CLH report

Proposal for Harmonised Classification and Labelling

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

Substance Name: Tralkoxydim

EC Number: **Not assigned**

CAS Number: **87820-88-0**

Index Number: **Not yet assigned**

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Part A.

1 PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

1.1 Substance

Table 1: Substance identity

Substance name:	**Tralkoxydim**
EC number:	**Not assigned**
CAS number:	**87820-88-0**
Annex VI Index number:	**Not yet assigned**
Degree of purity:	**≥ 96%**
Impurities:	**Confidential**

1.2 Harmonised classification and labelling proposal

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

	CLP Regulation	**Directive 67/548/EEC (Dangerous Substances Directive; DSD)**
Current entry in Annex VI, CLP Regulation	Not listed	Not listed
Current proposal for consideration by RAC	Carc. 2 – H351 (Suspected of causing cancer) Acute Tox. 4 - H302 (Harmful if swallowed) STOT-RE 2 - H373 (May cause damage to organs (liver) through prolonged or repeated exposure). Aquatic Chronic 2 – H411 (Toxic to aquatic life with long	Carc Cat 3; R40 Xn; R22 - 48/22 N; R51 -53

	lasting effects)	
Resulting harmonised classification (future entry in Annex VI, CLP Regulation)	Carc. 2 – H351 Acute Tox. 4 - H302 STOT-RE 2 - H373 Aquatic Chronic 2 – H411	Carc Cat 3; R40 Xn; R22 - 48/22 N; R51 -53

1.3 Proposed harmonised classification and labelling based on CLP Regulation and/or DSD criteria

Table 3: Proposed classification according to the CLP Regulation

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

2.14.	Oxidising solids	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
2.15.	Organic peroxides	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
2.16.	Substance and mixtures corrosive to metals	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
3.1.	Acute toxicity - oral	Acute Tox 4 H302	Not applicable	Not classified	Not applicable
	Acute toxicity - dermal	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
	Acute toxicity - inhalation	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
3.2.	Skin corrosion / irritation	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
3.3.	Serious eye damage / eye irritation	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
3.4.	Respiratory sensitisation	Not classified	Not applicable	Not classified	Data lacking
3.4.	Skin sensitisation	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
3.5.	Germ cell mutagenicity	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
3.6.	Carcinogenicity	Carc 2 H351	Not applicable	Not classified	Not applicable
3.7.	Reproductive toxicity	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
3.8.	Specific target organ toxicity –single exposure	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
3.9.	Specific target organ toxicity – repeated exposure	STOT-RE 2 H373	Not applicable	Not classified	Not applicable
3.10.	Aspiration hazard	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
4.1.	Hazardous to the aquatic environment	Aquatic Chronic 2 H411: toxic to	Not applicable	Not classified	

		aquatic life with long lasting effects			
5.1.	Hazardous to the ozone layer	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification

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

Labelling: Signal word: Warning
Picotgrams: GHS08 GHS07 GHS09
Hazard statements: H351 H302 H373 H411
Precautionary statements: to be assigned by supplier, not listed in Annex VI

Proposed notes assigned to an entry:

None

Table 4: Proposed classification according to DSD

Hazardous property	Proposed classification	Proposed SCLs	Current classification [1)]	Reason for no classification [2)]
Explosiveness	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
Oxidising properties	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
Flammability	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
Other physico-chemical properties *[Add rows when relevant]*	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
Thermal stability	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
Acute toxicity	R22	Not applicable	Not classified	Not applicable
Acute toxicity – irreversible damage after single exposure	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
Repeated dose toxicity	R48/22	Not applicable	Not classified	Not applicable
Irritation / Corrosion	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
Sensitisation	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
Carcinogenicity	Carc Cat 3: R40	Not applicable	Not classified	Not applicable
Mutagenicity – Genetic toxicity	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
Toxicity to reproduction – fertility	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
Toxicity to reproduction – development	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
Toxicity to reproduction – breastfed babies. Effects on or via lactation	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
Environment	R51-53	Not applicable	Not classified	

[1)] Including SCLs
[2)] Data lacking, inconclusive, or conclusive but not sufficient for classification

Labelling: Indication of danger: Xn, N
R-phrases: R22-40-48/22 -R51/53
S-phrases: S36-37-60-61

2 BACKGROUND TO THE CLH PROPOSAL

2.1 History of the previous classification and labelling

Tralkoxydim is not currently listed in Annex VI of Regulation EC 1272/2008 (CLP Regulation). Following evaluation of the data this proposal seeks to propose classification for acute toxicity (oral) and carcinogenicity.

At the time of submission there were not registrations for this substance under REACH.

2.2 Short summary of the scientific justification for the CLH proposal

Tralkoxydim is a cyclohexanedione oxime herbicide which was approved for Annex I listing under Council Directive 91/414/EEC, with the UK as Rapporteur Member State. In accordance with Article 36(2) of the CLP Regulation, tralkoxydim should now be considered for harmonised classification and labelling. Therefore, this proposal considers all human health and environmental end points. This Annex VI dossier presents a classification and labelling proposal based on the information presented in the assessment of tralkoxydim under Directive 91/414/EEC. The assessment made under that Directive is attached to the IUCLID 5 dossier.

2.3 Current harmonised classification and labelling

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

Not currently listed.

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

Not currently listed.

2.4 Current self-classification and labelling

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

Classification:
Carc. 2 H351
Acute Tox. 4 H302
Aquatic Chronic 2 H411

Labelling:
Signal word: Warning
Hazard Satements: H351 H302 H411
Picotgrams: GHS08 GHS07 GHS09

2.4.2 Current self-classification and labelling based on DSD criteria

Classification:
Carc Cat3; R40
Xn; R22 - 48/22
N; R51 -53

Labelling:
Indication of danger: Xn, N
R-phrases: R22-40-R51/53
S-phrases: S36-37-60-61

3 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

Tralkoxydim is a cyclohexanedione oxime herbicide which has been approved for Annex I listing under Council Directive 91/414/EEC, with the UK as Rapporteur Member State. In accordance with Article 36(2) of the CLP Regulation, tralkoxydim should now be considered for harmonised classification and labelling

Part B.

SCIENTIFIC EVALUATION OF THE DATA

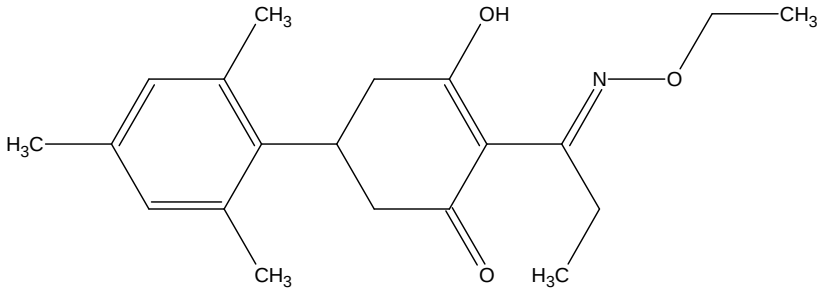
1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 5: Substance identity

EC number:	Not assigned
EC name:	Not assigned
CAS number (EC inventory):	Not applicable
CAS number:	87820-88-0
CAS name:	2-Cyclohexen-1-one,2-[1-(ethoxyimino)propyl]-3-hydroxy-5-(2,4,6-trimethylphenyl)-
IUPAC name:	2-[1(E)-N-ethoxypropanimidoyl]-3-hydroxy-5-mesitylcyclohex-2-en-1-one
CLP Annex VI Index number:	Not yet assigned
Molecular formula:	$C_{20}H_{27}NO_3$
Molecular weight range:	329.4

Structural formula:



1.2 Composition of the substance

Table 6: Constituents (non-confidential information)

Constituent	Typical concentration	Concentration range	Remarks
Tralkoxydim	96%	≥ 96%	

Current Annex VI entry: Not listed

The structure of tralkoxydim indicates that E/Z isomerism is possible about the C-N bond. In the solid state, tralkoxydim exists in a twisted enol form (E-isomer) as indicated by the name and structure presented. In non-polar solvents it exists as two tautomeric forms in rapid equilibrium: a planar enol form and an aminoenone form, with the latter predominating. In polar solvents tralkoxydim equilibrates between the two tautomers and the Z-oxime isomer.

Table 7: Impurities (non-confidential information)

Impurity	Typical concentration	Concentration range	Remarks
Confidential			

Current Annex VI entry: There are 6 process impurities which are all individually present in concentrations < 1%. These impurities have been taken into account in the proposed classification and labelling of Tralkoxydim and are not considered to be of additional concern. The impurities are considered to be confidential so are not listed in this report. Further information can be found in the technical dossier.

Table 8: Additives (non-confidential information)

Additive	Function	Typical concentration	Concentration range	Remarks
None				

Current Annex VI entry:

1.2.1 Composition of test material

The minimum purity of the active substance tralkoxydim is 96%. The toxicological studies detailed in this report used tralkoxydim with a purity of 92.4 – 99.4%. After careful and detailed review by the UK CA and those authorities responsible for the assessment under Directive 91/414/EEC, the technical specification of the current technical material and the material used in these studies are considered to be comparable.

In the solid state it is clear that tralkoxydim forms the enol structure described above. In solution, tralkoxydim shows a degree of instability making its structural identity in natural systems more elusive. Whilst there are no individual data on the isomeric content of the tested material, the appropriate form of the substance in natural systems has been taken into account by the toxicological and environmental studies performed. It is therefore considered that the tested material and the information presented in this dossier cover the substance as manufactured.

1.3 Physico-chemical properties

Table 9: Summary of physico - chemical properties

REACH ref Annex, §	Property	IUCLID section	Value	Reference/Comment
VII, 7.1	Physical state at 20 ^{o}C and 101.3 KPa	4.1	White solid	Reference: 1 Method: Purity: 99%
VII, 7.2	Melting /freezing point	4.2	106^{o}C The substance slowly decomposes on melting.	Reference: 1 OECD 102 Purity: 99%
VII, 7.3	Boiling Point	4.3	Not applicable - the substance slowly decomposes on melting.	Reference: 1 OECD 102 Purity: 99%
VII, 7.4	Relative density	4.4 density	1.16	Reference: 1 OECD 109 Purity: 99.4%
VII, 7.5	Vapour pressure	4.6	3.7 x 10^{-10} kPa	Reference: 1 OECD 104 Purity: 99%
VII, 7.6	Surface tension	4.10	70 mN/m at 20^{o}C	Reference: 1 OECD 115 Purity: 92.4%
VII, 7.7	Water solubility	4.8	6.1 mg/l at 22^{o}C and pH 6.5 9820 mg/l at 22 ^{o}C and pH 9.0	Reference: 1 OCED 105 (Flask) Purity: 99%
VII, 7.8	Partition coefficient n-octanol/water (log value)	4.7 partition coefficient	2.1 at 20^{o}C (pH not provided but assumed to be < 7) Due to the pKa value, log Kow is anticipated to decrease with increasing pH.	Reference: 1 OCED 107 (Shake flask) Purity: 99%
VII, 7.9	Flash Point	4.11	Not applicable the substance is a solid	
VII, 7.10	Flammability	4.13	Not highly flammable and does not liberate gases in hazardous amounts in contact with water or air.	Reference: 1 EEC A10, 12 and A13 Purity: 99%
VII, 7.11	Explosive Properties	4.14	Not classified. The substance does not contain any groups indicative of explosive	Reference: 1

			properties.	
VII, 7.12	Self-ignition temperature		The substance melted at 105°C and did not ignite at or below this temperature.	Reference: 1 EEC, A16 Purity: 99%
VII, 7.13	Oxidising Properties	4.15	Examination of the chemical structure indicates that it does not contain any chemical groups typical of oxidising agents. Thus the substance can be regarded as incapable of reacting exothermically with a combustible material.	Reference: 1
VII, 7.16	Dissociation constant	4.21	pKa = 4.3 at 25oC	Reference: 1 OECD 112 Purity: 99%

2 MANUFACTURE AND USES

2.1 Manufacture

Tralkoxydim is manufactured for use as an agricultural herbicide in the EU.

2.2 Identified uses

Tralkoxydim is used as an agricultural herbicide with particular activity against pernicious grass weeds such as wild-oats, blackgrass and ryegrass in cereal crops including wheat and barely.

3 CLASSIFICATION FOR PHYSICO-CHEMICAL PROPERTIES

Table 10: Summary table for relevant physico-chemical studies

Method	Results	Remarks	Reference
Not applicable			

3.1 Physico Chemical Properties

3.1.1 Summary and discussion of Physico chemical properties

3.1.2 Comparison with criteria

3.1.3 Conclusions on classification and labelling

As detailed in table 9 tralkoxydim does not meet the criteria for classification for physico-chemical properties.

4 HUMAN HEALTH HAZARD ASSESSMENT

4.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

4.1.1 Non-human information

The following summary is derived from the assessment made for the review under Directive 91/414/EEC.

Tralkoxydim was extensively absorbed following oral administration in rats and hamsters (100%). There are no data following inhalation and dermal exposure. The substance was widely distributed around the body with the highest levels being found in the liver, kidneys, blood and fat, although the residual levels found in these tissues were very low (<0.001-0.008 µg equiv/g at sacrifice on day 7 after repeat dosing with 1mg tralkoxydim). Tralkoxydim was extensively metabolised in rats and hamsters with only trace amounts of the parent material remaining in excreta. Six metabolites were identified in rats, the major urinary and biliary/faecal metabolite in both sexes was tralkoxydim acid followed by tralkoxydim alcohol. In rats the primary metabolic pathway is oxidation of one of the methyl groups on the phenyl ring to form tralkoxydim alcohol followed by further oxidation to form tralkoxydim acid, via an intermediary aldehyde metabolite. Tralkoxydim is rapidly excreted via urine and faeces in both male (95-100%) and female (84-87%) rats. However, there were some sex differences as the main route of excretion in males was via the urine (59-66% of the dose in 48 hours) whereas in females urinary and faecal excretion were comparable

(42-47% versus 37-48% of the dose within 48 hours respectively). A study in bile canulated rats showed that the majority of the absorbed dose was eliminated via bile in both sexes (78% in males and 64% in females within 48 hours of dosing) and a signification proportion of the biliary excretion was reabsorbed to be subsequently excreted in urine. Male rats appear to reabsorb more than females. In hamsters the profiles and routes of excretion are similar for both sexes (63-67% in urine and 12-17% in faeces within 48 hours of dosing).

Reference 2

4.1.2 Human information

None available

4.1.3 Summary and discussion on Toxicokinetics

Refer to section 4.1.1

4.2 Acute toxicity

Table 11: Summary table of relevant acute toxicity studies

Acute Oral		
Species/Dose	**LD_{50}**	**Observations and remarks**
Rat 5/sex/dose Males and Females 500,750,1000 and (males only) 1800 mg/kg Purity 97.8% OECD 401	M: 1258 mg/kg F: 934 mg/kg	Deaths occurred 1 to 2 days after dosing. Clinical signs of toxicity included decreased activity, lachrymation, dehydration, hypothermia, piloerection, pinched sides, upward curvature of the spine, reduced righting reflexes, depressed respiration, miosis, prostration and urinary incontinence. Two females at 1000 mg/kg showed severely repressed respiration. (Southwood J (1994) - Reference 2)
Mouse 5/sex/dose Males and Females 500, 1000 and 2000 mg/kg Purity 97.8% OECD 401	M: 1231 mg/kg F: 1100 mg/kg	Deaths occurred 1 to 3 days after dosing. Clinical signs of toxicity included decreased activity, dehydration, reduced righting reflex, tremors, hypothermia, reduced breathing rate and urinary incontinence (Southwood J (1994) - Reference 2)
Rabbit 3 males/dose 48.9, 96.6, 193, 293, 495 or 519 mg/kg Purity 97.8% OECD 401	> 519 mg/kg	There were no deaths and no signs of toxicity during the study. 519 mg/kg was the maximum concentration achievable at the dose rate of 1 ml/kg due to the viscosity of the solution. (Barber JE (1986) - Reference 2)
Acute Inhalation		
Species/Dose	LC50	Observations and remarks
Rat 5/sex/group 0, 443and 3467 mg/m3 for 4 hours (nose only) (Note 3467 mg/m3 was the maximum achievable concentration) Purity 97.3% OECD 403	> 3.5 mg/l (3467 mg/m3)	There were no deaths during the study. Clinical signs included wet fur, piloerection, hunched posture, stains around the snout and chromodacrorrhea. Abnormal respiratory noises were noted in males from 443 mg/m3 and in females at 3467 mg/m3. (Mclean Head LK & Bennett IP (1994) – Reference 2)
Acute Dermal		
Species/Dose	LD50	Observations and remarks
Rat	> 2000 mg/kg	There were no deaths. Clinical signs of toxicity included urinary incontinence, upward curvature of the spine, signs of

5/sex/dose 2000 mg/kg Purity 96.4% OECD 402		diarrhoea and chromodacryorrhea. (Pritchard VK (1994) - Reference 2)

4.2.1 Non-human information

4.2.1.1 Acute toxicity: oral

The LD_{50} values from the acute oral toxicity studies range from 934-1258 mg/kg.

4.2.1.2 Acute toxicity: inhalation

In an acute inhalation study the LC_{50} was found to be > 3.5 mg/l.

4.2.1.3 Acute toxicity: dermal

The LD_{50} from the acute dermal toxicity study was > 2000 mg/kg.

4.2.1.4 Acute toxicity: other routes

No data available

4.2.2 Human information

No data available

4.2.3 Summary and discussion of acute toxicity

See section 4.2.4.

4.2.4 Comparison with criteria

The LD_{50} values from the acute oral toxicity studies range from 934-1258 mg/kg. These are within the range of 200-2000 mg/kg for classification as Xn; R22 under Directive 67/548/EEC and 300-2000 mg/kg for classification in Acute Tox. 4; H302 under CLP.

In an acute inhalation study the LC_{50} was found to be > 3.5 mg/l. The target particulate concentration in the study was 5 mg/l (the cut-off for classification under both Directive 67/548/EEC and CLP) but the actual concentration tested (which was the maximum achievable concentration) was 3.5 mg/l. Given the results of this study no classification is proposed.

The LD_{50} from the acute dermal toxicity study was > 2000 mg/kg which is above the classification cut-off (2000 mg/kg) under both Directive 67/548/EEC and CLP therefore no classification is proposed.

4.2.5 Conclusions on classification and labelling

Directive 67/548/EEC: Xn: R22
CLP: Acute Tox. 4 H302

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

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

The clinical signs that were apparent after single oral, dermal and inhalation exposure included decreased activity, lachrymation, dehydration, hypothermia, piloerection, pinched sides, upward curvature of the spine, reduced righting reflexes, depressed respiration, miosis, prostration and urinary incontinence).

4.3.2 Comparison with criteria

Substances that have produced significant 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 toxicity in humans following single exposure are classified in STOT-SE 1 or 2. Classification is supported by evidence associating single exposure to the substance with a consistent and identifiable toxic 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 irritation.

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

4.3.3 Conclusions on classification and labelling

Directive 67/548/EEC: Not classified based on available data
CLP: Not classified based on available data

4.4 Irritation

4.4.1 Skin irritation

Table 12: Summary table of relevant skin irritation studies

Method	Results	Remarks	Reference
Rabbits (New Zealand White) 6 females (500mg tralkoxydim moistened to paste with olive oil) Purity 97.8% OECD 404	Very slight or well-defined erythema (average scores from 24-72 hours ≤ 1 in all animals) Very slight, slight or moderate oedema (average scores from 24-72 hours ≤ 1 in all animals)	The skin reactions had resolved within 7 days of application, apart from in 1 animal with slight desquamation	Barber JE 1(994) - Reference 2

4.4.1.1 Non-human information

See table above

4.4.1.2 Human information

No data available

4.4.1.3 Summary and discussion of skin irritation

Very slight or well-defined erythema (average scores from 24-72 hours ≤ 1 in all animals) and very slight, slight or moderate oedema (average scores from 24-72 hours ≤ 1 in all animals) were observed. All reactions had resolved within 7 days of application.

4.4.1.4 Comparison with criteria

As the scores for erythema and oedema were ≤ 1 in all animals tested and only slight desquamation persisted in 1 animal until the end of the study no classification is proposed.

4.4.1.5 Conclusions on classification and labelling

Directive 67/548/EEC: Not classified based on available data

CLP: Not classified based on available data

4.4.2 Eye irritation

Table 13: Summary table of relevant eye irritation studies

Method	Results	Remarks	Reference
Rabbits(New Zealand White) Females 100 mg tralkoxydim Purity 97.8% OECD 405	There were no effects on the cornea or iris (all scores were 0). Slight to moderate conjunctival redness and chemosis were observed (average scores from 24-72 hours ≤ 1 in all animals).	All eye responses had resolved by day 4.	Barber JE (1994) – Reference 2

4.4.2.1 Non-human information

Refer to table 13.

4.4.2.2 Human information

No data available.

4.4.2.3 Summary and discussion of eye irritation

There were no effects on the cornea or iris (all scores were 0). Slight to moderate conjunctival redness and chemosis were observed (average scores from 24-72 hours ≤ 1 in all animals). All effects had resolved by day 4.

4.4.2.4 Comparison with criteria

Tralkoxydim did not produce effects on the cornea or iris and scores for conjunctival redness and chemosis were ≤ 1 in all treated animals. Therefore, tralkoxydim does not meet the criteria for classification.

4.4.2.5 Conclusions on classification and labelling

Directive 67/548/EEC: Not classified based on available data

CLP: Not classified based on available data

4.4.3 Respiratory tract irritation

4.4.3.1 Non-human information

The potential for tralkoxydim to cause respiratory tract irritation has not been directly investigated. However, results from the acute inhalation study show no signs of irritation to the respiratory tract.

4.4.3.2 Human information

No data are available.

4.4.3.3 Summary and discussion of respiratory tract irritation

No effects on the respiratory tract have been observed.

4.4.3.4 Comparison with criteria

No effects on the respiratory tract have been observed therefore tralkoydim does not meet the criteria for classification.

4.4.3.5 Conclusions on classification and labelling

Directive 67/548/EEC: Not classified based on available data
CLP: Not classified based on available data

4.5 Corrosivity

Table 14: Summary table of relevant corrosivity studies

Method	Results	Remarks	Reference
Refer to table 12			

4.5.1 Non-human information

Refer to section 4.4.1

4.5.2 Human information

No data available.

4.5.3 Summary and discussion of Corrosivity

Refer to section 4.4.1

4.5.4 Comparison with criteria

Refer to section 4.4.1.

4.5.5 Conclusions on classification and labelling

Tralkoxydim does not meet the criteria for classification as corrosive when tested in standard skin and eye irritation studies. Consequently, no classification is proposed

Directive 67/548/EEC: Not classified based on available data
CLP: Not classified based on available data

4.6 Sensitisation

4.6.1 Skin sensitisation

Table 15: Summary table of relevant skin sensitisation studies

Species	Method	Doses	No. sensitised/total no.	Result
Guinea Pig Males 20 test and 10 control Purity 97.8%	OECD 406 Guinea Pig Maximisation Test Southwood J (1985) – Reference 2	Intradermal Induction: 5% Topical Induction: 75% Challenge Application: 75% (all doses were formulated in corn oil)	Test Animals: 0/20 No skin reactions in the negative control group.	Negative

A positive control group was not included and there is no additional information to assess the reliability of the study

4.6.1.1 Non-human information

Refer to table 15.

4.6.1.2 Human information

No data available

4.6.1.3 Summary and discussion of skin sensitisation

Tralkoxydim gave a negative response in a guinea pig maximisation study (0/20 response) when tested at a challenge concentration of 75%.

4.6.1.4 Comparison with criteria

A substance is classified as a skin sensitisor if, in a guinea pig maximization study, a positive response is observed in 30% of treated animals. As 0/20 animals gave a response following

treatment with tralkoxydim it can be concluded that it does not meet the criteria for classification in accordance with Directive 67/54/EEC or CLP.

4.6.1.5 Conclusions on classification and labelling Directive 67/548/EEC: Not classified based on available data.

Directive 67/548/EEC: Not classified based on available data CLP: Not classified based on available data.

4.6.2 Respiratory sensitisation

Table 16: Summary table of relevant respiratory sensitisation studies

Method	Results	Remarks	Reference
No data			

4.6.2.1 Non-human information

No data available.

4.6.2.2 Human information

No data available.

4.6.2.3 Summary and discussion of respiratory sensitisation

No data available.

4.6.2.4 Comparison with criteria

No data available.

4.6.2.5 Conclusions on classification and labelling

Directive 67/548/EEC: Not classified –no data available. **CLP: Not classified – no data available.**

4.7 Repeated dose toxicity

Table 17: Summary table of relevant repeated dose toxicity studies

Oral Studies

Dose schedule	Dose levels	Observations and remarks (effects of major toxicological significance)
Rats (diet) 90 days 20/sex/group Purity 97.8% OECD 408 Milburn GM, Chart IS, Gore CW et al 1994 in Reference 2	0, 50, 250 or 2500 ppm corresponding to 0, 4.2, 20.5 or 204.8 mg/kg/day (males) and 0, 4.6, 23.0 and 219.3 mg/kg/day (females)	No treatment related deaths or clinical signs of toxicity. Reduction in body weight gain (11% males and 10% females) and food consumption (8% males and 11% females) at 2500 ppm. Reduced red blood cell parameters at 2500 ppm (haemoglobin (ca. 5%), haematocrit (ca. 5%), RBC (ca. 3%)) in both sexes. Increased white blood cell count in males only (6.58, 7.73, 7.96 and 8.24 x 10^9//l at 0, 50, 250 and 2500 ppm). Mainly due to lymphocytosis which was apparent in males (5.2, 5.86, 5.95 and 6.59 x 10^9/l) but also in females (4.37, 4.34, 4.64 and 5.35 x 10^9/l) at 0, 50, 250 and 2500 ppm respectively. In the absence of any histopathological effects these differences are not considered to be toxicologically significant. At 2500 ppm decreased absolute (11% males and 18% females) and relative (10 % males and 6% females) kidney weight. Increased relative liver weight (10% males and 9% females). No treatment related macroscopic or microscopic findings noted. NOAEL = 250 ppm (20.5 and 23 mg/kg/day males and females respectively)
Mouse (diet) (C57) 28 days 5/sex/group Purity 97.8% Non-guideline study Tinston DJ (1989a & b) in Reference 2	Study 1: 0, 50, 250 or 1250 and 5000 ppm corresponding to 0, 10, 50, 250 and 1000 mg/kg/day in males and females.	No mortalities or clinical signs of toxicity. Reduced body weight gain in males (10% and 16%) and in females (20% and 31%) at 1250 ppm and 2500 ppm respectively. No effects on food consumption. Increased absolute liver weight (39, 66, 67 and 61% in males and 9, 57, 27 and 2% in females) and increased relative liver weights (38, 74, 87 and 93% in males and 20, 63, 59 and 49% in females) at 50, 250, 1250 and 5000 ppm respectively. Microscopic findings in the liver included hyperplasia and fibrosis of bile ducts, associated with acute inflammatory reactions ranging from slight to moderate in all animals from 50 ppm. A yellow/brown pigment accumulation (possibly protoporphyrin) in the bile ducts and Kupffer cells ranging from minimal to marked was observed in all animals from 50 ppm. Areas of focal or multifocal necrosis (in 0, 1, 4, 0 and 1 males and in 0, 4, 1, 2 and 2 females at 0, 50, 250, 1250 and 5000 ppm). Small crystalline aggregates were found in the lumen of the bile ducts, macrophages and hepatocytes at 1250 ppm and above. This varied from aggregates of very fine crystals (seen in the bile ducts) to larger crystalline bodies (more frequently seen in the macrophages and hepatocytes). NOAEL not determined due to the effects seen at the lowest dose of 10 mg/kg/day.

Mouse (diet) (C57) 28 days 5/sex/group Purity 97.8% Non-guideline study Tinston DJ (1989a & b) in Reference 2	Study 2: 0, 2, 10 and 25 ppm corresponding to 0, 0.4, 2, and 5 mg/kg/day in males and females.	No mortalities or clinical signs of toxicity. No effects on body weight gain or food consumption. Increased absolute liver weights (6% in males and 16% in females) and relative liver weights (6% in males and 9% in females) at 25 ppm. Microscopic findings in the liver included hyperplasia and fibrosis of bile ducts ranging from slight to moderate in 3/5 males and from minimal to moderate in 5/5 females at 25 ppm. A brown pigment accumulation (possibly protoporphyrin) was observed in the bile ducts and Kupffer cells ranging from minimal to slight in 3/5 males and 4/5 females at 25 ppm. Areas of multifocal necrosis were also observed in 3/5 females at 25 ppm. NOAEL (based on liver effects) = 10 ppm (2 mg/kg/day)
Mouse (diet) a) C57B1/10 b) AP c) Balb/c 28 days 10/sex/group Non-guideline study Stonard MD (1989a & b) – Reference 2	0, 25 or 125 ppm corresponding to 0, 5 or 25 mg/kg/day in males and females	There were no treatment related deaths or clinical signs of toxicity. No adverse effects on bodyweight gain or food consumption. Generally increased ALP (ranging from 5-112%) was noted in all strains and both sexes at 125 ppm along with increased ALT (ranging from 178-1172%) and cholesterol (ranging from 24-225%) in all strains and both sexes from 25 ppm. The AP strain of mice were least affected. Necropsy revealed enlarged and dark discoloured livers (in all strains) at 125 ppm and to a lesser extent at 25 ppm. The effect was more pronounced in males than females. Increased relative liver weight in all strains, ranging from 5-9% in males and 1-8% in females at 25 ppm and from 16-40% in males and 23-45% in females at 125 ppm. Absolute liver weights were increased by a similar amount. Microscopic findings in the liver included bile duct hyperplasia ranging from minimal to moderate in all strains and both sexes from 25 ppm. This effect was more severe in the C57BL/10 strain of mice (8/9 males and 10/10 females at 25 ppm compared to 1/10 males and 0/10 females in AP and 1/10 males and 0/10 females in Balb/c) and (0/10 males and 10/10 females at 125 ppm compared to 6/10 males and 8/10 females in AP and 7/10 males and 10/10 females in Balb/c). Biliary fibrosis and portal inflammations were also noted from 25 ppm at a comparable level. Necrosis was noted in C57BL/10 mice (2/10 males and 6/10 females at 25 ppm and 9/10 males and 7/10 females at 125 ppm) and in AP (4/10 males and 5/10 females) and Balb/c (5/10 males and 10/10 females) at 125 ppm only. Pigment accumulation in the hepatocytes and Kupffer cells was noted in mice of both sexes in each strain and at both doses. NOAEL not determined due to the effects seen at the lowest dose of 5 mg/kg/day.

Dose schedule	Dose levels	Observations and remarks (effects of major toxicological significance)
Hamster (diet) 28 day with Toxicokinetics 12/sex/dose Purity 98.2% Milburn GM (2002b) – reference 4	0 and 17000 ppm	At 17000ppm there were reductions in male body weight (11%) and in food consumption (33%). Liver weight was increased in males and females (absolute 29% and 33%, relative 46% and 37%). Significant increases cytochrome P450 and EROD were observed in males and females. In addition, testosterone hydroxylation was increased in males as follows: Testosterone 16 α hydroxylation; 0.016 and 0.127 nmol/min/mg protein Testosterone 16 β hydroxylation; 0.047 and 0.326 nmol/min/mg protein
Hamster (diet) 90 day with Toxicokinetics 12/sex/dose Purity 98.2% Milburn GM (2002a) – reference 4	0 and 500, 4000, 8000 and 12000 ppm 0, 30, 240, 480 and 720 mg/kg/day (males and females)	Liver weight was increased in males and females from 8000 ppm. At 12000 ppm absolute increase 34% males and 16% in females, relative 44% in males and 16% in females. Absolute and relative ovary weight was decreased in females from 8000 ppm by between 20 - 25%. Significant increases cytochrome P450 and EROD were observed from 4000 ppm. In addition, testosterone hydroxylation was significantly increased in males from 4000 ppm as follows; Testosterone 16 α hydroxylation; 0.023, 0.034, 0.058, 0.120 and 0.209 (nmol/min/mg protein) at 0, 500, 4000, 8000 and 12000 ppm respectively. Testosterone 16 β hydroxylation; 0.068, 0.108, 0.214, 0.308, and 0.520 (nmol/min/mg protein) at 0, 500, 4000, 8000 and 12000 ppm respectively.
Hamster (diet) 90 days 20/sex/group (main study) 10/sex/group (satellite study) Purity 92.4% Stonard MD (1994) – Reference 2	0, 250, 800, 2000 or 5000 ppm corresponding to 0, 30, 96, 240, 600 mg/kg/day in males and females. A satellite study was initiated with diets containing 0, 10000 or 20000 ppm equivalent to 1200 or 2400 mg/kg/day in males and females.	There were no treatment related deaths or clinical signs of toxicity. Reduced body weight gain in the satellite study only (30% and 59% in males, 26% and 24% in females at 10000 ppm and 20000 ppm respectively. Slight reduction in red blood cell parameters (RBC, haemoglobin, haematocrit ca. 4%) from 250 ppm in males and 2000 ppm in females. In the satellite group a reduction is these parameters was again noted reaching 11-14% in males and 7-10% in females at 20000ppm. In the main study the white blood cell count was reduced in males (7.94, 6.56, 7.31, 6.09 and 6.46 x 10^9/l) and in females (7.46, 5.67, 6.78, 5.56 and 5.94 x 10^9/l) at 0, 250, 800, 2000 and 5000 ppm. In the satellite study there were reductions in white blood cell count (37% and 31%) and lymphocyte count (40% and 28%) in males only from 10000 ppm. Increased absolute (15% males and 19% females) and relative (20% males and females) liver weight at 5000 ppm in the main study. Relative liver weights were also increased in the satellite study (35 and 49% in males and 20 and 34 % in females at 10000 and 20000 ppm). Relative kidney weights were also increased in males by 6% and 27% at 10000 ppm and 20000 ppm respectively. Microscopic findings in the liver included increased hepatocytes eosinophilla in 1/11 males at 5000 ppm and all males and females from 10000 ppm in the satellite study. Loss of hepatocytes vacuolation was observed in 7/11 males at 5000 ppm in the main study and in all animals from 10000 ppm in the satellite study. A NOAEL was not determined.

Dog 90 days OECD 409 4/sex/dose Purity 97.8% Brammer A, Chart IS, Doe JE, Gore CW, Pate I, Rowlands MA, Soames AR and Stonnard MD (1994) in Reference 2	0, 0.5, 5 and 50 mg/kg/day males and females (capsules)	No treatment related deaths or clinical signs of toxicity were noted. No treatment related effects on bodyweight gain or food consumption. Red blood cell parameters (haemoglobin, haematocrit, and red blood cell counts) were decreased (≤ 10%) at 50 mg/kg/day. White blood cell counts were increased in both sexes at 50 mg/kg/day due, in males, due to neutrophilia and, in females, to lymphocytosis. Increased liver weights in males compared to controls at 0.5 mg/kg/d (8%), 5 mg/kg/day (25%) and 50 mg/kg/day (64%) and in females at 5 mg/kg/d (6%) and 50 mg/kg/day (51%). At 50 mg/kg/day macroscopic evaluation revealed enlarged pale livers with a marked reticular pattern in all dogs. Microscopic findings in the liver included vacuolation of hepatocytes and fatty changes with a periportal distribution ranging from slight (4/4 males and 3/4 females) to marked (1/4 females only) in all animals at 50 mg/kg/day. Increased adrenal weights in both sexes at 50 mg/kg/day (39% males and 24% females). Microscopic findings in all animals at 50 mg/kg/day included vacuolation of cells in the zona fasciculate with a multifocal distribution. Decreased epididymides weight in males (21%) at 50 mg/kg/day. In addition, slight unilateral atrophy of the seminiferous epithelium was noted in 1 male of this group.
Dog 1 year 4/sex/dose OECD 452 Purity 94.9% Stonard MD (1994b) – Reference 2	0, 0.5, 5 and 50 mg/kg/day (capsules)	No treatment related mortalities or clinical signs of toxicity. No treatment related effects on bodyweight or food consumption. Red blood cell parameters (haemoglobin, haematocrit, and red blood cell counts) were reduced in males (ca. 11%) at 50 mg/kg/day. White blood cell counts were increased in both sexes at 50 mg/kg/day. Increased AP (249% m and 313% f) and ALT (78% m and 230% f) and decreased albumin (20% m and 20% f), total protein (8% m and 13% f), cholesterol (29% m and 48% f) and triglycerides (48% m and 64% f) at 50 mg/kg/day. Increased relative liver weights in males at 5 mg/kg/day (8%) and at 50 mg/kg/day (54%) and in females at 50 mg/kg/day (65%). Increased absolute liver weights in males (51%) and in females (65%) at 50 mg/kg/day. Macroscopic findings at 50 mg/kg/day included enlarged, mottled livers with accentuated lobular patterns and swollen lobes. Microscopic findings included moderate fatty changes in hepatocytes in 1/4 males at 5 mg/kg/day and in 4/4 males (moderate in 2/4 males and marked in 2/4 males.) and 2/4 females (moderate in 1/4 and marked in 1/4) at 50 mg/kg/day. Increased relative adrenal weights in males at 5mg/kg/day (18%) and at 50 mg/kg/day (70%) and in females at 50 mg/kg/day (75%). Absolute adrenal weights were increased at comparable levels. Microscopic findings in all animals at 50 mg/kg/day included vacuolation of cells in the zona fasciculate, this was also observed in 3/4 females at 5 mg/kg/day. Increased relative thyroid weight in males (47%) and females (42%) at 50 mg/kg/day. Absolute thyroid weights were increased at comparable levels. There were no microscopic findings in the thyroid. In the testis, unilateral tubular degeneration observed in 1/4 males at 0.5 mg/kg/day and 1/4 males at 50 mg/kg/day. Bilateral tubular degeneration was noted in 1/4 males at 0.5 mg/kg/day and 1/4 males at 5 mg/kg/day.

Dermal Studies

Dose schedule	Dose levels	Observations and remarks (effects of major toxicological significance)
Rat 21 days 5/sex/dose OECD 410 Purity 94.9% Leah AM (1989a & b) – Reference 3	0, 10, 100 or 1000 mg/kg/day	No treatment related mortalities or clinical signs of toxicity. No treatment related effects on bodyweight or food consumption. Slight reduction in white blood cell count in females at 100 mg/kg/day (22%) and 1000 mg/kg/day (28%). No other significant treatment related effects were reported. NOAEL > 1000 mg/kg/day

4.7.1 Non-human information

4.7.1.1 Repeated dose toxicity: oral

Rat

In the 90 day study in the rat the main effects were observed at 2500 ppm (204.8 and 219.3 mg/kg/day in males and females respectively) and included effects on bodyweight gain (c.a. ↓10%), minor haematological changes (↓≤ 5%) and increased liver and kidney weights (c.a. 10%). These effects were not accompanied by any supporting histopathology.

Mouse

The main target organ of toxicity in the mouse is the liver. Increased liver weights were observed from 25 ppm (5 mg/kg/day) along with microscopic changes including necrosis and hyperplasia and fibrosis of the bile ducts. Increased pigmentation, thought to be due to porphyrin, was also observed. Such effects were observed in the 3 strains of mice tested (C57B1/10, AP and Balb/c) so this is not unique to one strain of mouse.

However, further studies have been conducted which show that this effect, when seen following treatment with tralkoxydim, can be considered specific to the mouse. Further information is provided in section 4.7.1.6 and Annex I.

Hamster

In the 90 day hamster study effects were observed from 250 ppm (30 mg/kg/day). The main effects observed following treatment with tralkoxydim included minor haematological changes (RBC, haemoglobin, haematocrit reduced by <5% at ≤ 600 mg/kg/day and ≤ 15% at 2400 mg/kg/day),

increased liver and kidney weights from 600 mg/kg/day and microscopic effects in the liver from 600 mg/kg/day.

In a separate 90 day study and a 28 day study tralkoxydim was shown to induce liver enzymes at high doses (17000 ppm in the 28 day study and 4000 ppm in the 90 day study). At such high doses, and in the presence of liver enzyme induction, testosterone hydroxylase was induced.

Dog

In the dog studies, significant effects were observed from 50 mg/kg/day in the 90 day study. The main effects were haematological changes (↓ ≤10%), increased liver weight (64% males and 51% females) with associated histopathology including fatty changes and vacuolation in all animals.

In a 1 year study effects were observed from 5 mg/kg/day with increased liver weights in males (8%) and 1/4 males with fatty changes in hepatocytes. The effects were more marked at 50 mg/kg/day with increased liver weights (54% and 65% m and f) and fatty changes in hepatocytes (4/4 males and 2/4 females).

4.7.1.2 Repeated dose toxicity: inhalation

No data are available

4.7.1.3 Repeated dose toxicity: dermal

Dermal administration of tralkoxydim in a short-term repeated dose study did not result in any adverse effects.

4.7.1.4 Repeated dose toxicity: other routes

No data are available

4.7.1.5 Human information

No data are available

4.7.1.6 Other relevant information

Investigation into hepatic porphyria in mice

Administrations of low doses (5 mg/kg/day) of tralkoxydim were found to cause severe effects in the livers of mice**.** These effects were attributed to the accumulation of porphyrin but were not observed in the repeat dose studies conducted in rats, dogs or hamsters. To investigate this, further studies were conducted. These included a number of studies to determine the mode of action in mice, investigations into the species differences in tralkoxydim induced hepatic porphyria and investigations into the relevance to humans using cultured human hepatocytes.

These studies have shown that the hepatic porphyria in mice is due to the accumulation of N-methyl protoporphyrin IX and the inhibition of ferrochelatase activity (which is the terminal enzyme in the haem biosynthetic pathway) in the mouse liver. It is proposed that tralkoxydim is metabolised in the mouse liver by a specific pathway which results in the N-alkylation of haem, following the transfer of a methyl group form the C-ethyl moiety of tralkoxydim. The increase in N-methyl protoporphyrin IX leads to inhibition of the ferrochelatase enzyme which disturbs the normal control of the haem biosynthetic pathway. This in turn leads to stimulation of haem biosynthesis through enhanced ALAS activity which leads to an increase in porphyrin production and an accumulation of protoporphyrin in the liver.

Tralkoxydim has been shown to be porphyrinogenic in mice but similar effects were not observed at all in rats, or in hamsters or guinea pigs at significant levels or at low doses. It is proposed that the inability of tralkoxydim to induce hepatic porphyria in rats or hamsters and guinea pigs at significant levels, is likely due to its inability to inhibit ferrochelatase activity in these species. It is therefore inferred that this difference is due to differences in the metabolic pathway in mice which results in the formation of N-methyl protoporphyrin IX.

Tralkoxydim was also found to have no effect on ferrochelatase activity in cultured human hepatocytes. It is also proposed that human hepatocytes do not have a significant haem demand compared to the mouse. *In vitro* studies in cultured rat and mice hepatocytes mirrored the *in vivo* results in these species.

A summary of the studies that have been conducted to provide the above understanding, and a description of the haem biosynthetic pathway, are provided in Annex I to this report.

This proposed mode of action and the apparent lack of relevance to humans was accepted when the substance was reviewed under the 91/414 process.

4.7.1.7 Summary and discussion of repeated dose toxicity

The main target organ of toxicity following oral administration is the liver. In rats and hamsters increased liver weights (ca 10% in rats and 20% in hamsters), with some minor histopathology in the hamster liver only (hepatocyte eosinophillia in one male and loss of hepatocyte vacuolation) were observed at high doses (≥ 205 mg/kg/day in rats and ≥ 600 mg/kg/day in hamsters).

In dogs, significant effects were noted from 50 mg/kg/day in a 90 day study and included increased liver weights (64% in males and 51% in females), slight fatty changes and vacuolation of hepatocytes in all animals. These effects were also noted in a 1 year study at 5 mg/kg/day in males (increased liver weight 8%, and 1/4 males with fatty changes in hepatocytes) and at 50 mg/kg/day in males and females (increased liver weights (ca. 70%) and 4/4 males and 2/4 females with moderate to marked fatty changes in hepatocytes).

In the mouse, significant liver effects were observed from 5 mg/kg/day in a number of 28 day studies. These effects included an increase in porphyrin, necrosis and hyperplasia and fibrosis of the bile ducts. Additional mechanistic studies have been conducted and it is proposed that the porphyrinogenic activity in mice following treatment with tralkoxydim is due to a species specific metabolic pathway which results in the formation of N-methyl protoporphyrin IX.

Dermal administration of tralkoxydim in a short-term repeated dose study did not result in any adverse effects.

4.7.1.8 Summary and discussion of repeated dose toxicity findings relevant for classification according to DSD

Refer to section 4.7.1.7.

4.7.1.9 Comparison with criteria of repeated dose toxicity findings relevant for classification according to DSD

A substance is classified with R48 under Directive 67/548/EEC when it has produced or has been shown to have the potential to produce serious damage (clear functional disturbance or morphological change which has toxicological significance) following repeated exposure by the oral, dermal or inhalation routes. This can be on the basis of human data or evidence from studies in animals that cause such adverse effects at or below given guidance values (≤ 5 mg/kg/day or ≤50 mg/kg/day in a 90 day oral study in the rat).

There are no data in humans on tralkoxydim

The main target organ of toxicity for tralkoxydim following oral administration to animals is the liver.

The effects in rats and hamsters occur above the relevant guidance values for classification with R48.

In dogs, toxicologically significant effects (slight to marked fatty changes in the liver) were noted at 50 mg/kg/day in a 90 day study and from 5 mg/kg/day in a 1 year study. Such effects at these dose levels are considered to show that classification with R48/22 is appropriate.

In the mouse, significant liver effects were observed from 5 mg/kg/day in a number of 28 day studies. These effects included an increase in porphyrin, necrosis and hyperplasia and fibrosis of the bile ducts. Additional mechanistic studies have been conducted and it is proposed that the porphyrinogenic activity in mice following treatment with tralkoxydim is due to a species specific metabolic pathway which results in the formation of N-methyl protoporphyrin IX. This has not been seen to occur in rats, or in hamsters and guinea pigs at significant levels or at low doses. In addition, *in vitro* investigations show that such activity is not observed in isolated human hepatocytes. There is also evidence to propose that human hepatocytes have a low haem demand compared to the mouse. Therefore, these effects in mice, do not support classification with R48 in accordance with Directive 67/548/EEC.

4.7.1.10 Conclusions on classification and labelling of repeated dose toxicity findings relevant for classification according to DSD

Directive 67/548/EEC – Xn; R48/22

4.8 Specific target organ toxicity (CLP Regulation) – repeated exposure (STOT RE)

4.8.1 Summary and discussion of repeated dose toxicity findings relevant for classification as STOT RE according to CLP Regulation

Refer to section 4.7.1.7.

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

A substance is classified with STOT-RE under CLP when it has produced or has been shown to have the potential to produce significant toxicity in humans or be harmful to human health following repeated exposure by the oral, dermal or inhalation routes. This can be on the basis of human data or evidence from studies in animals that cause such adverse effects at or below given guidance values (≤ 10 mg/kg/day or ≤100 mg/kg/day in a 90 day oral study in the rat).

There are no data in humans on tralkoxydim.

The main target organ of toxicity for tralkoxydim following oral administration to animals is the liver.

The effects in rats and hamsters occur above the relevant guidance values for classification with STOT RE.

In dogs, toxicologically significant effects (slight to marked fatty changes in the liver) were noted at 50 mg/kg/day in a 90 day study and from 5 mg/kg/day in a 1 year study. Such effects at these dose levels are considered to show that classification with STOT-RE 2 is appropriate.

In the mouse, significant liver effects were observed from 5 mg/kg/day in a number of 28 day studies. These effects included an increase in porphyrin, necrosis and hyperplasia and fibrosis of the bile ducts. Additional mechanistic studies have been conducted and it is proposed that the porphyrinogenic activity in mice following treatment with tralkoxydim is due to a species specific metabolic pathway which results in the formation of N-methyl protoporphyrin IX. This has not been seen to occur in rats or in hamsters and guinea pigs at significant levels or at low doses. In addition, *in vitro* investigations show that such activity is not observed in isolated human hepatocytes. There is also evidence to propose that human hepatocytes have a low haem demand. Therefore, these effects in mice, do not support classification with STOT-RE.

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

CLP – STOT RE 2 H373 May cause damage to organs through prolonged exposure.

4.9 Germ cell mutagenicity (Mutagenicity)

Table 18: Summary table of relevant in vitro and in vivo mutagenicity studies

<table>
<tr><th colspan="4">In Vitro Data</th></tr>
<tr><th>Method</th><th>Organism/strain</th><th>Concentrations tested</th><th>Result</th></tr>
<tr><td>Bacterial Mutation Assay (Ames)
Purity 98.2%
OECD 471
Callander RC (2003) – Reference 3</td><td>S. typhimurium (TA1535, TA1537, TA98 and TA100) and E.coli (WP2P and WP2P uvrA)</td><td>100-5000 µg/plate</td><td>Negative ± S9 metabolic activation</td></tr>
<tr><td>Mammalian cell gene mutation test
Purity 98.2%
OECD 476
Clay P (2003) – Reference 3</td><td>L5178Y TK +/- mouse lymphoma cells</td><td>200-3300 µg/ml
(the maximum concentration equivalent to 10mM)</td><td>Negative ± S9 metabolic activation</td></tr>
<tr><td>Mammalian chromosome aberration test
Purity 98.2%
OECD 473
Fox V (2003) – Reference 3</td><td>Human lymphocytes</td><td>100-3300 µg/ml</td><td>Negative + S9 metabolic activation

Negative - S9 metabolic activation
An increase in cells with chromosome aberrations was observed in the first study (experiments 1 and 2 in table below) from 750 µg/ml. The values were outside of the historical control range. However, inconsistent results were obtained with the duplicate cultures used in these experiments. As a consequence they were repeated (experiments 3 and 4 below). The dose related effects seen in experiments 1 and 2 were not reproduced.
<u>Table 5.7.1.1.1: Mean chromosome aberrations (% excluding gaps) without S9.</u>
<table>
<tr><td rowspan="2">Concentration (µg/ml)</td><td colspan="4">Experiment number</td></tr>
<tr><td>1</td><td>2</td><td>3</td><td>4</td></tr>
<tr><td>0</td><td>1</td><td>0</td><td>1</td><td>2</td></tr>
<tr><td>100</td><td></td><td>0.5</td><td></td><td>0</td></tr>
<tr><td>250</td><td>1</td><td></td><td>0</td><td></td></tr>
<tr><td>750</td><td></td><td>3.50</td><td></td><td>2.5</td></tr>
<tr><td>1500</td><td></td><td>5.97</td><td></td><td>1.5</td></tr>
</table></td></tr>
</table>

			2000 | 3.47 | | 1 | 3000 | 5.0 | | 1 | Duration of treatment Experiments 1 and 3 - 3 hours Experiments 2 and 4 - 20 hours
In vivo Data			
Method	**Organism/strain**	**Concentrations tested**	**Result**
Mouse Micronucleus Test Purity 97.8% OECD 474 Sheldon et al (1994) – Reference 3	Mouse (5/sex/group)	300 and 480 mg/kg (i.p.) in corn oil Single dose animals terminated at 24, 48 and 72 hours	**Negative** Initial Test: 2/5 males receiving 480 mg/kg were killed in extremis at the 72 hour sampling point. There were no deaths at 24 and 48 hours. A small increase in polychromatic erythrocytes containing micronuclei was seen in males at 24 hours following dosing with 480 mg/kg (mean incidence of micronuclei 4.4, 3.6 and 6.6 at 0, 300 and 480 mg/kg respectively). Repeat Test: 300mg/kg: 1 female killed in extremis at the 48 hour sampling point. 480 mg/kg: 3/5 males and 4/5 females were found dead or were killed in extremis at the 24 hour sampling time. 5/5 males and 1/5 females were found dead or were killed in extremis at the 48 hour sampling time. No increase in polychromatic erythrocytes containing micronuclei in any of the surviving animals. For comparison, the mean incidence of micronuclei in males at 24 hours were 2.8, 4.4 and 1 at 0, 300 and 480 mg/kg respectively).
Unscheduled DNA synthesis assay Purity 97.8% OECD 486 Trueman (1994) – Reference 3	Rat hepatocytes	0, 250, 500 or 1000 mg/kg (gavage) in corn oil	**Negative** Cytotoxicity was observed at the highest concentration tested.

4.9.1 Non-human information

4.9.1.1 In vitro data

Three standard *in vitro* studies have been performed on tralkoxydim. There was no evidence of genotoxicity in the Ames test or in the mammalian cell gene mutation test. A small concentration related increase in cells with chromosome aberrations was noted, in the absence of S9, in an *in vitro* cytogenetics test in human lymphocytes. However, there was inconsistency between the duplicate cultures used in the first test and the increase was not reproduced in an additional experiment under the same conditions

4.9.1.2 In vivo data

Two *in vivo* mutagenicity studies are available. Tralkoxydim produced negative results in a rat liver UDS assay. In an initial mouse micronucleus study a small increase in the frequency of polychromatic erythrocytes containing micronuclei was seen in male mice receiving 480 mg/kg (a dose that caused mortality), at the 24 hour sampling time. However, the mean incidence of micronuclei in the concurrent controls was also high (4.4 compared to 3.4 and 2.2 at 48 and 72 hours respectively). In a repeat study, using the same doses and same conditions, no increase was observed. However, a high level of mortality was observed in the second study (3/5 males and 4/5 females of the 480 mg/kg group died or were killed in extremis at the 24 hour sampling time).

4.9.2 Human information

No data available.

4.9.3 Other relevant information

No data available.

4.9.4 Summary and discussion of mutagenicity

Negative results were obtained in the available *in vitro* and *in vivo* studies. The increase in chromosome aberrations in the in vitro cytogenetic assay and the increase in PCE containing micronuclei in the *in vivo* bone marrow micronucleus study were small, could not reproduced in repeat studies and were only seen at a dose level causing mortality.

4.9.5 Comparison with criteria

Substances known to induce heritable mutations or which are regarded as if they induce heritable mutations in the germ cells of humans are classified in Category 1A or 1B accordingly. This is based on human data or positive result from *in vivo* studies. As there are no human data classification in Cat 1A is not appropriate. As the *in vivo* studies produced negative results classification in Cat 1B is not appropriate.

Substances which cause concern for humans owing to the possibility that they may induce heritable mutations in the germ cells of humans are classified in Category 2. This is based on positive evidence obtained from experiments in mammals and/or in some cases from in vitro experiments.

Tralkoxydim produced negative results in 3 *in vitro* and 2 *in vivo* studies therefore classification in Category 2 is not appropriate.

4.9.6 Conclusions on classification and labelling

Directive 67/548/EEC: No classification based on available data.
CLP: No classification based on available data.

4.10 Carcinogenicity

Table 19: Summary table of relevant carcinogenicity studies

Dose schedule	Dose levels	Observations and remarks (effects of major toxicological significance)
Rat (Alpk) 2 years 64/sex/dose OECD 453 Purity 92.4% Stonard MD (1994c) – Reference 3	0, 50, 500 or 2500 ppm corresponding to 0, 2.3, 23.1 or 117.9 mg/kg/day in males and 0, 3.0, 30.1 or 162.8 mg/kg/day in females	There were no adverse treatment related effects on survival rates. *Non-tumour findings* General decrease in body weight gain was noted in males and females at 2500 ppm. At the end of the study the decrease was not significant in males (0.3%) but was in females (16%). Food consumption was also reduced at this level, particularly during the 1st year. Throughout the study general reductions in red blood cell parameters (c.a. 4-7% reduction in haemoglobin, haematocrit, and red blood cell count) were noted in both sexes at 2500 ppm. However, at the end of the study there was a slight increase in these parameters in males (5-6%) and a further decrease in females (ca. 10-13%). Increases in white blood cell counts were noted throughout the study (ranging from 11-19% in males and 1-57% in females) due to increased lymphocytes (15-31% in males and 15-89% in females) at 2500 ppm. Increases in relative liver weight at 2500 ppm (8% males and 3% females). At this level there was an increased incidence of clear cells in the liver of both sexes (15/52, 11/53, 16/52 and 32/52 males, 1/52, 0/52, 1/51 and 12/52 females at 0, 50, 500 and 2500ppm respectively) and an increase in the amount of haemosiderin in the kupffer cells of the females. Increase in the number of males with enlarged testis (0, 0, 3 and 4 males at 0, 50, 500 and 2500ppm respectively) along with an increase in testis with white areas (1, 1, 2, 8 males at 0, 50, 500 and 2500ppm respectively). An increase in unilateral and bilateral Leydig cell hyperplasia was noted in males at 2500 ppm (combined incidence 4/52, 5/53, 4/52 and 14/52 at 0, 50, 500 and 2500 ppm respectively. There was also an increased incidence of tubular atrophy of the testes and reduced numbers of spermatozoa in the epididymides accompanied by the presence of an increased number of early nucleated sperm precursor cells at 2500 ppm. Unilateral and bilateral retinal atrophy was increased in females at 2500 ppm (combined incidence 4/52, 4/52, 3/51 and 34/52 at 0, 50, 500 and 2500 ppm respectively). *Neoplastic findings* Increased incidence of Leydig cell tumours were observed in males at 2500 ppm (combined incidence of unilateral and bilateral tumours 3/52(5.8%), 5/53(9.4%), 6/52(11.5%) and 15/52 (28.8%) at 0, 50, 500 and 2500 ppm respectively). Contemporary historical control data* ranged from 3.8% to 19.2%. Increased incidence of brain astrocytoma (2/52, 1/53, 2/52 and 3/52 (5.8%) and spinal cord astrocytoma (0/52, 0/53, 0/52 and 1/52 (1.9%)) in males at 2500ppm. Only 1 control female was found to have a brain astrocytoma. Contemporary historical control data* range from 0 to 5.8% for brain astrocytoma and 0 to 1.9% for spinal cord astrocytoma. Increased incidence of uterine adenocarcinomas in females at 2500 ppm (1/52, 1/52, 0/53 and 3/52 (5.8%) at 0, 50, 500 and 2500 ppm respectively).

<table>
<tr><td></td><td></td><td>Contemporary historical control data* range from 0 to 5.8%.

*Historical control data are taken from a period of time before, during and after (1984-1990) the in-life phase of this study (1985-1987). See table 5.8.1.1.1 below.</td></tr>
<tr><td>Hamster

79 weeks

72/sex/dose

OECD 451

Purity 97.6%

Stonard MD (1989e) – Reference 3</td><td>0, 250, 2500 or 7500 ppm corresponding to 0, 14.9, 153 or 438.6 mg/kg/day in males and 0, 14.8, 148.3 or 427.9 mg/kg/day in females.

Three concurrent control groups were used</td><td>At termination of the study survival rates at all doses and in the controls were ≤ 50% in both sexes. Mortality was particularly high in females with survival rates of 6%, 10%, 3%, 0%, 10%, 14% compared to 50%, 42%, 39%, 42%, 44% and 39% in males at 0, 0, 0, 250 , 2500 and 7500 ppm respectively

Non neoplastic findings

Gross necropsy revealed a number of findings in both control and treated animals which were thought to have contributed to the premature deaths of many animals in the study. These findings are not considered to be treatment related.

<u>Neoplastic findings</u>

Increase in benign adrenal cortical adenomas in females 0, 1.4%, 1.4%, 2.8%, 5.6% and 5.6% at 0, 0, 0, 250, 2500 and 7500 ppm respectively).</td></tr>
<tr><td>Hamster

80 weeks

51/sex/dose

OECD 451

Purity 98.2%

Milburn GM (2002) – Reference 3</td><td>0, 500, 2500 or 12000 ppm corresponding to 0, 29.5, 150.3 or 700.3 mg/kg/day in males and 0, 27.8, 138.9 or 672.2 mg/kg/day in females.</td><td>There were no treatment related effects on mortality (survival rates were >50% at termination of study).

Bodyweight gain was reduced throughout the study in males receiving 12000 ppm (maximum of 23%). Food consumption was reduced in the first week (11% males and 5% females) and in the latter stages in males (8%).

Absolute liver weights were increased at 12000 ppm in males (22%) and females (36%). Relative liver weights were also increased at 12000 ppm in males (28%) and females (37%). There was no associated histopathology.

Kidney weight increased in males (absolute 8%, relative 12%) at 12000 ppm. There was no associated histopathology.

Testis weight increased (absolute 6% and relative 11%) at 12000 ppm. No associated histopathology but there was a slight increase in testicular tubular degeneration ranging from minimal to marked at this dose level (unilateral 9/51, 12/51, 15/51 and 17/51 and bilateral 7/51, 6/51, 4/51 and 4/51 at 0, 500, 2500 and 12000 ppm respectively).

<u>Neoplastic findings</u>

Dose related increase in the combined incidence of benign and malignant sex cord stromal tumours in the ovaries 2/49 (4.1%), 4/50 (8.0%), 6/48 (12.5%) and 7/51 (13.7%) at 0, 500, 2500 and 12000 ppm respectively. Benign tumours 1/49, 2/50, 4/48, 5/51 and malignant tumours 1/49, 2/50, 2/48 and 2/51 at 0, 500, 2500 and 12000 ppm respectively. These comprised of benign and malignant thecal cell, granulosa cell and, to a lesser extent, interstitial cell tumours (Table 5.8.1.2.1)

Table 5.8.1.2.1 Summary of ovarian neoplastic findings
<table>
<tr><td>Dose (ppm)</td><td>0</td><td>500</td><td>2500</td><td>12000</td></tr>
<tr><td>Tumour Type</td><td></td><td></td><td></td><td></td></tr>
<tr><td>Thecal cell</td><td>1 (M)</td><td>1 (M)</td><td>2 (B+M)</td><td>3 (2B+1M)</td></tr>
<tr><td>Granulosa cell</td><td>1 (B)</td><td>1 (M)</td><td>1 (B)</td><td>3 (B)</td></tr>
<tr><td>Interstitial cell</td><td></td><td>1 (B)</td><td></td><td></td></tr>
<tr><td>Granulosa/thecal</td><td></td><td>1 (B)</td><td>2 (B) (a)</td><td></td></tr>
</table></td></tr>
</table>

		cell				
		Solid granulosa/tubular			1 (M)	
		Granulosa/interstitial				1 (M)
		(a) – bilateral M = malignant B = benign * See below for information on historical controls (table 5.8.1.2.2). Benign adrenal cortical adenomas were noted in males (17, 7, 11 and 7) and in females (7, 7, 4 and 8) at 0, 500, 2500 and 12000 ppm respectively.				

4.10.1 Non-human information

4.10.1.1 Carcinogenicity: oral

Rat

In the 2 year chronic toxicity/carcinogenicity study in the rat the main non-neoplastic findings at 2500 ppm included reductions in red blood cell parameters (4-7%) increased lymphocyte counts, mild liver toxicity (increased ALT, increased clear hepatocytes, increased liver weights), testicular effects (enlarged testis, Leydig cell hyperplasia, tubular atrophy and reduced spermatozoa in the epididymides) and retinal atrophy in females.

Neoplastic findings

Leydig Cell Tumours

There was a significant increase in the incidence of Leydig cell tumours in male rats of the 2500ppm group (3/52 (5.8%), 5/53(9.4%), 6/52(11.5%) and 15/52 (28.8%) at 0, 50, 500 and 2500 ppm respectively). It is recognised that differentiation between Leydig cell tumours and focal Leydig cell hyperplasia may be difficult as the lesions appear to represent a continuous spectrum from small collections of hyperplastic cells to large tumours replacing almost the entire testis. In addition, Leydig cell tumours are known to be spontaneous age-related tumours that occur more frequently with advancing age and the tumours in this study were found to be of late onset (identified in animals killed at week 82 and thereafter). However, the incidence of Leydig cell tumours (28.8%) at 2500 ppm in this study was above the contemporary historical control range for this strain of rat (range 3.8 – 19.2%) and the incidence in the concurrent control group was low (5.8% (3/52). In addition, there are no supporting mechanistic data to demonstrate that these tumours are of no relevance to humans.

Uterine Adenocarcinoma

There was a slight increase in the incidence of uterine adenocarcinoma in females at 2500 ppm (1/52, 1/52, 0/53 and 3/52 (5.8%) at 0, 50, 500 and 2500 ppm respectively). However, there is no dose response relationship and the incidence is at the upper level of the contemporary historical control range (0 – 5.8%). These findings therefore appear to be incidental to treatment.

Brain/spinal cord astrocytoma

There was an increased incidence of brain (2/52, 1/53, 2/52 and 3/52 (5.8%) and spinal cord (0/52, 0/53, 0/52 and 1/52 (1.9%) astrocytoma. The incidences were at the upper level of the contemporary historical control ranges (5.8% and 1.9% respectively) as shown in table 19.1.

Table 19.1. Contemporary historical control data in Alpk:ApfSD rats

Start date of study	Males				Females
	Leydig Cell	Brain astrocytoma (BA)	Spinal cord astrocytoma (SCA)	BA + SCA	Uterine adenocarcinoma
1984	5/104 (4.8%)	Group 1: 2/52 (3.8%) Group 2: 3/52 (5.8%)	Group 1: 0/52 (0.0%) Group 2: 0/52 (0.0%)	5/104 (4.8%)	3/104 (2.9%)
1984	2/52 (3.8%)	0/52 (0.0%)	0/0 [(a)]	0/52 (0.0%)	0/52 (0.0%)
1985	2/52 (3.8%)	1/52 (1.9%)	0/0 [(a)]	1/52 (1.9%)	2/52 (3.8%)
1985 (b)	3/52 (5.8%)	2/52 (3.8%)	0/52 (0.0%)	2/52 (3.8%)	1/52 (1.9%)
1986	4/52 (7.7%)	1/52 (1.9%)	0/52 (0.0%)	1/52 (1.9%)	1/52 (1.9%)
1987	4./52 (7.7%)	1/52 (1.9%)	0/52 (0.0%)	1/52 (1.9%)	3/52 (5.8%)
1987	10/52 (19.2%)	0/52 (0.0%)	0/0 [(a))]	0/52 (0.0%)	2/51 (3.9%)
1988	4/52 (7.7%)	0/52 (0.0%)	0/0 [(a))]	0/52 (0.0%)	2/52 (2.8%)
1989	5/56 (8.9%)	0/56 (0.0%)	0/56 (0.0%)	0/56 (0.0%)	1/56 (1.8%)
1990	6/52 (1.5%)	3./52 (5.8%)	0/52 (0.0%)	3/52 (5.8%)	2/52 (3.8%)
1990	3/52 (5.8%)	3/52 (5.8%)	1/52 (1.9%)	4/52 (7.7%)	0/52 (0.0%)

(a) Spinal cord not examined (b) data from current study

Hamster

High mortality was observed in the first chronic study in hamsters at all doses and in controls, with <50% survival rates in both sexes at termination. Mortality in females was particularly high (survival rates at termination of study were ≤ 14%). A slight increase in benign adrenal cortical adenomas was noted in females, but given the high mortality and the fact that an increase in these tumours was not observed in the second hamster study, they are not considered to be treatment related.

In the second hamster study survival rates were higher (≥ 88% in males and ≥ 67% in females at termination of the study). Non-neoplastic findings at 12000 ppm (700.3 mg/kg/day males and 672.2 mg/kg/day in females) included minor effects on bodyweight gain, increased liver weights in both sexes and increased kidney and testes weights in males only. An apparent increase in the incidence of unilateral tubular degeneration in the testis was also noted.

Neoplastic findings

Ovarian Tumours

An increase in the combined incidence of benign and malignant sex cord stromal tumours in the ovaries was noted in the second study (2/49 (4.1%), 4/50 (8.0%), 6/48 (12.5%) and 7/51 (13.7%) at 0, 500, 2500 and 12000 ppm respectively). The individual incidence of benign tumours was 1/49, 2/50, 4/48, 5/51 and the incidence of malignant tumours was 1/49, 2/50, 2/48 and 2/51 at 0, 500, 2500 and 12000 ppm respectively.

Historical control data from a number of sources have been provided. These include data from the company's own laboratory, data form another UK laboratory and data from literature sources. These are explained below.

The historical control data from the Company's laboratory is taken from 2, 80 week studies conducted in the year preceding the in life phase of the current study (1999-2002). These show a combined incidence of malignant and benign tumours of 2/51 (1benign and 1 malignant) and 1/52 (benign) respectively.

Data from another UK laboratory are also available but these are significantly older than the current study (ranging from 3 to 11 years before) or were from studies of greater duration (10 weeks more). There is also insufficient data on the strain, supplier, diet and survival rates. It is also unclear how many animals were examined. These data are therefore of equivocal relevance to the current study.

Table 19.2 – Historical control data from another UK Laboratory for sex chord stromal tumours

Date	1988	1989	1990	1993	1993	1996	1996
Duration (weeks)	80	78	77	90	90	91	91
Ovaries examined (a)	99	100	50	50	50	60	58
Thecal cell	0	3	3	1	0	2	4
Granulosa cell	3	0	0	2	2	0	0
Granulosa/thecal cell	0	0	0	2	1	0	0
Granulosa-stromal cell	2	1	0	0	0	0	0

(a) – not clear how many animals have been examined

Published historical control data are also available but the same discrepancies apply making it of limited value in the interpretation of the current study. These show an incidence ranging from 0.7-10.5%). Refer to table 6.106 in reference 3.

There is no statistical increase in any individual histological tumour type in the current study and the individual incidences are within those seen in historical controls. However, none of the studies

show a combined incidence equal to 6/48 (12.5%) or 7/51 (13.7%) as seen from 2500ppm in the current study.

Given the nature of these tumours and the incidence from 2500 ppm these tumours can not be dismissed.

4.10.1.2 Carcinogenicity: inhalation

No data available

4.10.1.3 Carcinogenicity: dermal

No data available.

4.10.2 Human information

No data available

4.10.3 Other relevant information

No data available.

4.10.4 Summary and discussion of carcinogenicity

The carcinogenic potential of tralkoxydim has been investigated in rats and hamsters.

In the rat a dose related increased incidence of Leydig cell tumours was noted in male rats reaching 28.8% at 2500 ppm compared to 5.8% in controls. This was above the range seen in contemporary historical controls and no mechanistic data are available to dismiss the relevance to humans. In addition, the strain of rat used (Alpk) is not known to have a high background incidence of Leydig cell tumours. These tumours are therefore considered treatment related.

An increased incidence of brain and spinal cord astrocytoma was noted in males at 2500 ppm. These tumours are rare and, as the incidence was at the upper level observed in contemporary historical control data, it is prudent to consider that they are treatment related. However, the increase in such tumours was observed at the top dose and in male rats only.

In hamsters, a dose related increased incidence of combined benign and malignant sex cord stromal tumours in the ovaries was observed. High dose levels were used in the hamster study and there was no increase in any individual histological tumour type. However, the combined incidence is above the level seen in historical controls at the same and another UK laboratory and in published data. These tumours are therefore considered treatment related.

In short term hamster studies (table 17) tralkoxydim was shown to induce liver enzymes at high doses (17000 ppm in a 28 day study and 4000 ppm in a 90 day study). At such high doses, and in the presence of liver enzyme induction, testosterone hydroxylase was also induced. Such increases are indicative of a hormonal disturbance (induction of specific enzymes responsible for the metabolism of steroid hormones).

4.10.5 Comparison with criteria

In accordance with the criteria in CLP, classification in category 1A for carcinogenicity is not justified given that there is no evidence of tralkoxydim having caused cancer in humans.

On consideration of all available data, there are a number of factors which indicate that classification in Category 2 would be appropriate based on the overall level of concern. Considering the results of *in vitro* and *in vivo* studies, tralkoxydim is not considered to be genotoxic. In the hamster, the tumours are predominantly benign in nature, are predominantly unilateral (only 1 bilateral), and manifest as a wide variety of histological subtypes. The increase in brain and spinal cord astrocytomas was only observed at the top dose and in male rats only. In addition, the incidence was at the upper level observed in historical controls.

In view of these considerations, the available evidence is deemed to best match the criteria for classification in Category 2.

Similarly, according to Directive 67/548/EEC, classification as a Category 3 carcinogen is judged to be appropriate following the same reasoning.

4.10.6 Conclusions on classification and labelling

Directive 67/548/EEC: Carc Cat 3; R40

CLP: Carc. 2 H351: Suspected of causing cancer

4.11 Toxicity for reproduction

Table 20: Summary table of relevant reproductive toxicity studies

Dose schedule	Dose levels	Observations and remarks (effects of major toxicological significance)
Rat 15 males and 30 females/dose 3-generation study OECD 416 Purity 94.9% Wickramaratne GA (1994a & b) in Reference 3	0, 50, 200 and 1000 ppm corresponding to 0, 4.5, 18.2 and 91 mg/kg/day in males and females	*Parental toxicity*: No evidence of treatment related deaths or clinical signs of toxicity. Bodyweight gain during the pre-mating period was reduced in females receiving 1000 ppm compared to controls (final body weights reduced by (1.8%) F0, (4.9%) F1 and (5.6%) F2). Food consumption was also reduced in the F1 (4.9%) and F2 (4.2%) females at this dose level. A slight reduction in male bodyweight gain and food consumption was noted at 1000ppm during the premating period. Overall there was no significant effect on bodyweight gain during pregnancy but, at 1000 ppm, the initial body weight at the start of gestation was reduced in the F1 (7.5%) and F2 (4.1%) females compared to controls. *Fertility effects:* There was no evidence of an effect on mating, fertility or implantation. 1 control pup in the F1A generation and 1 control and 2 pups receiving 200 ppm in the F3A offspring were found dead or killed in extremis. There was no evidence of a treatment related effect. There were no clinical signs of toxicity in the pups but at 1000 ppm there were reductions in the mean body weight gain (final body weight reduced by between 3-8%) in all 3

		generations and the mean total litter weight was persistently lower than control values (17% in F1A, 14% in F2A and 10% in F3A).
Rat 24 females/dose OECD 414 Purity 96.4% Moxon ME, Pigott GH, Banham PB and Pate I (1989) in reference 3	0, 3, 30 or 300 mg/kg/day (gavage) Dosed on days 7-16 of gestation	*Effects in dams* Four dams in the 300mg/kg/day were killed between days 14-18 of gestation. These animals showed marked weight loss and were in poor clinical condition (coat staining, sings of urinary incontinence, piloereciton, hunched posture and subdued behaviour). Two of these dams had completely resorbed their litters in uetero, all implantations in the other dams appeared live. Of the surviving dams there was a reduction in overall bodyweight gain (46%) and in food consumption (40%) compared to controls. In addition, 3 had totally resorbed their litters. Post implantation losses also increased (23.9% compared to 2.9% in controls). This was due to early (8 at 300 mg/kg/day and 6 in control) and late (45 at 300 mg/kg/day and 3 in control) intra-uterine deaths. Reductions in the number of live foetuses (43%), gravid uterus weight (36.9%). *Effects in the foetus* Reductions in mean litter weight (44.9%) and mean foetal weight (30.1%) at 300 mg/kg/day. At 300 mg/kg/day there was an increase in the number of foetuses with external and visceral defects including subcutaneous oedema of torso (4 foetuses) and neck (3 foetuses), anasarca (6 foetuses) and cleft palate (1 foetus). No such effects were observed in any of the other groups. A number of major skeletal defects were observed at 300 mg/kg/day including misshapen and/or fused centra in the lumbar, sacral and, caudal vertebrae. These effects were observed in a number of foetuses from different litters A number of minor skeletal defects were noted including reduced ossification in the skull, hyoid, arches of the cervical vertebrae and pubes. At 30 mg/kg/day the only major skeletal defect observed included a misshapen 2nd and 3rd sacral vertebrae in 1 foetus. A number of minor skeletal defects and variations were noted including reduced ossification in the skull and arches of the cervical vertebrae. In addition, short 13th ribs were observed. There were dose related increases in the number of foetuses with 2nd cervical centra and odontoid not ossified from 30 mg/kg/day. An increased incidence of extra ribs and reduced ossification of the calcaneum was also observed but this was not dose related.
Rats 24 females/dose Developmental Toxicity Study OECD 414 Purity 96.4% Moxon ME, Pigott GH, Banham PB and Pate I (1989a) – Reference 3	0, 0.5, 1, 3 or 200 mg/kg/day (gavage) Dosed on days 7-16 of gestation.	Effects in dams At 200 mg/kg/day 3 animals were killed and 1 was found dead between days 14 and 17 of gestation. These animals showed marked weight loss and were in poor clinical condition (signs of urinary incontinence, piloerection, hunched posture and vaginal bleeding). Of the surviving dams there was a reduction in overall bodyweight gain weight gain (16%) and in food consumption. Reductions in mean gravid uterus weight (9.6%) were observed. The number of late intra-uterine deaths was slightly elevated (9 compared to 2 in controls) but this was due to the occurrence of 6 deaths in one female. The overall post-implantation loss value is comparable to that seen in the control group (6.2% in controls and 7% at 200 mg/kg/day). *Effects in foetuses* At 200 mg/kg/day there were reductions in mean foetal weight (10.6%) and mean litter weight (13%). At 200 mg/kg/day various major skeletal defects were noted including

		misshapen centra in the lumbar vertebrae and misshapen and fused centra in the sacral vertebrae. These effects were observed in 14 foetuses from 8 litters. Reduced ossification in the skull and sternbrae were also observed. At 3 mg/kg/day there was a single incidence of misshapen 2nd and 3rd sacral vertebral centra and incomplete alignment of the 2nd and 8th thoracic vertebrae in 1 foetus.
Rabbit 18 females/dose OECD 414 Purity 97.8% Killick ME, Wickramaratne GA, Banham PB and Pate I (1986) in reference 3	0, 2.5, 20 or 100 mg/kg/day (gavage) Dosed on days 7-19 of gestation	*Effects in dams* At 100 mg/kg/day 9 animals were killed, 8 following abortion (days 21-28) and 1 in extremis on day 18 of gestation. Post mortem revealed effects in the gastro intestinal tract. No other clinical signs were observed in these animals. At 100 mg/kg/day weight loss was observed between days 7-10 but the overall weight gain and food consumption were comparable. At 100 mg/kg/day there was a reduction in the mean number of implantations (16%) and number of liver foetuses (60%) compared to controls. In surviving dams an increase in the number of late intra uterine deaths (13% above controls) was observed. Mean litter weight was reduced (15.6%,) due to the reduced number of live foetuses, but the mean foetus weight was slightly increased (12.9%). *Effects in foetuses* The male sex ratio was significantly different at 20 mg/kg/day (39%) and at 100 mg/kg/day (64.4%). The higher value is outside the upper limit of the contemporary historical controls which range from 37.3% to 53.5% in studies conducted between 1982 and 1987. The major skeletal defects noted at 100 mg/kg/day included cebocephaly and exencephaly in 2 pups from different litters. In another foetus the 4th-7th right thoracic arches were misaligned due to an extra arch between the 6th and 7th vertebrae.

4.11.1 Effects on fertility

4.11.1.1 Non-human information

A multigeneration study has been conducted in rats. Minor signs of parental toxicity included small reductions in bodyweight gain and food consumption but there were no effects on mating, fertility or implantation.

No histopathological findings in the reproductive tissues of the animals in the multigeneration study were observed. However, repeat dose studies in the rat, hamster and dog reveal effects on the male gonads (refer to section 4.7 and 4.10).

In the 2-year study in the rat (table 19) an increase in the number of males with enlarged testis from 500 ppm (equivalent to 23.1mg/kg/day) along with an increase in testis with white areas at 2500 ppm (equivalent to 117.9 mg/kg/day) was observed. Unilateral and bilateral Leydig cell hyperplasia (4/52, 5/53, 1/52 and 10/52 at 0, 50, 500 and 2500 ppm respectively), an increased incidence of tubular atrophy of the testes and reduced numbers of spermatozoa in the epididymides accompanied by the presence of an increased number of early nucleated sperm precursor cells were also noted at 2500 ppm.

In the 90 day dog study (table 17) decreased epididymides weight (21%) was noted along with slight unilateral atrophy of the seminiferous epithelium in 1 male of the 50 mg/kg/day group. In addition, in the 1 year study, unilateral tubular degeneration was observed in 1/4 males at 0.5 and 50

mg/kg/day. Bilateral tubular degeneration was noted in 1/4 males at 0.5 mg/kg/day and 5 mg/kg/day.

In the 80 week hamster study (table 19) testis weight increased (absolute 6% and relative 11%) at 12000 ppm (700.3 mg/kg/day). No associated histopathology but there was a slight increase in testicular tubular degeneration ranging from minimal to marked at this dose level (unilateral 9/51, 12/51, 15/51 and 17/51 and bilateral 7/51, 6/51, 4/51 and 4/51 at 0, 500, 2500 and 12000 ppm respectively).

In addition, in short term hamster studies (table 17), tralkoxydim was shown to induce liver enzymes at high doses (17000 ppm in a 28 day study and 4000 ppm in a 90 day study). At such high doses, and in the presence of liver enzyme induction, testosterone hydroxylase was also induced. Such increases are indicative of a hormonal disturbance (induction of specific enzymes responsible for the metabolism of steroid hormones).

4.11.1.2 Human information

No data available.

4.11.2 Developmental toxicity

4.11.2.1 Non-human information

Rat

In the first rat developmental toxicity study marked signs of maternal toxicity were noted in those females receiving 300 mg/kg/day tralkoxydim (including death, 45% reduction in body weight gain and 40% reduction in food consumption). There were also significant reductions in little size (43%) and increased post-implantation loss characterised by late intra-uterine deaths. There was an increase in external/visceral defects including a number of foetuses with anasarca (massive body oedema) and one foetus with cleft palate. A high number of foetuses (61 foetuses form 14 litters) possessed major skeletal defects ranging from complete fusion of several centra in the sacral region to small projections on one or 2 adjacent centra. There was a single incidence of misshapen sacral vertebrae (2^{nd} and 3^{rd} vertebrae) at 30 mg/kg/day. The report states that this finding was different to the effect observed at 300 mg/kg/day as it was misshapen rather than fused and has been shown to occur spontaneously in untreated rats of this age and strain (see below of discussion of historical control data).

A number of minor skeletal variations were observed at 30 mg/kg/day and above. These variations reflected reduced ossification which became marked at 300 mg/kg/day.

In the second rat developmental toxicity study marked signs of maternal toxicity were noted in those females receiving 200 mg/kg/day tralkoxydim (including death, reductions in body weight gain (16%) and reduction in food consumption). There were also significant reductions in mean foetal weight (10.6%) and litter weight (13.0%). The external/visceral defects noted in the previous study were not observed in this study at 200 mg/kg/day or less. However, the same major skeletal defects were observed in 5% of foetuses (14 foetuses from 8 litters) and included misshapen or fused centra (ossified connections between adjacent vertebral centra) of the lumbar and sacral vertebrae. The specific changes reported at 200 mg/kg/day were found to be less extreme than those seen at 300 m g/kg/day in that they did not include the extreme fusion of several adjacent

vertebrae. There was a single incidence of slightly misshapen sacral vertebrae (again the 2nd and 3rd vertebrae) in the 3 mg/kg/day group. The report again states that this finding was different to those observed in the higher dose group and in the absence of any other effects this finding was considered to be incidental.

Various minor skeletal variations were observed and were indicative of reduced ossification which became marked at 200 mg/kg/day and above.

The historical control data (table 20.1) show that misshapen or fused vertebrae are very rare with only 4 cases of misshapen vertebrae and 1 case of fused vertebrae being reported in 3 out of 10 studies. These historical control studies were reported between 1986 and 1988 (the in life dates were not provided), the current study was conducted in 1985 and reported in 1989.

Table 20.1 - . Historical control data for fused and misshapen vertebrae (specific vertebrae not specified)

Study Date	No litters (foetuses)	No of foetuses (litters)	
		Vertebral fusions	Misshapen vertebrae
1986	20 (218)	0	1
1987	71 (803)	0	0
1987	24 (282)	0	0
1987	24 (302)	0	0
1987	23 (276)	0	0
1988	24 (281)	0	0
1988	24 (297)	0	0
1988	22 (277)	0	0
1988	11 (117)	1(1)	1 (1)
1988	158 (1877)	0	2 (2)

The major skeletal defects and wide range and high incidence of minor skeletal defects and variants observed at 200 and 300 mg/kg/day are considered to be due to the severe toxicity occurring at these levels. The single incidence of vertebral change observed at 30 mg/kg/day in the first study and 3 mg/kg/day in the second study has been shown to occur in historical controls. In addition, both affected foetuses at 3 and 30 mg/kg/day were the smallest in their respective litters and showed other minor defects and variants evident of delayed development (ossification differences).

A number of minor skeletal defects and variations were observed at doses not causing maternal toxicity. These effects reflected reduced ossification, but are not considered severe enough to support classification.

Rabbit

In the developmental toxicity study in the rabbit severe maternal toxicity was evident following treatment with 100 mg/kg/day tralkoxydim (including lethality, weight loss during dosing, high rates of abortion and changes in the gastro intestinal tract). There was also a reduction in the number of implantations, live foetuses, foetuses per litter and an increase is the number of late intra-uterine deaths. The male sex ratio was reduced (39%) at 20 mg/kg/day but this was within historical controls (37.3 – 53.5) and, given that there was no change in the mean litter size, it is unlikely that there is a selective effect of male foetuses. The increase (64.4%) in the male sex ratio at 100 mg/kg/day is above the limit seen in historical controls. However, the high abortion rate at this dose level makes it difficult to interpret this result and it is therefore not considered to be treatment related. Major skeletal defects were noted in 3 foetuses from 3 litters at 100 mg/kg/day including cebocephaly and exencephaly.

4.11.2.2 Human information

No data are available.

4.11.3 Other relevant information

No data available

4.11.4 Summary and discussion of reproductive toxicity

Fertility

In a multigeneration study in rats, minor signs of parental toxicity were observed including reductions in body weight gain and food consumption. In addition, the body weight gains of the offspring were also persistently lower but overall there were no treatment related effects on the reproductive parameters.

Whilst effects in the male reproductive organs were observed in rats, hamsters and dogs in the repeated dose studies, such effects were not observed in the multigeneration study in rats and no adverse effects on fertility were noted at doses of up to 91 mg/kg/day.

In addition, whilst repeat dose studies have shown that tralkoxydim caused minor perturbations in hormone levels, no adverse effects on fertility were observed in the multigeneration study in the rat at doses of up to 91 mg/kg/day.

Development

A number of developmental toxicity studies have been conducted in both rats and rabbits. In both of the rat studies severe maternal toxicity was evident (at 200 and 300 mg/kg/day) and was accompanied by severe foetal toxicity. There was an increase in external/visceral defects including a number of foetuses with anasarca (massive body oedema) and one foetus with cleft palate in the 1st study at 300 mg/kg/day. These effects were not observed in the second study at 200 mg/kg/day. An increase in major skeletal defects were noted in both studies at 300 mg/kg/day and 200 mg/kg/day including misshapen and fused vertebrae along with a high incidence of minor skeletal variations. The effects seen at 200 and 300 mg/kg/day can be considered to have occurred due to the toxicity of the substance at these levels and are not a developmental effect. There was a single incidence of misshapen sacral vertebrae (2nd and 3rd vertebrae) at 30 mg/kg/day in the 1st study and

at 3 mg/kg/day in the 2nd study. This is a rare effect, as demonstrated by the historical control data in table 5.9.2.1.2. However, there are inconsistent results in the 2 studies (the effect was observed at 30 mg/kg/day but not at 3 mg/kg/day in the 1st study whereas in the 2nd study it was found to occur at 3 mg/kg/day) and there is some evidence of foetal toxicity at 30 mg/kg/day (evidenced by reduced ossification and the increased incidence of short ribs).

In the rabbit severe maternal toxicity was evident at 100 mg/kg/day. There was no clear evidence of any developmental effects.

4.11.5 Comparison with criteria

As no human data are available classification in Category 1A is not appropriate. Classification in Category 1B is based on clear evidence of an adverse effect on sexual function and fertility or on development in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effects. Classification in Category 2 is appropriate when the evidence is not sufficiently convincing to place the substance in Category 1.

Whilst effects on the male reproductive organs were observed in repeat dose studies such effects were not noted in a multigeneration study and there was no effect on any of the fertility parameters. Therefore, no classification for fertility is proposed.

The effects seen at 200 and 300 mg/kg/day can be considered to have occurred due to the toxicity of the substance at these levels and are not a developmental effect. There was a single incidence of misshapen sacral vertebrae (2nd and 3rd vertebrae) at 30 mg/kg/day in the 1st study and at 3 mg/kg/day in the 2nd study. This is a rare effect, as demonstrated by the historical control data in table 20.1. However, there are inconsistent results in the 2 studies (the effect was observed at 30 mg/kg/day but not at 3 mg/kg/day in the 1st study whereas in the 2nd study it was found to occur at 3 mg/kg/day) and there is some evidence of foetal toxicity at 30 mg/kg/day (evidenced by reduced ossification and the increased incidence of short ribs). In conclusion, this effect observed in 1 foetus, at different dose levels in different studies, is not considered to support classification for developmental toxicity in accordance with the criteria.

No classification is proposed in accordance with Directive 67/548/EEC following the same reasoning.

4.11.6 Conclusions on classification and labelling

Directive 67/548/EEC: Not classified based on available data

CLP: Not classified based on available data

4.12 Other effects

4.12.1 Non-human information

No data available.

4.12.1.1 Neurotoxicity

No data available

4.12.1.2 Immunotoxicity

No data available.

4.12.1.3 Specific investigations: other studies

4.12.1.4 Human information

4.12.2 Summary and discussion

4.12.3 Comparison with criteria

4.12.4 Conclusions on classification and labelling

5 ENVIRONMENTAL HAZARD ASSESSMENT

A detailed summary of the available studies[1] has been reviewed and their robustness determined under Directive 91/414/EEC. The key information pertinent to determining a classification position is presented below. Further data on the studies included can be found in the Draft Assessment Report (DAR) references 5 and 6.

Tralkoxydim has a measured dissociation constant (pKa) of 4.3 at the standard analytical temperature of 25^{o}C. This indicates that above pH 4.3 the dissociated form of tralkoxydim will dominate and at an environmentally relevant pH > 6.3, 99 % of tralkoxydim will be ionised.

The isomeric composition of tralkoxydim used for the studies described in this section was not determined. For studies simulating environmental conditions, the pH was above 6.3 and it is assumed that the proportion of isomers reflected the ratio under naturally occurring environmental conditions.

[1] Studies included in this section refer primarily to aquatic fate. Additional studies are available for fate properties in soil. These are not relevant for the purpose of classification and labelling and are therefore not included.

5.1 Degradation

5.1.1 Table 21: Stability

The stability in air is not considered relevant for this type of dossier given that air is not considered an environmental compartment of concern for tralkoxydim (see Section 4.2.2).

Hydrolysis

A hydrolysis study is available using tralkoxydim. The study was not conducted to GLP or a guideline. However, the study was considered valid and acceptable for the DAR following Directive 91/414/EEC.

The study using phenyl ^{14}C radiolabelled tralkoxydim was run at pH 5, 7 and 9 at 25^{o}C. Hydrolysis was observed to be pH dependant – significant hydrolysis was observed at low pH while negligible hydrolysis was observed at the higher pH. Assuming first order degradation, the following DT_{50} values were calculated at 25^{o}C; pH 5 9 days; and, pH 7 140 days. Adjusting the values to 12^{o}C using Equation 25 in Technical Guidance Document (2003) (reference 7) to reflect the average EU outdoor temperature results in the following DT_{50} values; pH 5 25.5 days; and, pH 7 396 days.

From the study, one hydrolysis product was identified (R163434) at pH 5 and a maximum of 76.8 % Applied Radioactivity (AR). R163434 was not observed to undergo hydrolysis at pH 5 or 7.

Up to five additional non-identified hydrolysis products were observed at <5 % AR across all pHs.

Aqueous photolysis

Two studies are available and considered suitable for classification.

Study 1

An aqueous photolysis study following US EPA guidelines (161-2) and using phenyl ^{14}C and cyclohexene ^{14}C radio labels. The study involved subjecting sterile aqueous samples at 25^{o}C and pH 9 (to avoid hydrolysis) with 120 mg/l test substance to continuous irradiation. The artificial illumination was calculated to be the equivalent to 0, 5, 10, 20 and 30 days of summer sunlight in Florida, USA at 25-35^{o}N. This is equivalent of extreme southern Europe.

Similar results were observed for both radio labels so a single first order DT_{50} value of 5.8 Florida summer days was calculated. Two degradants were observed at > 10 % AR; R159368 and R158378 at a maximum of 22 % AR. Overall the study shows that tralkoxydim is susceptible to direct photodegradation in pure water under conditions of strong sunlight conditions and 25^{o}C.

Study 2

The quantum yield and phototransformation of tralkoxydim in water was evaluated following UBA guidelines. The method assessed the number of degraded molecules and light absorbance by the substance to determine the substance quantum yield. This was used to determine environmental half-lives. The study used the Frank and Klöpffer simulation model to estimate the following half-lives for the top 0-1 m of the River Neckar in Germany at ~ 50th degree latitude (central Europe) in May with typical cloudiness; pH 4 11 days; pH 7 490 days; and pH 9 350 days.

5.1.2 Biodegradation

5.1.2.1 Biodegradation estimation

A QSAR estimate using EPIWIN v.3.11[(reference 8)] gives Biowin 2 = 0.7865, Biowin 3 = 2.3092 and Biowin 6 = 0.0869. This indicates that tralkoxydim does not meet the REACH Screening criteria [(reference 9)] for persistence[(reference 10)]. This means that the substance is anticipated to have the following half-lives; < 60 days in marine water; < 40 days in freshwater or estuarine water; < 180 days in marine sediment; and, < 120 days in freshwater or estuarine water sediment.

It should be noted that it is unclear if the substance meets the domain of the QSAR model.

5.1.2.2 Screening tests

A ready biodegradation study is not available.

5.1.2.3 Simulation tests

Following SETAC methods and EPA Guideline 162-4, aerobic water / anaerobic sediment degradation of tralkoxydim was assessed using media from two natural water/sediment systems. The 'Dakota' system comprised a loam sediment and had a water pH of 8.05 (study start) to pH 8.28 (study completion). The 'Virginia Water' system comprised a sand sediment and had a water pH of 7.93 (study start) to pH 8 (study completion). The study was run over 139 days in the dark at 20^{o}C using phenyl ^{14}C and cyclohexene ^{14}C radio labels.

Dakota system

In the aquatic phase, tralkoxydim was observed to decrease to 62.8 % AR by day 28 and 34.6 % AR by day 135 based on the ^{14}C-phenyl label. Based on the ^{14}C-cyclohexenone label, 62.2 % AR was observed at day 28 and 33.7 % AR by day 135. Carbon dioxide measurements peaked at day 135 at 2.1 % AR (^{14}C-phenyl label) and 1.0 % AR (^{14}C-cyclohexenone label). Three water phase degradants (R163434, R158378 and R173642) were observed with a maximum for R173642 of 4.5 % AR by day 135 based on the ^{14}C-cyclohexenone label.

In sediment, tralkoxydim peaked at 20 % AR (^{14}C-phenyl label) and 18.3 % AR (^{14}C-cyclohexenone label) by day 135. Three degradants (R163434, R158378 and R173642) were observed in sediment with a maximum for R173642 of 4.5 % AR by day 135 based on the ^{14}C-cyclohexenone label.

Based on single first-order kinetics the tralkoxydim DT_{50} in the aquatic phase was calculated to be 92.9 days based on dissipation (primary degradation). Based on total system dissipation, the DT_{50} was calculated to be 161.3 days.

Virginia Water system

In the aquatic phase, tralkoxydim was observed to decrease to 45.5 % AR by day 28 and 11.7 % AR by day 135 based on the ^{14}C-phenyl label. Based on the ^{14}C-cyclohexenone label, 45.2 % AR was observed at day 28 and 11.6 % AR by day 135. Carbon dioxide measurements peaked at day 135 at 5.8 % AR (^{14}C-phenyl label) and 3.2 % AR (^{14}C-cyclohexenone label).

Three metabolites (R163434, R158378 and R173642) were observed with a maximum for R158378 of 5.9 % AR by day 70 based on the ^{14}C-phenyl label.

In sediment, tralkoxydim peaked at 13.9 % AR (^{14}C-phenyl label) by day 28 and 15.6 % AR (^{14}C-cyclohexenone label) by day 28. Three degradants (R163434, R158378 and R173642) were observed in sediment with a maximum for R158378 of 33.6 % AR by day 100 based on the ^{14}C-phenyl label.

Based on single first-order kinetics the tralkoxydim DT_{50} in the aquatic phase was calculated to be 43.9 days based on dissipation (primary degradation). Based on total system dissipation, the DT_{50} was calculated to be 60.1 days. These shorter DT_{50} values are anticipated to reflect the slightly lower pH of the Virginia Water system and the potential for hydrolysis.

5.1.3 Summary and discussion of degradation

A proposed degradation pathway for tralkoxydim is presented in figure 1.

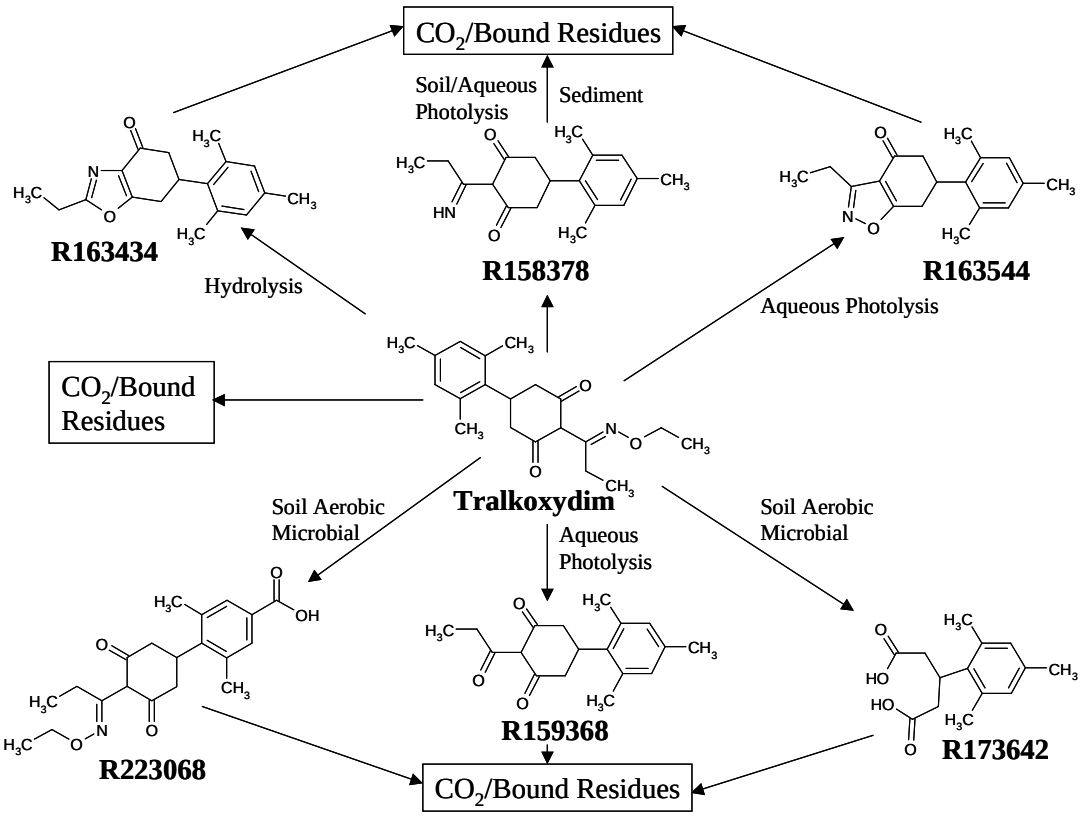
The hydrolysis study shows that tralkoxydim is susceptible to hydrolysis under acidic conditions but is hydrolytically stable under alkaline conditions. Under neutral to alkaline conditions ~pH7 and above, considered representative of the majority of European aquatic systems, and an environmentally relevant temperature (12^{o}C), tralkoxydim is considered to have a DT_{50} of 396 days. This means tralkoxydim is considered hydrolytically stable for the purpose of classification.

The aquatic photolysis data shows that tralkoxydim is susceptible to direct photodegradation in pure water under acidic conditions. Considering the second study following UBA guidelines, which is most closely representative of European conditions, at pH 7 a DT_{50} of 490 days was calculated. This means tralkoxydim is not considered to meet the criteria of 70 % degradation within 28 days.

The simulation study shows that tralkoxydim dissipates in the aquatic environment and undergoes primary degradation to a certain extent. However, the study DT_{50} values (total system dissipation DT_{50} 60.1 – 161.3 days) mean that tralkoxydim does meet the criteria of 70 % degradation in the aquatic environment within 28 days.

Based on these studies, tralkoxydim is not considered to undergo rapid and ultimate degradation under environmental conditions and is considered not readily biodegradable for the purpose of classification and labelling

Figure 1 - A proposed degradation pathway for tralkoxydim



5.2 Environmental distribution

5.2.1 Adsorption/Desorption

Two adsorption/desorption studies are available using radio labels. While the studies did not follow a specific guideline, they were considered valid and acceptable for the DAR following Directive 91/414/EEC.

Study 1

Using a ^{14}C-cyclohexenone radio label, four UK soils were used (clay, loamy sand, sandy loam and coarse sand) with pH values ranging from 5.4 to 6.8. Adsorption was observed to be inversely related to soil pH – the highest K_{oc} value was 314 l/kg for the lowest pH soil and the lowest K_{oc} value was 35 l/kg for the highest pH soil.

Study 2

Using a ^{14}C-phenyl radio label, two US soils were used (loamy sand and silty clay loam) with pH 6.2 to 6.8. While only two soils were used, again adsorption appeared to be inversely related to soil pH – the highest K_{oc} value was 100 l/kg for the lowest pH soil and the lowest K_{oc} value was 51 l/kg for the highest pH soil.

Adsorption does not appear to be related to soil clay or organic matter. At the study pH range, it is considered that tralkoxydim was mostly available in its ionised form which is considered negatively charged and therefore not considered to be influenced by negatively charged organic matter.

Overall, the studies indicate tralkoxydim is unlikely to adsorb strongly to solid matrices and is likely to be mobile with mobility increasing with pH.

5.2.2 Volatilisation

The vapour pressure of tralkoxydim is 3.7 x 10^{-10} kPa at 20 ^{o}C.

Based on measured data, the calculated Henry's Law Constant at 20^{o}C ranges from 1.8 x 10^{-5} Pa.m^{3}.mol^{-1} at pH 5.2 to 1.2 x 10^{-5} Pa.m^{3}.mol^{-1} at pH 9.

On this basis tralkoxydim is considered unlikely to partition to the air.

5.2.3 Distribution modelling

Not relevant to this type of dossier.

5.3 Aquatic Bioaccumulation

5.3.1 Aquatic bioaccumulation

5.3.1.1 Bioaccumulation estimation

Following OECD Guideline 107, tralkoxydim has a measured log K_{ow} value of 2.1 at 20^{o}C (assumed pH 7). This value is below 3 indicating a limited bioaccumulation potential.

It is noted that tralkoxydim has a pKa of 4.3 at 25^{o}C. This means the logK_{ow} is anticipated to decrease with increasing pH as tralkoxydim becomes predominantly available in its ionised form with increasing pH from pH 4.3 and consequently more water soluble.

Measured BCF_{fish} data are available and are preferred.

5.3.1.2 Measured bioaccumulation data

A fish accumulation study is available for tralkoxydim (97.6 % purity) using *Lepomis macrochirus* (bluegill sunfish). While the study did not follow a specific guideline, it was conducted to GLP, and was considered valid and acceptable for the DAR following Directive 91/414/EEC. The study used flow-through conditions, ^{14}C-cyclohexene radio labelled tralkoxydim and unlabelled tralkoxydim. The uptake phase was 19 days followed by a 56 day depuration phase. The test pH ranged from 7.2 – 8.1. On this basis, it is considered that the dissociated form of tralkoxydim predominated and that this is representative of environmentally relevant conditions.

Based on the radio label the following fish bioconcentration factors (BCF_{fish}) were determined: whole fish BCF_{fish} 32; muscle BCF_{fish} 13; and, viscera BCF_{fish} 185.

Based on the relevant BCF_{fish} for whole fish, for the purpose of classification and labelling, tralkoxydim is not considered bioaccumulative under Directive 67/584/EEC criteria of >100, and not bioaccumulative under Regulation (EC) No. 1272/2008 criteria of >500.

5.3.2 Summary and discussion of aquatic bioaccumulation

Based on the measured log K_{ow} values (<3) and measured BCF_{fish} (32 $l/kg_{wet\ fish}$) tralkoxydim is considered to have a low bioaccumulation potential under environmentally relevant conditions.

5.4 Aquatic toxicity

5.4.1 Fish

5.4.1.1 Short-term toxicity to fish

Tralkoxydim

Two GLP 96 hour acute toxicity to fish studies are available using tralkoxydim (92.4 % purity) and two fish species; *Oncorhynchus mykiss* (rainbow trout); and *Lepomis macrochirus* (bluegill sunfish). While the studies did not follow a specific guideline, they were conducted to GLP, and were considered valid and acceptable for the DAR following Directive 91/414/EEC.

Study 1

Tralkoxydim concentrations were considered stable with analytical concentrations 95 - 96 % of nominal concentrations. Test pH ranged from 7.4 to 7.9. Based on measured concentrations the 96 hour LC_{50} for *Oncorhynchus mykiss* was >7.2 mg a.s./l.

Study 2

Tralkoxydim concentrations were considered stable with analytical concentrations 81 % of nominal concentrations. Test pH ranged from 7.1 to 7.9. Based on measured concentrations the 96 hour LC_{50} for *Lepomis macrochirus* was >6.1 mg a.s./l.

Degradants

Two static GLP 96 hour acute toxicity to fish studies are available following OECD Guideline 203 using aquatic degradants.

Study 1

Using R173642 (100 % w/w) and *Oncorhynchus mykiss* (rainbow trout) analytical measurements were 108 % of nominal concentrations and results were based on measured concentrations. The 96 hour LC_{50} was >120 mg/l.

Study 2

Using R223068 (99 % w/w) and *Pimephales promelas* (fathead minnow) analytical measurements were 48 – 86 % of nominal concentrations and results were based on measured concentrations. The 96 hour LC_{50} was 44 mg/l (95 % C. I. 41 – 48 mg/l).

Additional supporting toxicity to fish data

A GLP 28-day sub-lethal fish toxicity study is available for tralkoxydim (97.4 % purity) following OECD Guideline 204 and using *Oncorhynchus mykiss* (rainbow trout). Test pH ranged from 7.2 to 7.6. Under continuous flow conditions analytical concentrations were 74 – 82 % of nominal concentrations and results were based on mean measured concentrations. The 28 day LC_{50} was >7.4 mg/l and the 28 day NOEC was 4.6 mg/l.

5.4.1.2 Long-term toxicity to fish

There are no long-term fish toxicity data.

5.4.2 Aquatic invertebrates

5.4.2.1 Short-term toxicity to aquatic invertebrates

Tralkoxydim

One GLP static 48 hour acute toxicity to *Daphnia magna* (water flea) study is available following US EPA and ASTM Guidelines using tralkoxydim (97.8% purity). Test pH ranged from 8.1 to 8.3. Analytical measurements were within ± 20 % of nominal concentrations and results were based on measured concentrations. The 48 hour EC_{50} was >175 mg a.s./l.

Degradants

Three static GLP 48 hour acute toxicity to *Daphnia magna* studies are available following OECD Guideline 202 and EPA Guidelines using aquatic degradants.

Study 1

Using R173642 (100 % w/w) analytical measurements were 108 % of nominal concentrations and results were based on measured concentrations. The 48 hour EC_{50} was 85 mg/l (95 % C. I. 72 – 100 mg/l).

Study 2

Using R223068 (99 % w/w) analytical measurements were 92 % of nominal concentrations and results were based on measured concentrations. The 48 hour EC_{50} was >110 mg/l.

Study 3

Using R158378 (100 % w/w) analytical measurements were not included and results are based on nominal measured concentrations. The 48 hour EC_{50} was >5 mg/l.

5.4.2.2 Long-term toxicity to aquatic invertebrates

A semi-static 21-day long-term *Daphnia magna* toxicity study following OECD Guideline 202 (1984 version Part II, now OECD Guideline 211) is available using tralkoxydim (97 % purity). Test pH ranged from 7.42 to 8.4. Results were based on mean measured concentrations. The 21 day EC_{50} was >8.1 mg/l and the 21 day NOEC based on reproduction was 2.1 mg/l.

5.4.3 Algae and aquatic plants

Algae

Results are based on growth rate and not biomass as values based on biomass are not considered robust for the purpose of classification.

Tralkoxydim

Three GLP static algal growth inhibition studies are available following OECD Guideline 201 using three different species. Test pH data is not available but considering the test guideline, it is assumed that the initial pH would be ≥ 7.5 and that this would not have decreased significantly during the test.

Study 1

The 96 hour study used tralkoxydim (91.5 % purity) and *Pseudokirchneriella subcapitata* (green alga formerly *Selenastrum capricornutum*). The exposure concentration range was nominally 0.56 - 7.5mg/l. Analytical concentrations were 68 - 86 % of nominal concentrations and results were based on measured concentrations. The 96 hour E_rC_{50} was >5.1 mg a.s./l and the 96 hour NOE_rC was 5.1 mg a.s./l.

Study 2

The 120 hour study used tralkoxydim (90.3 % purity) and *Anabaena flos-aquae* (blue-green alga). The exposure concentration range was nominally 18 - 180 mg/l. Analytical concentrations were 100 - 106 % of nominal concentrations and results were based on measured concentrations. The 120 hour E_rC_{50} was >180 mg a.s./l and the NOE_rC was 56 mg a.s./l.

Study 3

The 120 hour study used tralkoxydim (90.3 % purity) and *Pseudokirchneriella subcapitata* (green alga formerly *Selenastrum capricornutum*). The exposure concentration range was nominally 0.75 - 96 mg/l. Analytical concentrations were 96 - 104 % of nominal concentrations and results were based on measured concentrations. The 72 hour E_rC_{50} was 21 mg a.s./l. The 96 hour E_rC_{50} was 20 mg a.s./l. The 120 hour E_rC_{50} was 16 mg a.s./l (95 % C. I. 9.3 – 31 mg/l) and the 120 hour NOE_rC was 6 mg a.s./l.

Degradants

One static GLP 96 hour algal growth inhibition study following OECD Guideline 201 and using aquatic degradant R158378 (100 % w/w) is available. Analytical concentrations were not stated and results were based on nominal concentrations. The 96 hour E_rC50 was >5 mg/l and a NOEC could not be determined.

Aquatic plants

Tralkoxydim

One 14 day semi-static GLP growth inhibition study following EPA guidelines using *Lemna gibba* and tralkoxydim (90.3 % purity) is available. The test pH range was 4.7 to 5.0 in fresh solutions and 5.2 to 5.7 in expired solutions. This is lower than the range used in other ecotoxicity studies but very close to the M-Hoagland's nutrient medium guideline of 4.8 -5.2 in fresh media and considered acceptable. While the ionised form of tralkoxydim would dominate at such study pH range, the proportion of non-ionised tralkoxydim would have been higher than in other studies. In the absence of further information, it is assumed that the observed toxicity is due to the ionised form of tralkoxydim which would have predominated in test solutions and at a higher environmentally relevant pH range.

Analytical concentrations were 47 – 57 % of nominal concentrations and results were based on mean measured concentrations.

Based on the number of fronds the 14 day EC_{50} was 2.6 mg a.s./l (95 % C. I. 2.3 – 2.9 mg a.s./l). The 14 day $NOE_{frond\ no}C$ was 0.58 mg a.s./l.

Degradants

Two static *Lemna gibba* GLP 7 day growth inhibition studies following EPA guidelines and using aquatic degradants are available.

Study 1

Using R173642 (100 % w/w), analytical concentrations were 100 – 104 % of nominal concentrations and results were based on measured concentrations. Based on the number of fronds the 7 day hour EC_{50} was 110 mg/l (95 % C. I. 92 - >120 mg/l) and the 7 day $NOE_{frond\ no}C$ was 30 mg/l.

Study 2

Using R223068 (99 % w/w), analytical concentrations were 106 – 108 % of nominal concentrations and results were based on measured concentrations. Based on the number of fronds the 7 day hour EC_{50} was 53 mg/l (95 % C. I. 48 - 59 mg/l. The 7 day $NOE_{frond\ no}C$ was 30 mg/l.

5.4.4 Other aquatic organisms (including sediment)

There are no sediment organism toxicity data for tralkoxydim.

A *Chironomus riparius* toxicity study using degradant R158378 is available following OECD Guideline 218. As exposure was via spiked sediment, it is not considered relevant for the purpose of aquatic classification and labelling and therefore not included.

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

Tralkoxydim is susceptible to hydrolysis under acidic conditions but hydrolytically stable under alkaline conditions. Under neutral to alkaline conditions, considered representative of the majority of European aquatic systems, and an environmentally relevant temperature (12^{o}C), tralkoxydim is considered to have a DT_{50} of 396 days. This means tralkoxydim is considered hydrolytically stable for the purpose of classification.

Tralkoxydim is susceptible to direct photodegradation in pure water under acidic conditions. However, under conditions most closely representative of European conditions, at pH 7 a DT_{50} of 490 days was calculated. This means tralkoxydim is not considered to meet the criteria of 70 % degradation within 28 days.

The simulation study shows that tralkoxydim dissipates in the aquatic environment and undergoes primary degradation to a certain extent. However, the study DT_{50} values mean that tralkoxydim does not meet the criteria of 70 % degradation in the aquatic environment within 28 days.

Based on the above information, tralkoxydim is not considered to undergo rapid and ultimate degradation under environmental conditions and is considered not rapidly deegradable for the purpose of classification and labelling (>70 % mineralisation in the aquatic environment within 28 days).

The $logK_{ow}$ of tralkoxydim is anticipated to decrease with increasing pH. This mirrors the increase in water solubility with increased pH given that the dissociated form of tralkoxydim will increase with increasing pH. The highest measured $logK_{ow}$ value of 2.1 at an assumed pH of 7 is less than 3 indicating a limited bioaccumulation potential. Based on a fish bioconcentration study the following BCF_{fish} values were determined: whole fish BCF_{fish} 32; muscle BCF_{fish} 13; and, viscera BCF_{fish} 185. Considering the whole fish BCF_{fish} value for the purpose of classification and labelling, tralkoxydim is not considered bioaccumulative under the Directive 67/548/EEC criteria of >100, and not bioaccumulative under Regulation EC No. 1272/2008 criteria of >500.

Tralkoxydim is used as a herbicide to control weeds. Reflecting this, the most sensitive trophic level appears to be aquatic plants. In a 14 day growth inhibition study using *Lemna gibba* the 14 day EC_{50} based on frond number was 2.6 mg a.s./l (95 % C. I. 2.3 – 2.9 mg a.s./l). The 14 day $NOE_{frond\ no}C$ was 0.58 mg a.s./l. This means the lowest $L(E)C_{50}$ for tralkoxydim is considered to be 1 mg/l $<L(E)C_{50} \leq 10$ mg/l.

Following the recent 2nd ATP in Commission Regulation (EU) No 286/2011 the lowest available NOEC for consideration of chronic toxicity is 0.58 mg a.s./l and therefore in the range >0.1 to ≤1mg/l.

Based on acute toxicity data, tralkoxydim is not acutely toxic to fish or Daphnia (representative of invertebrates/crustacea). One algal growth inhibition study shows tralkoxydim is not toxic to *Ananaena flos-aquae* (blue green algae). Two algal growth inhibition studies are available for *Pseudokirchneriella subcapitata* (green alga formerly *Selenastrum capricornutum*). The first quotes a 96 hour E_rC_{50} of >5.1 mg/l based on the highest exposure concentration. The second with an extended exposure concentration range (considered within solubility given the likely pH range), quotes a 96 hour E_rC_{50} of 20 mg a.s./l with a LOE_rC above the previous study exposure range. This indicates that at an environmentally relevant pH, with increased solubility due to ionisation, tralkoxydim is toxic to algae within the range 10 mg/l $<L(E)C_{50} \leq 100$ mg/l.

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

Directive 67/548/EEC
N Dangerous for the Environment
R51 Toxic to aquatic organisms
R53 May cause long term effects in the environment

S61 Avoid release to the environment. Refer to special instructions/Safety Data Sheet

CLP Aquatic Chronic 2 H411 ‘Toxic to aquatic life with long lasting effects’, Pictogram GHS09. As the substance is not considered Aquatic Acute 1 or Aquatic Chronic 1, an M factor of 1 is not applicable.

6 OTHER INFORMATION

None available

7 REFERENCES

1. Pesticide Assessment Report (DAR) - public version - initial risk assessment provided by the rapporteur Member State United Kingdom for the existing active substance tralkoxydim of the third stage (part A) of the review programme referred to in Article 8(2) of Council Directive 91/414/EEC. Volume 3, Annex B, B.1-B.5 – March 2006

2. Pesticide Assessment Report (DAR) - public version - initial risk assessment provided by the rapporteur Member State United Kingdom for the existing active substance tralkoxydim of the third stage (part A) of the review programme referred to in Article 8(2) of Council Directive 91/414/EEC. Volume 3, Annex B, B.6, part 1 – March 2006

3. Pesticide Assessment Report (DAR) - public version - initial risk assessment provided by the rapporteur Member State United Kingdom for the existing active substance tralkoxydim of the third stage (part A) of the review programme referred to in Article 8(2) of Council Directive 91/414/EEC. Volume 3, Annex B, B6, part 2 – March 2006

4. Pesticide Assessment Report (DAR) - public version - initial risk assessment provided by the rapporteur Member State United Kingdom for the existing active substance tralkoxydim of the third stage (part A) of the review programme referred to in Article 8(2) of Council Directive 91/414/EEC. Volume 3, Annex B, B.6, part 3 –March 2006

5. Pesticide Assessment Report (DAR) - public version - initial risk assessment provided by the rapporteur Member State United Kingdom for the existing active substance tralkoxydim of the third stage (part A) of the review programme referred to in Article 8(2) of Council Directive 91/414/EEC. Volume 3, Annex B, B.8 – March 2006

6. Pesticide Assessment Report (DAR) - public version - initial risk assessment provided by the rapporteur Member State United Kingdom for the existing active substance tralkoxydim of the third stage (part A) of the review programme referred to in Article 8(2) of Council Directive 91/414/EEC. Volume 3, Annex B, B.9 – March 2006

7. European Chemicals Bureau Joint Research Centre (2003) Technical Guidance Document on Risk Assessment.

8. Reference: United States Environmental Protection Agency [USEPA] (2004) Estimation Programmes Interface Suite™ for Microsoft ® Windows, v3.11. United States Environmental Protection Agency, Washington , DC, USA. Available from http://www.epa.gov

9 Reference: European Chemicals Agency [ECHA] (2008) Guidance on information requirements and chemical safety assessment: chapter R.11: PBT assessment. May 2008.

10 If Biowin 2 <0.5 (does not biodegrade fast) and Biowin 3 <2.2 (ultimate biodegradation timeframe ≥ months) then substance considered persistent. If Biowin 6 <0.5 (does not biodegrade fast) and Biowin 3 <2.2 (ultimate biodegradation timeframe ≥ months) then substance considered persistent.

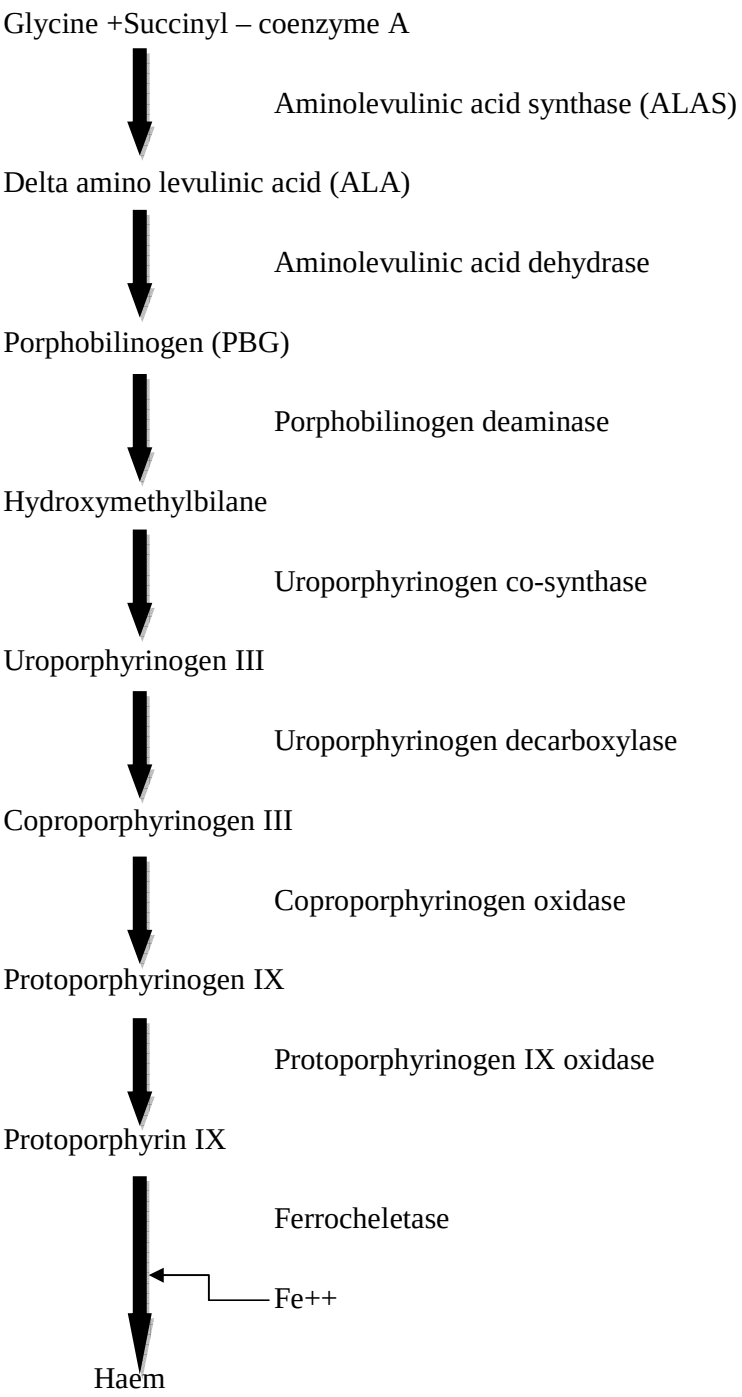
8 ANNEXES

Annex I – Investigation into postulated mode of action and the human relevance or porphyria in mice.

Annex I

Postulated mode of action and human relevance of porphyria in mice

1. The Haem Biosynthetic Pathway



2. Proposed mode of action in mice

Tralkoxydim has been shown to cause direct alkylation of haem following transfer of a methyl group from the C-ethyl moiety of tralkoxydim. This leads to the formation of N-methyl protoporphyrin IX in the mouse liver. N-methyl protoporphyrin IX is known to inhibit ferrocheletase activity. Ferrocheletase is the terminal enzyme in the haem biosynthetic pathway and is responsible for the insertion of iron. Due to a reduction in ferrocheletase activity less haem is produced and this causes an increase in aminolevulinic acid synthesase (ALAS) activity (which is the rate limiting enzyme in the process) and an increase in porphyrin production.

3. Mechanistic investigations supporting mode of action in the mouse

a) Mechanism of tralkoxydim induced hepatic chloestasis: studies in rats and mice (Brady AM and Lock (1994) – Reference 4)

Males rats (SD) and mice (CD-1) were orally administered tralkoxydim as a single or repeat dose in arachis oil. At sacrifice, the livers were removed and total porphyrin concentration, ferrochelatase and 5-aminolevulinic acid synthtase (ALAS) activities were evaluated.

In mice, after a single dose of 10 or 100 mg/kg tralkoxydim, a dose-related increase in total porphyrin content was seen from 4 hours and was markedly increased by 24 hours. At 100 mg/kg there was a rapid decrease in ferrocheletase activity and a rapid increase in ALAS activity.

In a second study mice received a single dose of 0.05 – 750 mg/kg tralkoxydim and were killed at 24 hours. Dose related increases in liver porphyrin levels were seen from 2 mg/kg. Hepatic ALAS activity was increased in a dose-related fashion at all doses tested and hepatic mitochondrial ferrochelatase activity was decreased at doses ranging from 10 -750 mg/kg.

In rats, no effects on porphyrin concentration or ferrochelatase activity were noted following single dosing with 10 – 750 mg/kg or following 4 daily doses of 0.5, 10 or 100 mg/kg. ALAS activity was slightly increased following single exposure at 400 and 750 mg/kg.

b) Identification of an inhibitor of ferrochelatase in the livers of mice dosed with tralkoxydim (Brady A M and Lock (1994a) – Reference 4)

Male mice (8/group) received a single oral dose of between 50 - 200 mg/kg tralkoxydim or 200 mg/kg 3,5-diethoxycarbonyl-1,4-dihydro-2,4,6-trimethylpyridine (DDC -a known porphyrinogenic agent which acts by eliciting the production of N-alkylated porphyrins in the liver which in turn inhibits ferrocheletase activity (Lavelle, 1987)). Radio labelled ALA hydrochloride was administered 2 hours earlier. Analysis of the livers of rats treated with tralkoxydim or DDC showed the presence of N-methyl protoporphyrin IX. The N-methyl protoporphyrin IX is believed to be the ferrochelatase inhibitor as a time-course study over 4 hours using mice dosed with 100 mg/kg tralkoxydim revealed a close correlation between induction of the ferrochelatase inhibitor and inhibition of ferrochelatase activity. N-methyl protoporphyrin IX was also found following treatment with DDC. The authors of the study conclude that N-methyl protoporphyrin IX is porphyrinogenic and its accumulation in the liver can cause porphyria in all species. However, it may be differences in the production of N-methyl protoporphyrin IX which are responsible for the

species differences observed following treatment with tralkoxydim. Analysis of the N-methyl protoporphyrin IX showed that only 2 out of 4 possible isomers of N-methyl protoporphyrin IX were present indicating that there is regioselectivity in the formation of the N-methyl protoporphyrin IX.

c) Origin of N-methyl protoporphyrin IX in the liver of mice following administration of radio labelled tralkoxydim (Brady A M and Lock (1994b) – Reference 4).

Male mice (20/group) received a single oral dose of 50 mg/kg of either (14C-mesityl), (14C-ethoxyimine) or (14C-ethyl) tralkoxydim. They were killed 4 hours after dosing and the livers removed and pooled for each group. The specific 14C labelling of N-methyl protoporphyrin IX was expressed as the ratio of tralkoxydim (nmol) to N-methyl protoporphyrin IX (nmol).

The N-methyl protoporphyrin isolated from mice treated with (14C-mesityl) or (14C ethoxyimine) tralkoxydim contained only trace amounts of [14C] radiolabel (ratio 0.009 and 0.001 respectively). However, for mice treated with (14C-ethyl) tralkoxydim, a significant amount of radiolabel was found (ratio of tralkoxydim to N-methyl protoporphyrin IX was 0.75).

A series of structural analogues of tralkoxydim were also tested to further investigate the importance of the C-ethyl moiety in the porphyrinogenic activity. These included (the ethoxyimine series, C-ethyl series, tralkoxydim oxazole and tralkoxydim isoxazole). Methoxyime-, propoxyimine- and benzyloxyime-tralkoxydim all caused markedly increased levels of hepatic porphyrin and decreased ferrochelatase activity. In the C-ethyl series of tralkoxydim analogues, only tralkoxydim was found to be conclusively porphyrinogenic. The C-methyl-, C-propyl- and C-benzyl tralkoxydim analogues were not porphyrinogenic and had no effects on ferrochelatase activity. Tralkoxydim oxazole and tralkoxydim isoxazole also lacked porphyrinogenic activity in mice. Since the C-ethyl moiety is retained in these analogues it is inferred that the oxime structure found in tralkoxydim may also be required for the methyl group transfer.

Ultimately, this suggests that the N-methyl protoporphyrin accumulation in the mouse liver results from a direct alkylation of haem by tralkoxydim with the C-ethyl moiety of tralkoxydim being responsible for the alkylation.

4. Investigations into the species differences in tralkoxydim induced hepatic porphyria

a) 14 Day species comparison feeding study

Dose schedule	Dose levels	Observations and remarks (effects of major toxicological significance)
Rats –SD Mice – C57BL Hamsters – SYR Guinea Pigs – Dunkin Hartley	Rats, hamsters and mice: 0, 50 or 500 ppm Equivalent to: Rats: 0, 6.4 or 61.3 mg/kg/	Reductions in bodyweight gain were observed in rats, mice and guinea pigs at the top doses. The liver was examined for evidence of porphyrin accumulation and cytochrome P-450 concentrations were recorded. In mice there was a marked dose related increase in porphyrin accumulation (4.1, 174.4 and 138.5 nmol/g at 0, 50 and 500ppm) and a dose related decrease in cytochrome P-450 (0.77, 0.44 and 0.33 at 0, 50and 500ppm). In guinea pigs there was an increase in

14 days 5 males/dose Purity 92.4% Stonard MD (1989f, g and 1994d) – Reference 4	Mice: 0, 9.7 or 108.0 mg/kg/day Hamsters: 0, 6.0 or 56.7 mg/kg/day. Guinea Pigs 0, 100 or 1000 ppm equivalent to 5.5 or 54.2 mg/kg/day	porphyrin concentration at 1000 ppm (0.96, 0.92 and 3.4 nmol/g at 0, 100 and 1000 ppm) and an increase in cytochrome P-450 (1.32, 3.30 and 3.85 at 0, 100 and 1000 ppm). There was no evidence of an effect on cytochrome P-450 or porphyrin concentrations in rats or hamsters. Macroscopic examination of the mouse livers revealed dark discolouration in all tralkoxydim treated animals. An increase in both absolute (30% and 28%) and relative liver weight (29% and 50%) was observed at 50 and 500 ppm. Histopathological examination of the mouse liver showed brown birefringenet pigment deposits distributed throughout the bile ducts, Kupffer cells and hepatocytes which were accompanied by biliary hyperplasia, fibrosis and associated portal inflammation and hepatocytes necrosis. There were no treatment related effects in rats, hamsters or guinea pigs.

b) Species differences in tralkoxydim-induced hepatic porphyria – investigation in rats (Brady AM and Lock (1994c) – Ref 4)

Male rats (3/dose) were orally dosed with 0, 0.5, 10 or 100 mg/kg/day tralkoxydim for 4 days. Total hepatic porphyrin content, ferrochelatase activity, cytochrome P-450 content, 7-ethoxycoumarin-O-deethylase (ECOD) and 7-ethoxyresorufin-O-deethlyase (EROD) activities were not affected by treatment.

In a second experiment, male rats (20/dose) were fed diets containing 0 or 2500 ppm (125 mg/kg/day) tralkoxydim for 6 months. Total hepatic porphyrin content, ferrochelatase activity and cytochrome P-450 content were not affected by treatment. ECOD and EROD activities were increased in the treated rats.

In a third experiment, male rats (3/dose) were dosed with 0.17μmol/kg 5-amino(4-14C) laevulinic acid followed 2 hours later by oral administration of 0, 50 or 200 mg/kg tralkoxydim or 200 mg/kg DDC as a positive control. The animals were killed 17 hours later and the extracted livers were examined chromatographically. Radiolabelled N-methyl protoporphyrin IX and haem were observed in the livers of rats treated with DDC. Only radiolabelled haem was observed in the livers of rats treated with tralkoxydim and the controls. Fractions were eluted from the region of the N-methyl protoporphyrin IX peak and screened against rat and mice liver ferrocheletase. Inhibition of both rat and mouse ferrocheletase was observed using the eluted sample from the DDC expose. No effects were seen with the sample taken from the tralkoxydim exposure. This indicates that N-methyl protoporphyrin IX is not formed in the liver of rats following exposure to tralkoxydim.

c) Species differences in tralkoxydim-induced hepatic porphyria in hamsters (Brady AM and Lock (1994d) – Ref 4)

Male hamsters were orally dosed with either single doses of 10 - 750 mg/kg or multiple daily doses of 0, 0.5, 10 or 100 mg/kg/day tralkoxydim for 4 days.

5-aminolaevuline acid synthetase (ALAS) was found to be increased in a dose-related fashion in the hamster liver 4 hours after single dosing with 10-750 mg/kg tralkoxydim. This reached a 3-fold increase at 400 and 750 mg/kg which is small in comparison to that observed in mice. Cytochrome P-450 content and ECOD and EROD activities were increased in hamsters treated with 100 mg/kg/day for 4 days. The increase in ALAS activity was proposed to be due to the increase in the induction of cytochrome P450.

Studies were performed to see if N-methyl porphyrins were produced in hamsters dosed with 0, 50 or 200 mg/kg tralkoxydim (DDC was used as a positive control and 14C radiolabelled ALA was administered). Radiolabelled N-methyl protoporphyrin IX and haem were observed in the liver extracts of hamsters treated with DDC. Radiolabelled haem was found in the liver extracts of all hamsters treated with tralkoxydim along with a very slight amount of N-methyl protoporphyrin IX from those hamsters treated with 200 mg/kg tralkoxydim. Again fractions eluted from the area of the N-methyl protoporphyrin IX peak were screened against mouse liver ferrocheletase. Inhibition was clearly evident with extracts from the DDC dosed hamsters. A slight inhibition was evident from the extracts of hamsters treated with 50 and 200 mg/kg tralkoxydim. This indicates that a slight amount of N-methylprotoporphyrin IX is produced in the livers of hamsters following treatment with tralkoxydim.

In vitro studies in rodent and human hepatocytes

a) Induction of porphyria in primary cultures of mouse and rat hepatocytes with tralkoxydim (Brady A M and Lock (1994e) – Ref 4)

Isolated mouse and rat hepatocytes were exposed to either 5-500 μm tralkoxydim (>97%), or 5-100 μm of DDC or 3,5-diethyloxycarbonyl-1,4-ethyl-1,4-dihyro-2,6-dimethylpyridine (EDDC) from the time of seeding for 4 days.

In mouse hepatocytes a marked increase in total porphyrin concentrations were noted with tralkoxydim, DDC and EDDC at all concentrations tested. Mitochondrial ferrochelatase activity was also inhibited with tralkoxydim appearing to be the least potent.

In rat hepatocytes only DDC and EDDC caused accumulation of porphyrin and inhibition of ferrochelatase activity.

5. Relevance to humans

Induction of porphyria in primary cultures of human hepatocytes: studies with tralkoxydim (Brady A M and Lock (1994f) – Ref 4).

Human and rat hepatocyte cultures were exposed to 10-500 μm tralkoxydim (>97%), 5-100 μm DDC or 5-100 μm EDDC from the time of seeding to the end of the culture period. In human hepatocytes, tralkoxydim did not induce inhibition of ferrochelatase activity or lead to an increase in total porphyrin. A marked inhibition of ferrochelatase activity was noted only following treatment with DDC and EDDC but there was no accumulation of porphyrin.

Cultured human hepatocytes (from 4 separate donors) were also treated with ALA (0.001 – 1000 μm), which confirmed the haem biosynthetic pathway was functional (accumulation of porphyrin was observed) in the cultured human hepatocytes. It is therefore inferred that the lack of porphyrin accumulation following treatment with DDC and EDDC reflects the absence of a significant haem demand in human hepatocytes and not an inability of the hepatocytes to synthesise porphyrins. A similar experiment in rat hepatocytes showed that the dose response and accumulation of porphyrin were comparable in both species.

Conclusion

Porphrinogenic effects have been observed following the administration of tralkoxydim to mice. Mechanistic studies have shown that tralkoxydim causes direct alkylation of haem following transfer of a methyl group from the C-ethyl moiety. This was shown to result in the formation of N-methyl protoporphyrin IX in the mouse liver, which is a known inhibitor of ferrocheletase. Ferrocheletase is the terminal enzyme in the haem biosynthetic pathway and is responsible for the insertion of iron. A decrease in ferrocheletase activity was observed in studies in the mouse. Due to a reduction in ferrocheletase activity less haem is produced and this causes an increase in aminolevulinic acid synthesase (ALAS) activity (which is the rate limiting enzyme in the process) and an increase in porphyrin production. The mechanistic studies in mice showed an increase in ALAS activity following exposure to tralkoxydim therefore supporting the proposed mode of action.

No such effects were seen when tralkoxydim was administered to rats. In hamsters and guinea pigs slight increases in the porphryin content of the liver, production of N-methyl protoporphyrin IX and a slight decrease in ferrocheletase activity have been observed. However, these were at very low levels compared to the effects seen in the mouse and were at relatively high doses compared to the mouse.

In vitro studies have shown that treatment with tralkoxydim does not inhibit ferrocheletase activity in cultured human hepatocytes (*in vitro* experiments in both mice and rats mirrored the *in vivo* results). In addition, the *in vitro* experiments also suggest that the haem demand in human hepatocytes is not significant when compared to the mouse. Therefore the relevance to humans is not considered to be significant.