b*

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

Within the classification criteria, valifenalate is considered 'not rapidly degradable'.

Valifenalate has a log K_{OW} value of 3.05-3.11, which is lower than the CLP cut-off log K_{OW} value of ≥4. An experimental bioconcentration study in fish is available however, and this gave a growth corrected and lipid normalised kinetic whole fish BCF of < 4 for valifenalate. This is also less than the CLP BCF trigger of 500, therefore, valifenalate is not considered to have the potential to bioconcentrate.

Chronic/long-term aquatic toxicity data on valifenalate are available for fish, invertebrates, algae and aquatic plants. Algae are the most chronically sensitive group. The lowest reliable chronic value is considered to be the 96-hour nominal NOEC of 0.106 mg valifenalate/L for *Skeletonema costatum*. Valifenalate is 'not rapidly degradable' and based on the lowest chronic endpoint it should be classified as Aquatic Chronic 2.

Table 73: Summary of relevant information on chronic aquatic toxicity

Taxonomic group	Species	Lowest representative NOEC/EC_{10}	End points	Reference
Fish	*Pimephales promelas*	12 mg/L	NOEC	*See Annex conf. 30.*
Aquatic invertebrates	*Daphnia magna*	3.2 mg/L	*NOEC*	*See Annex conf. 18.*
Aquatic plants	*Skeletonema costatum*	0.106 mg/L	*NOEC*	*Hicks, 2015b*

11.8 CONCLUSION ON CLASSIFICATION AND LABELLING FOR ENVIRONMENTAL HAZARDS

Aquatic Chronic 2; H411: Toxic to aquatic life with long-lasting effects

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier Submitter's proposal

The Dossier Submitter (DS) proposed to classify the substance as Aquatic Chronic 2; H411 based on lack of rapid degradation and a 96h nominal NOEC value of 0.106 mg/L for the marine diatom *Skeletonema costatum*.

Degradation

A hydrolysis study according to OECD TG 111 and in compliance with GLP was run at pH 4, 7 and 9 in the dark in aqueous buffered solutions. Valifenalate was stable at pH 4 (50°C), while at pH 7 and pH 9 a pseudo-first order kinetic hydrolysis reaction was observed. The following DT_{50} values of 90.94 d (25ºC), 7.62 d (50ºC), 5.21 d (55ºC) and 2.09 d (65ºC) at pH 7 and 4.15 d (25ºC) and 0.33 d (50ºC) at pH 9 were determined. The hydrolytic degradation of valifenalate increased with higher pH values. Two main compounds found were the unchanged parent substance valifenalate and IR5839 (3-(4-chlorophenyl)-3-({(2S)-2-[(isopropoxycarbonyl) amino]-3-methylbutanoyl} amino) propanoic acid, also referred to as IR5885 acid). For both of the compounds the diasteroisomeric ratio (S,R/S,S) was approximately 1:1.

Photochemical degradation in water was not expected to be significant since the molar absorption coefficient (ε) is <10 M-1 × cm-1 at λ >290 nm.

There was one ready biodegradability test available for valifenalate following EEC method C.4-D (1992) and OECD TG 301F (Manometric Respirometry) and in compliance with GLP using domestic activated sludge (adaptation not specified) that resulted in 3% (based on $ThOD_{NH4}$) and 2% (based on $ThOD_{NO3}$) degradation after 28 days.

A water/sediment study carried out according to OECD TG 308 and in compliance with GLP, was conducted using two aquatic systems (Pond and River systems) for 22 days. The radioactivity in the surface water decreased during all the study and it was 40.84% (Pond) and 43.74% (River) of applied radioactivity (AR) at the end of incubation period. The radioactivity in the sediment increased throughout the study reaching 50.64% AR (Pond) and 45.51% AR (River) at the end of incubation period. Valifenalate degraded in both aquatic systems: after 22 days it accounted for 5.92% AR (Pond) and 5.51% AR (River). In the whole system, the DT_{50} values were 4.5 days (Pond) and 4.71 days (River) and DT_{90} values, 14.9 days (Pond) and 15.64 days (River). Six compounds were found in the surface water and in the sediment extracts. The main degradation products were S2 and S3: S2 reached 52.80% AR (Pond) and 56.34% AR (River). S2 was identified as 3-(4-chlorophenyl)-3-({(2S)-2-[(isopropoxycarbonyl) amino]-3-methylbutanoyl} amino) propanoic acid (also referred to as IR5839 or IR5885 acid). The compound S3, that increased up to a maximum of 13.77% AR (Pond) and 8.16% AR (River), was identified as 4-chlorobenzoic acid (also referred to as PCBA). The fraction S6 slowly increased reaching 8.93% AR and 8.04% AR. It was represented by a pool of 4 compounds and none of these reached values higher than 3.13% AR. None of the other compounds, S4 and S5, ever reached levels higher than 5% AR. The non-extractable radioactivity (bound residue) increased to 8.99% AR (Pond) and 16.24% AR (River). The radioactivity in the ^{14}C-CO_2 traps was always lower than the detection limit in both the systems except at the last three sampling times when it reached values ranging between 0.77% AR and 1.24% AR. The ^{14}C-Mass Balance was always higher than 90% AR and ranged from 90.61% to 104.12% AR for Pond system and from 90.49% to 107.96% AR for River system.

In conclusion, the DS considered valifenalate not to be rapidly degradable for classification purposes.

Bioaccumulation

A bioconcentration study (OECD TG 305, GLP) was available for valifenalate. Rainbow trout (*Oncorhynchus mykiss*) was exposed to concentrations (93.5 and 893.5 µg/L) of the radiolabelled valifenalate for 14 days in a flow-through system, followed by 14-day depuration period in clean water. Due to the extremely low accumulation of valifenalate in fish at both dose levels, no relevant plateau levels and consequently no half-lives or accumulation/depuration kinetics could be determined. Based on the total radioactivity concentration in the exposure water and the residual radioactivity found in fish parts, ratios between fish and water (BCF) amounted to 1.3, 3.0 and 2.3 for edibles, non-edibles and whole fish, respectively, indicating lack of bioconcentration at both dose levels. The kinetic BCF (growth corrected and lipid-normalized) was < 4 for whole fish. Analyses of radioactivity of the test water showed mainly the presence of the parent compound at both dose levels throughout the entire exposure period. Besides the constant levels of parent compound ranging on average from 96.2 to 98.0% of the radioactivity recovered, three unknown radioactive fractions W0, W2 and W3/4 were

found in minor amounts (< 3% of the radioactivity recovered).

Furthermore, the measured octanol-water partition coefficient (log K_{OW}) determined according to OECD TG 117 (HPLC method) is 3.05 – 3.11 at 20°C and pH 7.

The DS concluded that valifenalate has a low potential to bioconcentrate and is therefore not considered a bioaccumulative substance for classification purposes.

Aquatic Toxicity

The DS provided aquatic toxicity data for the active substance regarded as reliable in the CLP Report, and a summary of the relevant information on aquatic toxicity is provided in the following table (the key endpoints used in hazard classification are highlighted in bold).

Data for sediment-dwelling invertebrates (marine amphipod *Leptocheirus plumulosus* and freshwater midge *Chironomus dilutes*) were reported in CLH report but were not used for classification because the endpoint values were presented in relation to sediment concentrations of valifenalate (mg/kg).

Table: *Summary of relevant information on aquatic toxicity of valifenalate*

Method	Species	Endpoint	Toxicity value (mg/L)	Reference
Short-term toxicity				
OECD TG 203	*Oncorhynchus mykiss*	96h LC_{50} (mortality)	>100 nom	Anonymous (2003b), final results: Anonymous (2003a)
OECD TG 203	*Brachydanio rerio*	96h LC_{50} (mortality)	>100 nom	Anonymous (2003), final results: Anonymous (2003)
US EPA OPPTS 850.1075	*Cyprinodon variegatus*	96h LC_{50} (mortality)	>15 mm	Anonymous (2005a)
US EPA OPPTS 850.1075	*Lepomis macrochirus*	96h LC_{50} (mortality)	>40 nom	Anonymous (2015a)
OECD TG 202	*Daphnia magna*	48h EC_{50} (immobilization)	>100 nom	Anonymous (2002), final results: Anonymous (2002)
US EPA OPPTS 850.1035	*Americamysis bahia*	**96h LC_{50}** (mortality)	**2.8 mm**	Anonymous (2005c)
US EPA OPPTS 850.1025	*Crassostrea virginica*	96h EC_{50} (shell deposition)	3.1 mm	Anonymous (2005d)
OECD TG 201	*Scenedesmus*	72h E_bC_{50}	>100 nom	Anonymous (2002b), final

	subspicatus	72h E_rC_{50} (growth)	>100 nom	results: Anonymous (2002)
US EPA OCSPP 850.4500	*Skeletonema costatum*	96h I_bC_{50} 96h I_rC_{50} 96h I_yC_{50} (growth)	>9.48 gmm >9.48 gmm >9.48 gmm	Hicks (2015b)
US EPA OCSPP 850.4500	*Navicula pelliculosa*	96h I_bC_{50} 96h I_rC_{50} 96h I_yC_{50} (growth)	>5.45 gmm >5.45 gmm >5.45 gmm	Bergfield (2015a)
US EPA OCSPP 850.4550	*Anabaena flos-aquae*	96h I_bC_{50} 96h I_rC_{50} 96h I_yC_{50} (growth)	>4.13 gmm >4.13 gmm >4.13 gmm	Aufderheide (2015b)
US EPA OCSPP 850.4400	*Lemna gibba*	7d EC_{50} (growth)	>5.02 gmm	Bergfield (2015b)
Long-term toxicity				
OECD TG 215	*Oncorhynchus mykiss*	28d NOEC (growth)	≥100 nom	Anonymous (2003c), final results: Anonymous (2003b)
EPA OPPTS 850.1400	*Pimephales promelas*	33d NOEC (growth)	12 nom	Anonymous (2005b)
OECD TG 211	*Daphnia magna*	22d NOEC (reproduction) 22d NOEC (mortality)	3.2 nom 10 nom	Anonymous (2003d), final results: Anonymous (2002)
OECD TG 201	*Scenedesmus subspicatus*	72h NOEC (growth)	≥100 n	Anonymous (2002b), final results: Anonymous (2002)
US EPA OCSPP 850.4500	*Skeletonema costatum*	**96h NOEC** (growth)	**0.106 gmm**	Hicks (2015b)
US EPA OCSPP 850.4500	*Navicula pelliculosa*	96h NOEC (growth)	5.45 gmm	Bergfield (2015a)
US EPA OCSPP 850.4550	*Anabaena flos-aquae*	96h NOEC (growth)	2.15 gmm	Aufderheide (2015b)
US EPA OCSPP	*Lemna gibba*	7d NOEC	5.02 gmm	Bergfield (2015b)

850.4400		7d EC_{10} (growth)	> 5.02 gmm	

Note: nom – nominal concentrations; mm – mean measured concentrations; gmm - geometric mean measured concentrations;

Acute toxicity

For acute aquatic toxicity, reliable toxicity data for the active substance were reported for fish, invertebrates, algae and aquatic plants, with invertebrates being the most sensitive trophic level. The lowest acute toxicity value is the 96h mean measured LC_{50} of 2.8 mg/L for saltwater mysid shrimp *Americamysis bahia* which is above the classification threshold value of 1 mg/L. Therefore, the DS proposed **not to classify** the valifenalate as acutely hazardous to the aquatic environment.

Chronic toxicity

For chronic aquatic toxicity, reliable toxicity data for the active substance were reported for fish, invertebrates, algae and aquatic plants, with algae being the most chronically sensitive group. The lowest chronic toxicity value is the 96h nominal NOEC of 0.106 mg/L for marine diatom *Skeletonema costatum.* The DS proposed to classify the substance as **Aquatic Chronic 2** based on the lowest chronic endpoint for algae and considering that the substance is not rapidly degradable and has low potential for bioaccumulation.

Comments received during consultation

Comments were received from three Member States (MS) and one company-manufacturer. Two MSs and the company-manufacturer agreed with DS proposal to classify the substance as Aquatic Chronic 2. The third MS agreed with the proposed classification but based on a different interpretation of the data. The MS pointed out the limitations of the key chronic toxicity study on algae *Skeletonema costatum* (Hicks, 2015) and that, due to these limitations of the key study, the MS was of the opinion that the study does not support the proposed classification. In the view of the MS, the classification should be based on the surrogate approach for the most acutely sensitive endpoints (saltwater mysid *Americamysis bahia*), which would result in the same classification as proposed by DS. The DS disagreed with the commenting MS and is of the opinion that the algae study should be used for classification. As regards the application of the surrogate approach, the view of the DS is that this approach is not warranted since a sufficient set of chronic studies is available.

Assessment and comparison with the classification criteria

Degradation

RAC agrees with the DS's proposal to consider valifenalate as not rapidly degradable. Valifenalate is hydrolytically stable at pH 4 but it undergoes hydrolysis with increasing alkalinity. Hydrolysis DT_{50} values at pH 7 are 90.94 d (25ºC), 7.62 d (50ºC), 5.21 d (55ºC) and 2.09 d (65ºC) and pH 9 are 4.15 d (25ºC) and 0.33 d (50ºC). Two main compounds were found, unchanged parent substance and IR5839. Data on hydrolysis might be considered for classification purposes only when the longest half-life determined within the pH range 4-9 is less than 16 days (corresponding to a degradation of > 70%

within 28 days). Accordingly, valifenalate is hydrolytically stable.

In a 28-day ready biodegradability study following OECD TG 301F (GLP), 3% degradation was observed, indicating that valifenalate is not readily biodegradable.

The results of the aerobic water/sediment simulation study showed degradation of the valifenalate in both aquatic systems (5.92% AR (Pond) and 5.51% AR (River) after 22 days). In addition, rapid loss of the valifenalate from the whole system was observed (DT_{50} values were 4.5 days (Pond) and 4.71 days (River) and DT_{90} values, 14.9 days (Pond) and 15.64 days (River)). Six degradation products were formed in water and sediment. The main metabolites were IR5839, PCBA and fraction S6. No information on toxicity of the metabolites to allow classification of the metabolites is available in the CLH report.

Overall, although valifenalate degrades quickly in the whole system of the water/sediment study, the substance does not pass the ready biodegradability test, the available abiotic and biotic degradation information does not indicate that valifenalate is ultimately degraded (> 70%) within 28 days (equivalent to a half-life < 16 days) or transformed to non-classifiable metabolites. Consequently, RAC considers the substance to be not rapidly degradable for the purposes of environmental classification.

Bioaccumulation

RAC agrees with the DS that valifenalate has a low potential to bioaccumulate in aquatic organisms. The basis for this is that measured BCF values of < 4 is below the CLP criterion of 500 and the measured log K_{ow} value of 3.05 – 3.11 is below the CLP criterion of 4.

Acute toxicity

RAC is of the opinion that adequate acute toxicity data are available for fish, invertebrates, algae and aquatic plants. Invertebrates are the most sensitive group and the lowest result is a 96h EC_{50} value of 2.8 mg/L for mysid shrimp *Americamysis bahia*. RAC notes that all acute toxicity endpoints ($L(E)C_{50s}$ and IC_{50}) for fish, invertebrates, algae and aquatic plants (see table) are above the threshold value of 1 mg/L. Consequently, RAC concludes that **valifenalate does not warrant classification for acute aquatic toxicity**.

Chronic toxicity

RAC is of the opinion that reliable long-term aquatic toxicity data are available for all three trophic levels. The lowest chronic effect value corresponds to a test with *Skeletonema costatum* with a 96h NOEC of 0.106 mg/L. As the value is >0.1 but <1 mg/L and the substance is considered not rapidly degradable, RAC concludes that following table 4.1.0(b)(i) of CLP, a classification as Aquatic Chronic 2 (H411) is warranted.

RAC notes that no chronic toxicity test data are available for the most sensitive species under acute testing (*Americamysis bahia*). Using table 4.1.0(b)(iii) of CLP, considering that Valifenalte is not rapidly degradable, the 96h LC_{50} of 2.8 mg/L indicates classification as Aquatic Chronic 2, which supports the outcome derived using chronic data.

In summary, RAC agrees with the DS that **valifenalate warrants classification as Aquatic Chronic 2 (H411).**

12 EVALUATION OF ADDITIONAL HAZARDS

12.1 Hazardous to the ozone layer

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

Pure valifenalate has a vapour pressure of 9.6×10^{-8} Pa and water solubility of 24.1 mg/L (both at 20°C) resulting in a calculated Henry's Law constant of 1.6×10^{-6} Pa m^3/mol (at 20°C and pH 5.4 ± 0.5). This combination of properties indicates no volatilisation and thus no significant amounts of valifenalate are to be expected in air. The Atkinson calculated oxidative photochemical degradation half life is 7.5 hours assuming a hydroxyl radical concentration of 5×10^5 molecules/cm^3 (*Fisk, 2003*).

12.1.2 Comparison with the CLP criteria

There is no available evidence concerning the properties of valifenalate and its predicted or observed environmental fate and behaviour indicating that it may present a danger to the structure and/or the functioning of the stratospheric ozone layer.

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

Valifenalate is not listed in Annex I to Regulation (EC) No 1005/2009.

No classification is warranted.

RAC evaluation of hazards to the ozone layer

Summary of the Dossier Submitter's proposal

Pure valifenalate has a vapour pressure of 9.6 × 10-^{8} Pa (20°C) and water solubility of 24.1 mg/L (20°C) resulting in a calculated Henry's Law constant of 1.6×10^{-6} Pa m^3/mol (20°C, pH 5.4 ± 0.5). This combination of properties indicates no volatilisation and, thus, no significant amounts of valifenalate are to be expected in air. The Atkinson calculated oxidative photochemical degradation half-life is 7.5 hours assuming a hydroxyl radical concentration of 5×10^5 molecules/cm^3 (Fisk, 2003).

Comments received during consultation

One comment was received from company-manufacturer which agreed with DS proposal not to classify the substance as hazardous to the ozone layer.

Assessment and comparison with the classification criteria

Transport of valifenalate in air is considered to be negligible due to its very low vapor pressure and Henry's constant, whilst its photochemical oxidative degradation in air is expected to be rapid. Therefore, exposure of stratospheric ozone to valifenalate is

expected to be negligible.

Thus, RAC agrees with the DS's proposal that **no classification is warranted for this hazard class.**

13 ADDITIONAL LABELLING

Not relevant.

14 REFERENCES

Aufderheide J (2015a): Valifenalate Technical: Whole Sediment Acute Toxicity to a Marine Amphipod (*Leptocheirus plumulosus*). ABC Laboratories. FMC Corporation, FMC Tracking No.: 2014ETX-VAL1348.

Aufderheide J (2015b): Valifenalate Technical: Growth Inhibition Test with the Cyanobacterium, *Anabaena flos-aquae*. ABC Laboratories, FMC Corporation, FMC Tracking No.: 2014ETX-VAL1345

Aufderheide J (2015c): Valifenalate Technical: Whole Sediment Acute Toxicity Test with Midge Larvae (*Chironomus dilutus*). ABC Laboratories, FMC Corporation, FMC Tracking No.: 2014ETX-VAL1347

Bergfield A (2015a): Valifenalate Technical: Growth Inhibition Test with the Freshwater Diatom, *Navicula pelliculosa*. ABC Laboratories, FMC Corporation, FMC Tracking No.: 2014ETX-VAL1344

Bergfield A (2015b): Valifenalate Technical: Growth Inhibition Test with the Freshwater Aquatic Plant, Duckweed, *Lemna gibba*. ABC Laboratories, FMC Corporation, FMC Tracking No.: 2014ETX-VAL1343

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Hicks S (2015b). Valifenalate Technical: Growth Inhibition Test with the Marine Diatom, *Skeletonema costatum*. ABC Laboratories, FMC Corporation, FMC Tracking No.: 2014ETX-VAL1346

15 ANNEXES

Annex I – Summary of the Study reports

Annex II – Mode of Action Analysis using the WHO/IPCS Mode of Action Framework

Annex III – Historical control data

Annex containing confidential information (Annex conf.)