CLH report

Proposal for Harmonised Classification and Labelling

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

Substance Name: Pinoxaden

EC Number: **Not yet assigned**

CAS Number: **243973-20-8**

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:	**Pinoxaden**
EC number:	**Not yet assigned**
CAS number:	**243973-20-8**
Annex VI Index number:	**Not yet assigned**
Degree of purity:	**≥ 97% w/w**
Impurities:	**Toluene (≤ 0.1% w/w)** **There are a number of other impurities which have been taken into consideration and are not considered to be of concern with regards to the classification. Full information is provided in the technical dossier.**

1.2 Harmonised classification and labelling proposal

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

	CLP Regulation
Current entry in Annex VI, CLP Regulation	**None**
Current proposal for consideration by RAC	**Acute Tox 4; H332 - Harmful if inhaled** **Skin Irrit 2; H315 - Causes skin irritation** **Eye Irrit 2; H319 - Causes serious eye irritation** **STOT SE 3; H335 - May cause respiratory irritation** **Skin Sens 1A; H317 - May cause an allergic skin reaction** **Aquatic Acute 1; H400 - Very toxic to aquatic life (Acute M-factor = 1)** **Aquatic Chronic 3; H412 - Harmful to aquatic life with long lasting effects**

Resulting harmonised classification (future entry in Annex VI, CLP Regulation)	**Acute Tox 4; H332 - Harmful if inhaled** **Skin Irrit 2; H315 - Causes skin irritation** **Eye Irrit 2; H319 - Causes serious eye irritation** **STOT SE 3; H335 - May cause respiratory irritation** **Skin Sens 1A; H317 - May cause an allergic skin reaction** **Aquatic Acute 1; H400 - Very toxic to aquatic life (Acute M-factor = 1)** **Aquatic Chronic 3; H412 - Harmful to aquatic life with long lasting effects**

1.3 Proposed harmonised classification and labelling based on CLP Regulation

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 relevant	Conclusive but not sufficient for classification
2.2.	Flammable gases	Not classified	Not applicable	Not relevant	Conclusive but not sufficient for classification
2.3.	Flammable aerosols	Not classified	Not applicable	Not relevant	Conclusive but not sufficient for classification
2.4.	Oxidising gases	Not classified	Not applicable	Not relevant	Conclusive but not sufficient for classification
2.5.	Gases under pressure	Not classified	Not applicable	Not relevant	Conclusive but not sufficient for classification
2.6.	Flammable liquids	Not classified	Not applicable	Not relevant	Conclusive but not sufficient for classification
2.7.	Flammable solids	Not classified	Not applicable	Not relevant	Conclusive but not sufficient for classification
2.8.	Self-reactive substances and mixtures	Not classified	Not applicable	Not relevant	Conclusive but not sufficient for classification
2.9.	Pyrophoric liquids	Not classified	Not applicable	Not relevant	Conclusive but not sufficient for classification
2.10.	Pyrophoric solids	Not classified	Not applicable	Not relevant	Conclusive but not sufficient for classification
2.11.	Self-heating substances and mixtures	Not classified	Not applicable	Not relevant	Conclusive but not sufficient for classification
2.12.	Substances and mixtures which in contact with water emit flammable gases	Not classified	Not applicable	Not relevant	Conclusive but not sufficient for classification
2.13.	Oxidising liquids	Not classified	Not applicable	Not relevant	Conclusive but not sufficient for classification

CLP Annex I ref	Hazard class	Proposed classification	Proposed SCLs and/or M-factors	Current classification [1)]	Reason for no classification [2)]
2.14.	Oxidising solids	Not classified	Not applicable	Not relevant	Conclusive but not sufficient for classification
2.15.	Organic peroxides	Not classified	Not applicable	Not relevant	Conclusive but not sufficient for classification
2.16.	Substance and mixtures corrosive to metals	Not classified	Not applicable	Not relevant	Conclusive but not sufficient for classification
3.1.	Acute toxicity - oral	Not classified	Not applicable	Not relevant	Conclusive but not sufficient for classification
	Acute toxicity - dermal	Not classified	Not applicable	Not relevant	Conclusive but not sufficient for classification
	Acute toxicity - inhalation	**Acute Tox 4; H332 – Harmful if inhaled**	**None**	Not classified	**Not applicable**
3.2.	Skin corrosion / irritation	**Skin Irrit 2; H315 – Causes skin irritation**	**None**	Not classified	**Not applicable**
3.3.	Serious eye damage / eye irritation	**Eye Irrit 2; H319 – Causes severe eye irritation**	**None**	Not classified	**Not applicable**
3.4.	Respiratory sensitisation	Not classified	Not applicable	Not classified	Data lacking
3.4.	Skin sensitisation	**Skin Sens 1A; H317 – May cause an allergic skin reaction**	**Not applicable**	Not classified	**Not applicable**
3.5.	Germ cell mutagenicity	Not classified	Not applicable	Not classified	Conclusive but not sufficient for classification
3.6.	Carcinogenicity	Not classified	Not applicable	Not classified	Conclusive but not sufficient for classification
3.7.	Reproductive toxicity	Not classified	Not applicable	Not classified	Conclusive but not sufficient for classification
3.8.	Specific target organ toxicity –single exposure	**STOT SE 3; H335 – May cause respiratory irritation**	**Not applicable**	Not classified	**Not applicable**

CLP Annex I ref	Hazard class	Proposed classification	Proposed SCLs and/or M-factors	Current classification [1)]	Reason for no classification [2)]
3.9.	Specific target organ toxicity – repeated exposure	Not classified	Not applicable	Not classified	Conclusive but not sufficient for classification
3.10.	Aspiration hazard	Not classified	Not applicable	Not classified	Conclusive but not sufficient for classification
4.1.	Hazardous to the aquatic environment	**Acute 1; H400 - Very toxic to aquatic life** **Chronic 3; H412 - Harmful to aquatic life with long lasting effects**	**Acute M-factor = 1**	**Not classified**	**Not applicable**
5.1.	Hazardous to the ozone layer	Not addressed	Not applicable	Not classified	Not addressed

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

Labelling:

Pictograms: GHS07, GHS09

Signal word: Warning

Hazard statements: Acute Tox 4; H332 (Harmful if inhaled)
Skin Irrit 2; H315 (Causes skin irritation)
Eye Irrit 2; H319 (Causes serious eye irritation)
STOT SE 3; H335 (May cause respiratory irritation)
Skin Sens 1A; H317 (May cause an allergic skin reaction)
Aquatic Acute 1; H400 (Very toxic to aquatic life)
Aquatic Chronic 3; H412 (Harmful to aquatic life with long lasting effects)

Precautionary statements: Not included in Annex VI of CLP

Proposed notes assigned to an entry:

None

2 BACKGROUND TO THE CLH PROPOSAL

2.1 History of the previous classification and labelling

Pinoxaden is a new active substance in scope of Regulation 1107/2009. There is no existing entry in Annex VI of CLP and the classification and labelling has not been considered previously. In accordance with Article 36(2) of CLP, the substance is subject to the harmonised classification and labelling procedure.

At the time of submission, the substance is not registered under REACH.

2.2 Short summary of the scientific justification for the CLH proposal

The conclusion on the peer review of the active substance under Regulation 1107/2009 was published in the EFSA journal in 2013 (EFSA Journal 2013;11(8):3269). This concluded that pinoxaden is harmful by inhalation, is a skin and eye irritant, may cause respiratory tract irritation and skin sensitisation. It also raised concern for reproductive toxicity (development) based on the observation of diaphragmatic malformations in one rabbit developmental toxicity study.

This CLH report presents a classification and labelling proposal based mainly on the information presented in the DAR of pinoxaden.

No classification for acute exposure via the oral or dermal route is warranted, with LD_{50} values being >5000 and >2000 mg/kg bw respectively. The inhalation LC_{50} to male rats was 4.63 mg/L and this meets the criteria for classification as **Acute Tox 4; H332 –harmful if inhaled**.

There was no evidence for specific target organ toxicity following single exposure to pinoxaden and therefore it is not propsed to classify pinoxaden with STOT-SE 1 or 2.

No signs of irritation were observed in the rabbit skin irritation test. However, on the basis of the irritation seen in the workforce at the manufacturing sites, it is proposed to classify pinoxaden as **Skin Irrit 2; H315 – Causes skin irritation.** Based on the corneal and conjunctival oedema scores, and on the basis of the irritation seen in the workforce at the manufacturing sites, it is proposed to classify pinoxaden as **Eye Irrit 2; H319 – causes serious eye irritation**. Based on information on the workforce at the manufacturing sites and information from the acute inhalation study, it is proposed to classify pinoxaden as **STOT-SE 3; H335 – may cause respiratory irritation.**

Negative results were obtained in a guideline compliant Guinea Pig Maximisation Test. However in a guideline Local Lymph Node Assay (LLNA), an EC 3 of 0.43% was observed. As such, it is proposed to classify pinoxaden as a strong skin sensitiser **Skin Sens 1A; H317 – May cause an allergic skin reaction.**

In repeated dose studies, significant toxic effects were observed on the kidney in rats and on the kidney and blood in mice, but only at dose levels well in excess of the specified guidance values for classification with STOT-RE. In the dog, gastro-intestinal effects and minor changes in clinical chemistry parameters were observed at dose levels close to the specified (rat) 90-day guidance value of 100 mg/kg bw/day, but in the absence of associated body weight reductions and histopathology findings in any organ, these effects are not regarded as *significant* toxic effects in the context of STOT-RE classification. On this basis, it is not proposed to classify pinoxaden for STOT-RE.

Pinoxaden tested negative in both bacterial and mammalian cells and for DNA damage/repair when assessed in isolated rat hepatocytes. Two *in vitro* cytogenetic assays were positive, with increased incidences of chromosomal aberrations in both the absence and presence of metabolic activation. These increases were associated with cytotoxicity and there was no evidence of significant clastogenic activity in the mammalian cell gene mutation assay from the analysis of small colonies. *In vivo*, pinoxaden was non-clastogenic in the mouse bone marrow micronucleus assay up to a dose (2000 mg/kg bw) causing bone marrow cytotoxicity. There was no evidence of DNA damage in rat liver in a UDS assay conducted at the limit dose of 2000 mg/kg bw. On this basis, it is not proposed to classify pinoxaden as a germ cell mutagen.

A slightly increased incidence of leiomyosarcoma of the non-glandular stomach was noted in male rats at the top dose of 250 mg/kg bw/day (2/60 - 3.3% vs 0% in controls – Lab HCD range: 0 – 0%). However, this increase was not considered to be a specific, treatment-related effect of pinoxaden due to the nature of the tumours and the lack of any association with pre-neoplastic findings. In the mouse, there was a slight increase in the incidence of lung adenoma and carcinoma in male mice at 300 and 750 mg/kg bw/day. However, the increase was small (just above the laboratory historical control range); showed no clear dose response relationship; occurred at doses causing lethality and poor survival; and might have been related to the unintended direct ingress of material/vehicle into the lung through gavage dosing/mis-dosing. It was therefore concluded that these tumours were not related to oral exposure to pinoxaden. This conclusion was confirmed by the absence of lung tumours (or any other tumours) in a second mouse carcinogenicity study conducted by dietary administration up to doses (574/706 mg/kg bw/day) exceeding the MTD. On this basis, it is not proposed to classify pinoxaden for carcinogenicity.

No effects on fertility or development were observed in the rat. In the rabbit, the weight of evidence from four prenatal developmental toxicity studies indicates that unspecific developmental toxicity (resorptions, post-implantation loss and reduced foetal weight) occurs at around 100 mg/kg bw/day pinoxaden in the presence of maternal toxicity. These foetal effects are considered to be the secondary, unspecific consequence of the observed maternal toxicity. A low incidence of malformations of the diaphragm was seen from a dose of 30 mg/kg bw/day (1 foetus in 1 litter at 30 mg/kg bw/day and 3 foetuses in 3 litters at 100 mg/kg bw) in one study. However, this was not repeated in three subsequent studies (using groups of 24 pregnant females and the relevant dose of 100 mg/kg bw/day) in which genetic and familial influences of sibling matings and non-randomised male donors were removed. Overall, the available evidence suggests that the diaphragmatic malformations seen in the first study might have arisen from matings between siblings or other related individuals. Failure to control for these factors in the first study brings into question the reliability of such findings. Overall, it is considered that pinoxaden has no teratogenic potential or specific developmental effects in the rabbit and it is not proposed to classify for reproductive toxicity.

In the environment, pinoxaden would be expected to hydrolyse very rapidly only when surface water pH was relatively high. Under most environmental conditions the hydrolysis rate would be more moderate. Pinoxaden undergoes limited photodegradation and is considered photolytically stable under environmentally relevant conditions for the purposes of classification.

A ready biodegradation test resulted in 12% degradation (based on theoretical carbon dioxide evolution) at day 29. On this basis, it is concluded that pinoxaden is not ‘readily biodegradable’. Mineralisation was only a minor element of dissipation of pinoxaden aquatic water/sediment systems. However, although pinoxaden does not undergo rapid ultimate degradation (>70% in 28 days) it does degrade rapidly in water/sediment systems (whole system DT50 <1 day) to entirely non-classifiable degradants - and so on this basis it is considered to be ‘rapidly degradable’ for the purposes of aquatic hazard classification.

The log Kow of 3.2 for pinoxaden is lower than the trigger value of 4 for Regulation EC 1272/2008 and it shows a low potential for bioconcentration.

Metabolite NOA 407854 (M2) is the only major metabolite identified in both the water and the sediment phases and it was shown to be persistent. A full set of acute fish, invertebrate and algae/aquatic plant data is available for M2. It is noted that this and the more minor M3 degradant are at least an order of magnitude less acutely toxic than the parent pinoxaden and it also poses a low chronic hazard, therefore these degradants are themselves considered unclassified.

A full set of valid acute fish, invertebrate and algae/aquatic plant data is available for pinoxaden. Pinoxaden is an herbicide and, as anticipated, algae / aquatic plants are the most sensitive trophic group. Based on available acute and chronic data for pinoxaden, acute toxicity (L(E)C50 values) are concluded to be >0.1 to ≤1.0 mg/L. In relation to chronic toxicity, data are available on fish and algae/aquatic plants and the lowest NOEC values are also >0.1 but ≤1 mg/L.

Based on acute aquatic toxicity data, a classification of category **Acute 1; H400 with an Acute M-factor of 1** is proposed. Based on chronic aquatic toxicity data, long-term NOECs for algae and aquatic plants are >0.1 but ≤1 mg/L and due to its rapid degradation to non-classified degradants pinoxaden is considered 'rapidly degradable' with a low bioaccumulation potential, therefore classification as category **Chronic 3; H412** is proposed.

2.3 Current harmonised classification and labelling

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

Pinoxaden is a new active substance. There is no current harmonised classification and labelling in Annex VI of CLP.

2.4 Current self-classification and labelling

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

Signal word: Warning

Hazard statements: Acute Tox 4; H332 (Harmful if inhaled)

Skin Irrit 2; H315 (Causes skin irritation)

Eye Irrit 2; H319 (Causes serious eye irritation)

STOT SE 3; H335 (May cause respiratory irritation)

Skin Sens 1A; H317 (May cause an allergic skin reaction)

Aquatic Chronic 3; H412 (Harmful to aquatic life with long lasting effects

3 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

Pinoxaden is a new pesticide active substance currently under review for approval to Regulation (EC) No 1107/2009 of the European Parliament and of the Council. In accordance with Article 36(2) of CLP it should be considered for harmonised classification and labelling.

Part B.

SCIENTIFIC EVALUATION OF THE DATA

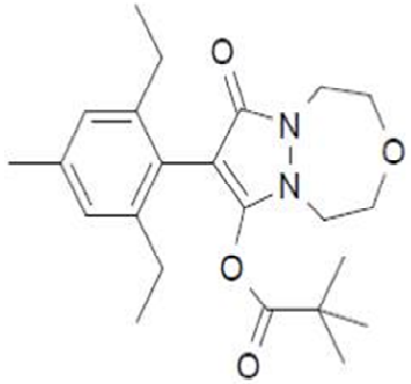
1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 4: Substance identity

EC number:	Not yet assigned
EC name:	Not yet assigned
CAS number (EC inventory):	Not yet assigned
CAS number:	243973-20-8
CAS name:	Propanoic acid, 2,2-dimethyl-, 8-(2,6-diethyl-4-methylphenyl)1,2,4,5-tetrahydro-7-oxo-7H-pyrazolo[1,2-d][1,4,5]oxadiazepin-9-yl ester
IUPAC name:	8-(2,6-diethyl-4-methylphenyl)-7-oxo-1,2,4,5-tetrahydro-7H-pyrazolo[1,2-d][1,4,5]oxadiazepin-9-yl 2,2-dimethylpropanoate
CLP Annex VI Index number:	Not relevant
Molecular formula:	$C_{23}H_{32}N_2O_4$
Molecular weight range:	400.5 g/mol

Structural formula:



1.2 Composition of the substance

Table 5: Constituents (non-confidential information)

Constituent	Typical concentration	Concentration range	Remarks
Pinoxaden	≥ 97%	≥ 97% < 100%	

Current Annex VI entry: No current entry

Table 6: Impurities (non-confidential information)

Impurity	Typical concentration	Concentration range	Remarks
Toluene	≤ 0.1% w/w		At this concentration it is not considered to impact on the classification.

There are a number of impurities present at quantities ≥ 1 g/kg. The impurities have been taken into consideration in the classification of this substance. It is concluded that there are no other impurities for toxicological or environmental consideration.

Table 7: Additives (non-confidential information)

Additive	Function	Typical concentration	Concentration range	Remarks
None				

Current Annex VI entry: No current entry

1.2.1 Composition of test material

The batches or pinoxaden tested were generally of higher purity than pinoxaden as manufactured. As such, some of the tested batches may not have contained some of the impurities found in the technical material. Further information was provided during the review process to show the equivalence of the batches. The available studies are considered appropriate to support the classification of pinoxaden itself.

1.3 Physico-chemical properties

All references taken from the DAR for Pinoxaden - Volume 3, Annex B, B.2: Physical and chemical properties – July 2006.

Table 8: Summary of physico - chemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
State of the substance at 20°C and 101,3 kPa	White, fine powder Beige powder	Das, 2001a Das, 2003a	Purity 99.5%. ASTMS methods Purity 98.1%. ASTMS methods
Melting/freezing point	120.5 to 121.6ºC	Das, 2001b	Purity 99.5% EEC method A 1
Boiling point	No boiling observed up to 360°C. Thermal decomposition (change in colour) observed at 335ºC.	Das, 2002a	Purity 99.5% EEC method A 2
Relative density	1.16 at 24ºC	Füldner, 2001	Purity 99.5% OECD 109
Vapour pressure	2.0×10^{-7} Pa at 20°C 4.6×10^{-7} Pa at 25ºC	Geoffroy, 2003a	Purity 99.5% OECD 104. Obtained from a vapour pressure curve
Surface tension	45.8 mN/m at 20ºC.	Martin, 2003	Technical. EEC method A5 (OECD 115) Suggests active substance is surface active.
Water solubility	200 mg/l in pure water at 25°C (quoted to two significant figures) Effect of pH not determined.	Das, 2001c	Purity 99.5% EEC Method A6. Effect of pH not determined as no dissociation observed.
Partition coefficient n-octanol/water	Log P_{ow} = 3.2 at 25°C effect of pH not determined as no dissociation observed.	Das, 2001e	Purity 99.5% EEC Method A8. Surface tension data suggests pinoxaden is surface active. Data acceptable despite potential surface activity.
Flash point	Not required as pinoxaden is a solid with mp > 40°C.		
Flammability	Flammability: substance melted but did not ignite.	Jackson, 2003a	Purity 98.1% EEC method A10
Explosive properties	No explosive reaction to friction, heat or shock.	Jackson, 2003d	Purity 98.1% EEC method A14
Self-ignition temperature	Auto flammability: No ignition detected below melting point.	Jackson, 2003c	Purity 98.1% EEC method A16
Oxidising properties	No reaction indicative of oxidising.	Jackson, 2003d	Purity 98.1%

Property	Value	Reference	Comment (e.g. measured or estimated)
			EEC method A17
Granulometry	No data		
Solubility in organic solvents and identity of relevant degradation products	All determined at 25°C Acetone 250 g/l Dichloromethane >500 g/l ethyl acetate 130 g/l hexane 1.0 g/l methanol 260 g/l octanol 140 g/l toluene 130 g/l	Das, 2003b	Purity 98.1% CIPAC MT 157.3
Dissociation constant	No pk_a observed experimentally.	Martin, 2001	Purity 99.5% OECD 112
Viscosity	No data		

2 MANUFACTURE AND USES

2.1 Manufacture

Pinoxaden in manufactured by Syngenta inside the EU.

2.2 Identified uses

Pinoxaden is placed on the market both inside and outside of the EU as an herbicide.

3 CLASSIFICATION FOR PHYSICO-CHEMICAL PROPERTIES

Table 9: Summary table for relevant physico-chemical studies

Method	Results	Remarks	Reference
Refer to Table 8			

Summary and discussion of physic-chemical properties

Refer to Table 8.

Comparison with criteria

In a standard flammability study (EEC A10) pinoxaden was found to be not flammable. Experience in handling and use indicates it is not pyrophoric and does not react with water to liberate flammable gases. Further, it was tested in a standard self-ignition temperature study (EEC A16) and no spontaneous ignition was observed.

Pinoxaden was tested in a standard explosivity study (EEC A14) where it was found to be not explosive under the influence of a flame and was not sensitive to impact or friction.

Pinoxaden was tested in a standard study (EEC A17) and was not oxidising.

Conclusions on classification and labelling

Not classified – Conclusive but not sufficient for classification

4 HUMAN HEALTH HAZARD ASSESSMENT

References are taken from the DAR for pinoxaden Volume 3, Annex B, B.6, part 1 and 2: Toxicology and Metabolism – July 2006

4.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

4.1.1 Non-human information

Information on the toxicokinetics of pinoxaden is available from four oral studies in rats.

4.1.2 Human information

No data are available.

4.1.3 Summary and discussion on toxicokinetics

The following summary is based upon that in the Pesticide Draft Assessment (DAR) made for review under Regulation (EC) 1107/2009.

Absorption and excretion: A single oral dose of 0.5 mg [phenyl-1-^{14}C]-pinoxaden/kg was rapidly and extensively absorbed by both sexes. Maximum blood [^{14}C]-concentrations were reached within 1 hour followed by a rapid decline to the limit of detection by 8 hours in males and 24 hours in females. Excretion was rapid with <90% of the dose eliminated within 72 hours. After seven days, *ca* 65% of the dose had been excreted in urine and 24 -29% in faeces, tissue residues being very low with none exceeding the limit of quantitation. Experiments in bile duct cannulated rats showed that biliary elimination (9-12% of dose) was of relatively minor importance compared to renal excretion. Bile duct cannulated rats also excreted only *ca* 6% or less of the dose in faeces showing that absorption of a 0.5 mg/kg dose exceeded 90%.

Following a single oral dose of 300 mg [phenyl-1-^{14}C]-pinoxaden/kg, blood [^{14}C]-concentrations increased rapidly in both sexes and an almost constant concentration was maintained between 1 and 8 hours after dosing. Radioactivity levels then decreased to the limit of quantitation by 48 hours in males and 72 hours in females. Excretion was rapid and predominantly in urine. Over seven days 66 - 79% of the dose was excreted in urine and *ca* 26% in faeces. The extent of absorption appeared similar to the low dose level. Seven days after dosing, tissue residues were low, being highest in liver and kidney (0.04 – 0.08 ppm pinoxaden equiv.), but were near to the limit of quantitation in all other tissues.

At both dose levels, the [^{14}C]-residues in excised tissues represented less than 0.01% of the dose.

Elimination: Following a single oral dose of 0.5 or 300 mg [phenyl-1-^{14}C]-pinoxaden/kg, radioactivity was eliminated from tissues with half-lives of 2 - 5 hours and 5 - 7 hours respectively. Residues were generally highest in blood and in organs of excretion, i.e. liver and kidneys. Lowest residues were present in brain, bone, testes, thymus, fat, ovaries and uterus. No marked sex difference was apparent in either the tissue distribution pattern or the half-lives of elimination.

Following the repeated daily oral dosing of female rats (as the sex showing higher tissue concentrations in preceding studies) with 0.5 mg [phenyl-1-^{14}C]-pinoxaden/kg, blood [^{14}C]-concentrations rapidly reached a plateau of *ca* 0.06 to 0.09 ppm pinoxaden equivalents. The highest tissue concentrations were observed at 24 hours after the seventh and fourteenth doses in liver and

kidneys (ca. 0.025 and 0.015 ppm pinoxaden equiv. respectively). Residues in other tissues were markedly lower (<0.003 ppm pinoxaden equiv.) or below the limit of determination. After the 14th and final dose, levels declined rapidly and reached the limit of determination by 50 hours. The mean half-life of blood residues was *ca* 7 hours, consistent with the determination after a single similar dose. Three days after the final dose, the radioactivity in all excised tissues amounted to less than 0.01% of the total administered dose, with a further 0.28% present in the carcass. No marked tissue accumulation of radioactivity was observed during repeated dosing.

Throughout the study, excreta were collected daily from one sub-group housed in metabolism cages. Excretion was rapid and by three days after the final dose, 70% and 22% of the total dose were excreted *via* urine and faeces. Neither routes nor rates of excretion changed during the repeated dosing period. Analysis of excreta collected over 24 hours after the first and final doses revealed no qualitative or quantitative differences in the metabolite profile in urine or faeces.

Following the repeated daily oral dosing of female rats with 300 mg [pyrazole-3,5-^{14}C1]-pinoxaden /kg, blood concentrations reached a plateau of *ca* 2 ppm pinoxaden equivalents after the second dose. The highest tissue levels of radioactivity were observed at 24 hours after the seventh dose in liver and kidneys (ca. 34 and 18 ppm pinoxaden equiv. respectively). All other tissues concentrations reached maximum levels within 7 days and were below the concentration in blood. After the 14th and final dose, blood concentration decreased rapidly reaching half their maximum concentration within 15 hours. All tissue residues declined rapidly with elimination half-lives in the range of 1 to 3 days.

Seven days after the final dose, 69% of the total dose was excreted in urine and 26% *via* faeces. More than 90% of the administered radioactivity was excreted within 24 hours of the final dose and excretion was complete by 7 days after the final dose when less than 0.01% of the total dose remained in the excised tissues and a further 0.18% in the carcass. Neither routes nor rates of excretion changed during the repeat dosing period. No qualitative or quantitative differences were observed in urinary or faecal metabolite patterns after single or multiple doses. There was no indication of any potential for tissue accumulation after multiple oral 300 mg/kg doses.

Biotransformation: An oral dose of pinoxaden was quantitatively metabolized by the rat as no unchanged parent was present in urine, bile or faeces. The major metabolite was the hydrolysis product M2, accounting for 62 - 70% of a 0.5 mg/kg dose and 77 - 91% of a 300 mg/kg dose. The hydroxylation product M4, was the only other metabolite above >10% of the administered dose, representing *ca* 13% of a 0.5 mg/kg dose and 7% of a 300 mg/kg dose. All other 33 metabolites were minor and each represented ≤ 1.2% of the administered dose. Metabolites generally represented products of hydrolysis, hydroxylation and conjugation. The biotransformation of pinoxaden was almost qualitatively and quantitatively independent of sex and of the dose level investigated. There was a difference in the ratio of M2 to M4 between the dose levels but there was no sex difference.

Based on the structures identified the metabolism of pinoxaden, i.e. 2,2-dimethyl-propionic acid 8-(2,6-diethyl-4-methyl-phenyl)-9-oxo-1,2,4,5-tetrahydro-9*H*-pyrazolo[1,2-*d*] [1,4,5]oxa-diazepine-7-yl ester proceeds via hydrolysis, hydroxylation, de-alkylation, ring cleavage and ring formation reactions, followed by conjugation with glucuronide, sulphate and sugars.

Summary: Oral doses of pinoxaden (0.5 to 300 mg/kg) were rapidly and extensively absorbed with >90% absorption at 0.5 mg/kg. Excretion of the absorbed dose was rapid occurring predominantly in urine with a lesser amount in bile. At the low dose level (0.5 mg/kg), tissue residues declined to below the limit of quantitation within 7 days; at the high dose level (300 mg/kg), only liver and kidney contained detectable concentrations by 7 days after dosing. There was no sex difference in

either tissue distribution or rate of elimination; at both dose levels, the residues in excised tissues represented less than 0.01% of the dose. Repeated administration to female rats for 14 days did not result in any significant accumulation in tissues; there was no change in the route or rate of excretion. Over the dose range 0.5 to 300 mg/kg, pinoxaden was completely metabolised in rat; metabolism proceeded via hydrolysis, hydroxylation, de-alkylation, ring cleavage and ring formation reactions, followed by conjugation with glucuronide, sulphate and sugars.

4.2 Acute toxicity

Information on the acute toxicity of pinoxaden is available from one oral study in rats, one inhalation study in rats and one dermal study in rats.

Table 10: Summary table of relevant acute toxicity studies

Method	Results LD_{50}/LC_{50}	Remarks	Reference
Oral OECD 401 GLP Hanlbm:Wistar rat 5/sex/dose Doses: 0, 5000 mg/kg bw Vehicle: 0.5% carboxymethyl cellulose (CMC) in 0.1% aqueous polysorbate 80 Pinoxaden - EZ005006, (97.2% purity)	**>5000 mg/kg bw**	**5000 mg/kg**: Mortality: 1/5 males (day 5), 0/5 females Clinical signs included: soft faeces (2M,1F), hunched posture (2M,2F). All surviving animals appeared normal by day 1. Reddish small intestine, large intestine and caecum in male found dead; no other remarkable necropsy observations.	2000a DAR B.6.2
Inhalation (dust aerosol, 4 h, nose-only) OECD 403 GLP Hanlbm:Wistar rat 5/sex/dose Concentrations: 2.2, 3.7, 5.4 mg/L MMAD = 2.2 – 2.7 µm Vehicle: None Pinoxaden technical - EZ005006, (97.2% purity)	Males: **4.63 mg/L** Females: **6.24 mg/L** Male & Female: **5.22 mg/L**	**5.4 mg/L**: Mortality: 3/5 males, 2/5 females. Clinical signs included: During exposure – salivation (5M,5F) and bradypnea (5M,5F); Following exposure – hunched posture (5M,5F), laboured respiration (5M,5F), rales (5M,5F) and ruffled fur (5M,5F). All recovered by Day 8. Marked, transient body weight loss between days 1-4 (M -22.0%, F -11.0%). Necropsy findings included: red discolouration lungs (2M,2F), incompletely collapsed lungs (1M), several dark foci thymus (1M). **3.7 mg/L**: Mortality: 2/5 males, 1/5 females. Clinical signs included: During exposure – salivation (5M, 5F) and tachypnea (5M, 5F); Following exposure - laboured respiration (5M, 5F), rales (5M, 5F), ruffled fur (5M, 5F), decreased spontaneous activity (5M, 5F) and swollen abdomen (1F). All recovered by Day 8. Marked, transient body weight loss between days 1-4 (M -21.9%, F -19.4%).	2001 DAR B.6.2.2

Method	Results LD_{50}/LC_{50}	Remarks	Reference
		Necropsy findings included: red discolouration lungs (3M). **2.2 mg/L**: No mortality. Clinical signs included: During exposure – hunched posture (5M,5F), laboured respiration (5M,5F), ruffled fur (5M,5F) and salivation (5M,5F); Following exposure - hunched posture (5M,5F), laboured respiration (1M,1F), rales (5M,5F), ruffled fur (5M,5F) and red secretion around nose (1F). All recovered by Day 8. Marked, transient body weight loss between days 1-4 (M -15.2%, F -6.0%). Necropsy findings included: red discolouration mandibular lymph node (2M).	
Dermal OECD 402 GLP Hanlbm:Wistar rat 5/sex/dose Doses: 0, 2000 mg/kg bw Vehicle: Test article was moistened with 0.5% carboxymethyl cellulose (CMC) in 0.1% aqueous polysorbate 80 24 h application, semi-occlusive Pinoxaden technical - EZ005006, (97.2% purity)	**>2000 mg/kg bw**	**2000 mg/kg bw**: No mortalities, no clinical signs or signs of irritation. Slight body weight loss 3/5 females during 1st week.	2000b DAR B.6.2.3

4.2.1 Non-human information

4.2.1.1 Acute toxicity: oral

In a guideline acute oral toxicity study in the Wistar rat (2000a), mortality was observed in 1/5 males and 0/5 females at 5000 mg/kg; the decedent male was found dead on day 5. Clinical signs observed on the treatment day were soft faeces in two males and one female in the test group and hunched posture in two males and two females in the test group. All surviving animals appeared normal by day 1. Necropsy examinations revealed reddish small intestine, large intestine and caecum in the test group male that was found dead; there were no other remarkable necropsy observations.

An acute oral LD_{50} of >5000 mg/kg bw was derived.

4.2.1.2 Acute toxicity: inhalation

In a guideline acute, nose-only inhalation toxicity study in the Wistar rat (2001), there were no deaths during the 4 hour exposure period. No deaths were observed in either gender at a concentration of 2.2 mg/L. At 3.7 mg/L 2/5 males and 1/5 females were found dead two days after the exposure. At 5.4 mg/L 3/5 males and 2/5 females were found dead; four rats (3 males and 1 female) died the day after exposure and a further female was found dead three days after the exposure.

The principal clinical signs consisted of effects on breathing, salivation and ruffled fur seen at all three concentration levels, hunched posture at the low- and high-concentration levels, decreased spontaneous activity at the mid- and high-concentration levels and restlessness at the mid-concentration level. The effects on breathing were reflected by the findings of laboured respiration and breath sounds (rales) at all three concentrations preceded by tachypnea at the mid-concentration or bradypnea at the high-concentration level. In addition, red secretion from the nose was seen in one low-concentration female and a swollen abdomen in one female survivor of the mid-concentration group.

Marked, transient losses in mean body weight were evident in male and female animals of Group 1 (2.2 mg/L) and in all male and female survivors of Groups 2 (3.7 mg/L) and 3 (5.4 mg/L).

Necropsy revealed dark red and/or reddish discoloration of the lungs in two of the three premature deaths at 3.7 mg/L and in four of the five premature deaths at 5.4 mg/L, and incompletely collapsed lungs in one male survivor at 5.4 mg/L. There were no other macroscopic pathology findings attributable to treatment. The mortality, clinical signs and transient losses in bodyweight listed above were considered treatment related.

Acute inhalation 4 hour LC_{50} values of 4.63 mg/L (90% CL 3.35–20.68 mg/L) for males, 6.24 mg/L (CL not determined) for females and 5.22 mg/L (95% CL 4.07–18.00 mg/L) for males and females combined were derived.

4.2.1.3 Acute toxicity: dermal

In a guideline acute dermal toxicity study in the Wistar rat (2000b), there were no mortalities. No clinical signs were observed during the study period. There were no signs of irritation at the application site. Slight body weight loss occurred in three test-group females during the first study week. Necropsy examinations revealed a slightly granulated surface of the right kidney in one male and a dilated left renal pelvis in one female; there were no other remarkable necropsy observations.

An acute dermal LD_{50} of >2000 mg/kg bw was derived.

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

Pinoxaden was not acutely toxic to rats by the oral or dermal route. However, by inhalation the 4h-LC50 of pinoxaden dust aerosol to male rats was 4.63 mg/L.

4.2.4 Comparison with criteria

Via the oral route, classification is required where the LD_{50} is < 2000 mg/kg bw. The LD_{50} for pinoxaden was >5000 mg/kg bw and therefore no classification is warranted

Via the dermal route, classification is required where the LD_{50} is < 2000 mg/kg bw. The LD_{50} was >2000 mg/kg bw and therefore no classification is warranted.

Via the inhalation route, the 4h-LC_{50} (aerosol) to male rats was 4.63 mg/L; this meets the criteria for classification as Acute Tox 4; H332 (i.e., $1.0 \leq ATE \leq 5.0$ mg/L).

4.2.5 Conclusions on classification and labelling

Acute Tox. 4 (H332) – Harmful if inhaled

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

There were no indications of specific organ toxicity in the single exposure acute studies.

In the acute oral study (2000a), clinical signs were non-specific (e.g. hunched posture and soft faeces) and necropsy findings were also non-specific.

In the acute dermal study (2000b) there were no specific toxic effects.

In the acute inhalation study (2001) clinical signs of toxicity were non-specific and necropsy findings were related to lethality. The principal clinical signs consisted of effects on breathing, salivation and ruffled fur at all three concentration levels, hunched posture at the low- and high-concentration levels, decreased spontaneous activity at the mid- and high-concentration levels and restlessness at the mid-concentration level. Necropsy of each animal revealed dark red and/or reddish discoloration of the lungs in the majority of the premature deaths at 3.7 mg/L and in four of the five premature deaths at 5.4 mg/L. It should be noted that it is already proposed to classify pinoxaden for acute inhalation toxicity.

Please refer to section 4.2 and table 10 for further information. Also, please refer to section 4.4.3 for discussion of respiratory tract irritation and classification with STOT-SE 3; H335.

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

There was no evidence for specific target organ toxicity following single exposure to pinoxaden.

Please refer to section 4.4.3 for information on respiratory tract irritation and classification with STOT-SE 3; H335.

4.3.2 Comparison with criteria

No classification required for STOT SE 1 or 2. Please refer to section 4.4.3 for discussion of respiratory tract irritation and classification with STOT-SE 3; H335.

4.3.3 Conclusions on classification and labelling

STOT-SE 3; May cause respiratory tract irritation

4.4 Irritation

4.4.1 Skin irritation

Information on the skin toxicity of pinoxaden is available from one skin irritation study in rabbits and human experience.

Table 11: Summary table of relevant skin irritation studies

Method	Results: Average scores	Remarks	Reference
OECD 404 GLP New Zealand White rabbits Dose: 0.5 g Vehicle: Moistened with water Pinoxaden technical - EZ 005006, (97.2% purity)	Scores at 24, 48 and 72 h Erythema: 0, 0, 0 Oedema: 0, 0, 0	3 animals tested No skin reactions	2000a DAR B.6.2.4

4.4.1.1 Non-human information

The skin irritation potential of pinoxaden has been investigated in a standard guideline study in rabbits (2000a). No signs of systemic toxicity were seen in any animal during the course of the study. No skin reactions were noted at the application site of any animal at any of the observation times. Pinoxaden is, therefore, not a skin irritant to the rabbit.

In a guideline 28-day dermal study in rats, slight erythema formation was observed at the application site in 2/10 males and 3/10 females at 100 mg/kg bw/day and also in 2/10 females at 10 mg/kg bw/day. However, this was not noted in animals receiving 1000 mg/kg bw/day and therefore, these dermal effects were not considered treatment-related. Refer to section 4.7.1.3 and table 15.

4.4.1.2 Human information

Since the commencement of large scale production of pinoxaden in 2005, incidents of skin irritancy (redness, itchiness and rashes) have been observed among the workforce at the manufacturing sites. The manufacturer considers these data to be accurate and reliable. Further details of these human experience data are provided below.

During the development stage, pinoxaden was synthesized and formulated at Munchwilen, Switzerland. It is now manufactured at the Syngenta site in Grangemouth, Scotland and formulated in Monthey, Switzerland, Omaha, US and by a third party in Canada.

The Health, Safety and Environment (HSE) Operations Group of Syngenta, which includes the Global Occupational Health (GOH) function, maintains a database of incidents involving chemical exposure of workers.

Since 2004 up to 2011, there have been a total of 35 adverse reactions out of a total of 306 employees. The cases can be summarised in a number of ways:

By Date of onset:

Year	2004	2005	2006	2007	2008	2009	2010	2011
no of cases	6	2	4	1	2	11	6	3

By Site:

Site	Muenchwilen	Grangemouth	Omaha	Monthey	UK Sales	3rd Party
no of cases	6	11	16	1	1	(8)

By effect:

Effect	Eye irritation	Skin irritation	Respiratory irritation	Resp & skin	Resp & eye	Eye & skin
no of cases	1	7	21	2	1	3

Effect by Year

Year	2004	2005	2006	2007	2008	2009	2010	2011
Cause(s)	Resp:6	Skin:1 Skin/Resp:1	Skin:4 Resp:5 (3rd party)	Skin:1	Resp:2	Resp:7 Resp/Eye:2 Resp/skin:1 Skin/eye:1 Skin: 3 (3rd party)	Resp:4 Skin:1 Skin/eye:1	Resp:2 Skin/eye:1

In all the dermal cases, the symptoms exhibited were minor and resolved completely without the need for medical intervention.

4.4.1.3 Summary and discussion of skin irritation

The skin irritation potential of pinoxaden has been investigated in a standard guideline study in rabbits. No signs of systemic toxicity were seen in any animal during the course of the study. No skin reactions were noted at the application site of any animal at any of the observation times. Pinoxaden is, therefore, not a skin irritant to the rabbit.

Slight erythema formation was observed at the application site in 2/10 males and 3/10 females at 100 mg/kg bw/day and also in 2/10 females at 10 mg/kg bw/day in a guideline 28-day dermal study in rats. However, these effects were not noted in animals receiving 1000 mg/kg bw/day pinoxaden and as such, these dermal effects were not considered treatment-related.

Incidences of skin irritation (redness, itchiness and rashes) have been observed among the workforce at the manufacturing sites. These were minor and resolved completely without the need for medical intervention.

4.4.1.4 Comparison with criteria

Based on animal data, classification for skin irritation is applicable where a) the mean score (from gradings over 24-72 hours after patch removal) from 2/3 animals is ≥ 2.3 - ≤ 4 for erythema/eschar or for oedema or b) where inflammation persists to the end of the observation period (generally 14 days) in at least 2 animals or c) if there is pronounced variability amongst animals with a very definite response related to exposure to the substance in a single animal (although the criteria in (a) and (b) are not met). No signs of irritation were observed in the rabbit skin irritation test and therefore these criteria are not met. Slight erythema formation was observed in a 28-day dermal study in the rat, but only at the low and mid-dose group, not in the high-dose group. As such, thes effects observed in the repeat dose study are not considered to be treatment related.

However, classification can also be based on human data (section 3.2.2.1 and 3.2.2.4) and where adequate and reliable information are available this shall take precedence. Therefore, given the incidences of skin irritation seen in the workforce at the manufacturing sites, it is proposed that pinoxaden should be classified as Skin Irrit 2, H315.

4.4.1.5 Conclusions on classification and labelling

Skin Irrit. 2; H315 – Causes skin irritation

4.4.2 Eye irritation

Information on the eye irritation of pinoxaden is available from one study in rabbits and human experience.

Table 12: Summary table of relevant eye irritation studies

Method	Results	Remarks	Reference
OECD 405 GLP New Zealand White rabbits Dose: 0.1 g Vehicle: None Pinoxaden technical - EZ 005006, (97.2% purity)	Mean actual scores for each of 3 rabbits at 24, 48 and 72 h Corneal opacity: 1,1,1 Iritis: 0,0,0 Conjunctivae (redness): 1,1.3,1.3 Conjunctivae (chemosis): 2, 2.7, 3	Three animals tested Full recovery by 28 days Moderate corneal opacity (score 2) in one animal on day 21 – resolved on day 28 Mild conjunctival redness and chemosis (score 1) in the same animal on day 21 – resolved on day 24	2000b DAR B.6.2.5

4.4.2.1 Non-human information

The eye irritation potential of pinoxaden has been investigated in a standard guideline study in rabbits (2000b). Slight corneal opacity was observed in all animals 1 to 72 hours after application but had disappeared in one animal on day 7, in a second animal on day 10 but in the third animal, corneal opacity increased in severity on day 7 and persisted as moderate or marked to day 21 and finally clearing at the 28 day reading. No abnormal findings were observed in the iris at any reading. Slight reddening of the conjunctiva with moderate to marked chemosis was observed in all animals at the 1 hour reading. Slight to moderate reddening persisted to the 7, 14 or 21 day reading for the three animals. Moderate to marked chemosis was observed in all animals 24 and 48 hours

after treatment. The chemosis diminished in two animals at the 72 hour reading and was clear by day 7; in the third animal, the chemosis persisted until 21 days after treatment.

All eye reactions were clear within 28 days after treatment.

4.4.2.2 Human information

Since the commencement of large scale production of pinoxaden in 2005, incidents of eye irritancy (4 cases out of a total of 306 employees) have been observed among the workforce at the manufacturing sites. The manufacturer considers these data to be accurate and reliable. Further details have been included in section 4.4.1.2.

4.4.2.3 Summary and discussion of eye irritation

The eye irritation potential of pinoxaden has been investigated in a standard guideline study in rabbits. Pinoxaden was irritating to the rabbit eye.

Incidences of eye irritancy have been observed among the workforce at the manufacturing sites.

4.4.2.4 Comparison with criteria

Under CLP, a substance should be classified for irreversible eye effects (Category 1) *if it produces in at least one animal effects on the cornea, iris or conjunctiva that are not expected to reverse or have not fully reversed within an observation period of normally 21 days* and/or *it produces at least in two of three tested animals a positive response of corneal opacity ≥3 and/or iritis >1.5*.

In the pinoxaden eye irritation study, one animal still showed moderate corneal opacity (score 2) and mild conjunctival redness and chemosis (score 1) on day 21. However, since the corneal opacity had reversed by day 28 and the conjunctival reactions had reversed by day 24, it is considered that pinoxaden does not cause irreversible eye effects. In addition, the corneal opacity (scores of 1, 1, 1) and iritis scores (0, 0, 0) were below the values required for Category 1 classification. So, classification of pinoxaden with Category 1 is not considered appropriate.

Under CLP, a substance should be classified for reversible eye effects (Category 2) *if, in at least two of three tested animals, a positive response is observed of corneal opacity ≥1 and/or iritis ≥1 and/or conjunctival redness ≥2 and/or conjunctival oedema ≥2; calculated as mean score following grading at 24, 48 and 72 hours and which are fully reversible.*

For the corneal opacity (scores of 1, 1, 1) and conjunctival oedema scores (2, 2.7, 3), pinoxaden meets the criteria for classification as Eye Irrit 2; H319. These effects were fully reversible within 28 and 24 days post-treatment respectively.

Incidents of eye irritancy have also been observed among the workforce at the manufacturing site.

Classification of pinoxaden as an eye irritant under CLP as Eye Irrit 2; H319 is proposed on the basis of animal data and reports of eye irritation in the workforce.

4.4.2.5 Conclusions on classification and labelling

Eye Irrit 2: H319 – Causes serious eye irritation

4.4.3 Respiratory tract irritation

Evidence for respiratory tract irritation is available from both an acute inhalation study in rats and from human experience.

4.4.3.1 Non-human information

Signs of possible respiratory irritation (laboured respiration, breath sounds, tachypnea, bradypnea, dark red and/or reddish discoloration of the lungs and one collapsed lung) were observed in the acute inhalation study (see Part B Section 4.2.1.2).

4.4.3.2 Human information

During the late development phase of pinoxaden in 2004 and subsequent commencement of large scale production of pinoxaden in 2005, incidents of respiratory tract irritation (short-lived episodes of coughing) have been observed among the workforce at the manufacturing sites. In most respiratory cases, the typical symptoms included sneezing or intermittent coughing, which resolved completely upon removal of the worker from the workplace. In 1 case, following exposure during formulation activities with pinoxaden, one worker was diagnosed with occupational asthma but the cause was inconclusive. In the past 2 years further coughing incidents and very isolated incidents of asthma-like symptoms (including wheezing) have been reported.

The manufacturer considers these data to be accurate and reliable. Further details have been included in section 4.4.1.2.

4.4.3.3 Summary and discussion of respiratory tract irritation

No specific respiratory irritation study has been conducted on experimental animals, however signs of possible respiratory irritation (laboured respiration, breath sounds, tachypnea, bradypnea, dark red and/or reddish discoloration of the lungs and one collapsed lung) were observed in the acute inhalation study. In addition, there are reliable and accurate data from a manufacturing site showing that some of the workforce, when handling technical pinoxaden experienced short-term coughing episodes. Based on these data, it is appropriate to classify pinoxaden as a respiratory tract irritant.

4.4.3.4 Comparison with criteria

Classification for respiratory tract irritation is primarily based on human data. This can include subjective observations, with symptoms such as coughing, pain, choking and breathing difficulties. Objective measurements may provide further evidence (e.g., electrophysiological responses, biomarkers of inflammation in nasal or bronchoalveolar lavage fluids). Whilst there are no validated animal models, further information may be available from single and repeat dose animal tests, including the observation of clinical signs of toxicity (dyspnoea, rhinitis etc.,) with histopathology (e.g., hyperemia, edema, inflammation, thickened mucous layer etc.,).

Pinoxaden has shown some evidence of causing respiratory tract irritation in humans (characterised by episodes of coughing, wheezing and sneezing at the manufacturing site). Whilst there is no more information in humans, supportive information is provided from the acute inhalation study in rats where signs of possible respiratory irritation (laboured respiration, breath sounds, tachypnea, bradypnea, dark red and/or reddish discoloration of the lungs and one collapsed lung) were observed. Therefore, it is proposed that pinoxaden should be classified with STOT SE 3, H335.

4.4.3.5 Conclusions on classification and labelling

STOT SE 3: H335 – May cause respiratory irritation

4.5 Corrosivity

4.5.1 Non-human information

Pinoxaden does not have a pH ≤ 2 or ≥ 11. There are no data from rabbit skin and eye irritancy studies to indicate that pinoxaden is corrosive to animal tissue.

4.5.2 Human information

No information available

4.5.3 Summary and discussion of corrosivity

There are no data to suggest that pinoxaden is corrosive to animal tissue.

4.5.4 Comparison with criteria

As pinoxaden does not have a pH ≤ 2 or ≥ 11 and has shown no signs of corrosivity in routine rabbit eye and skin studies, it should not be classified for this end point.

4.5.5 Conclusions on classification and labelling

No classification – conclusive but insufficient for classification

4.6 Sensitisation

One skin sensitisation study in the guinea pig and one local lymph node assay (LLNA) in the mouse on pinoxaden are available.

4.6.1 Skin sensitisation

Table 13: Summary table of relevant skin sensitisation studies

Method	Results	Remarks	Reference
OECD 406 - maximisation study GLP Guinea pig / Himalayan Spotted 30 animals (20 test, 10 control) Pinoxaden technical - EZ005006 (97.2% purity)	Negative 0/19 test animals (1 animal died) 0/10 controls	Induction: Intradermal: 5% in 0.5% CMC + 0.1% Tween 80, Topical: 50% in 0.5% CMC + 0.1% Tween 80 under an occlusive dressing for 48 hours. Challenge: 50% preparation in 0.5% CMC + 0.1% Tween 80. No dermal reaction following challenge in test or control animals.	2000c DAR B.6.2.6

Method	Results	Remarks	Reference
OECD 429 – Local lymph node assay (LLNA) GLP Mouse / CBA/J Rj 4 females/group Pinoxaden technical - AMS 1055/6 (99.6% purity) Vehicle: DMF	Stimulation index >3 at 1, 5, 10 and 25%. EC3 value 0.43% w/v. Conclusion: Pinoxaden is a strong skin sensitiser.	Assay 1: 25,10, 5%, vehicle control Assay 2 (invalid) Assay 3: 25, 5, 1, 0.1, 0.01%, vehicle control, positive control (25% HCA)	2010a

4.6.1.1 Non-human information

In a Magnusson and Kligman skin sensitisation study in guinea pigs (2000c), a 5% preparation had been shown to be well tolerated systemically and to be mildly/moderately irritant to the skin in a preliminary study and was therefore selected for the intradermal induction. A 50% preparation had been shown to be non-irritant in the preliminary study but to be the maximum practical concentration which could be applied to the skin. This concentration was used for the topical induction and challenge applications.

There were no positive skin reactions on the test flanks of the test-group animals, corresponding to a sensitisation rate of 0%. There were no skin reactions among the control animals or on the control flanks of the test-group animals.

The positive control substance 2-mercaptobenzothiazole gave the appropriate response.

In the LLNA on pinoxaden (2010a), a stimulation index >3.0 was determined at concentrations of 1, 5, 10 and 25%. An EC value of 0.43% was calculated. On the basis of this result, pinoxaden is considered to be a strong skin sensitiser.

4.6.1.2 Human information

There are no confirmed cases of skin sensitisation in humans but there is a single case of a manufacturing worker with a putative diagnosis of skin sensitisation based on the exclusion of other causative agents by skin patch testing.

4.6.1.3 Summary and discussion of skin sensitisation

On the basis of the results obtained in the LLNA, pinoxaden is considered to be a strong skin sensitiser. It is unclear why a negative result was obtained in a valid maximisation study in which the substance was tested up to 50%. The different results could be due to the different vehicles (CMC and Tween 80 in the maximisation study and DMF in the LLNA) or/and to the different species (guinea pig in the maximisation study and mouse in the LLNA).

4.6.1.4 Comparison with criteria

In the positive LLNA, the EC 3 value was 0.43%. In accordance with the classification criteria, if the EC3 value is < 2%, the substance should be classified as a Category 1A skin sensitiser (strong skin sensitiser). A generic concentration limit of 0.1% would apply to such a Category 1A

substance. A lower specific concentration limit does not need to be set for pinoxaden as this is not triggered by an EC3 value of 0.43%.

4.6.1.5 Conclusions on classification and labelling

CLP: Skin Sensitiser 1A; H317 – May cause and allergic skin reaction

4.6.2 Respiratory sensitisation

No information available

4.6.2.1 Non-human information

No information available

4.6.2.2 Human information

During the late development phase of pinoxaden in 2004 and subsequent commencement of large scale production of pinoxaden in 2005, incidents of respiratory tract irritation (short-lived episodes of coughing) have been observed among the workforce at the manufacturing sites. In most respiratory cases, the typical symptoms included sneezing or intermittent coughing, which resolved completely upon removal of the worker from the workplace. In 1 case, following exposure during formulation activities with pinoxaden, one worker was diagnosed with occupational asthma but the cause was inconclusive. In the past 2 years further coughing incidents and very isolated incidents of asthma-like symptoms (including wheezing) have been reported.

The manufacturer considers these data to be accurate and reliable. Further details have been included in section 4.4.1.2.

4.6.2.3 Summary and discussion of respiratory sensitisation

Very isolated incidents of asthma-like symptoms (including wheezing) have been reported among the workforce at the manufacturing sites. It is unclear whether these symptoms represent respiratory tract irritation or respiratory sensitisation. It should be noted that it is already proposed to classify pinoxaden for respiratory irritation. Overall, it is concluded that there is no clear evidence that pinoxaden has the potential to induce allergic respiratory sensitisation.

4.6.2.4 Comparison with criteria

As there is no clear evidence to confirm that pinoxaden causes allergic respiratory hypersensitivity, it should not be classified for this end point.

4.6.2.5 Conclusions on classification and labelling

No classification – data lacking

4.7 Repeated dose toxicity

The repeated dose toxicity of pinoxaden has been investigated extensively via the oral route in the rat (one 28-day, two 90-day studies and a chronic study – only the 1-yr findings reported here), mouse (one 90-day study and chronic studies- reported in the carcinogenicity section) and dog (one 28-day, one 90-day and 1-yr study). A repeat dose study via the dermal route in the rat is also available (one 28-day study) (see Part B Section 4.7.1.3).

Table 14: Summary table of relevant repeated dose toxicity studies

Method	Results	Reference
OECD 407 GLP Oral gavage, 28 day Rat/Wistar 5/sex/dose 0, 300, 600 and 1000 mg/kg bw/day Pinoxaden technical - Batch No. 1192-PH-10 (95.7% purity) Vehicle: 0.5% aqueous carboxymethylcellulose and 0.1% Tween 80 **Relevant guidance value for 28-day rat study = 300 mg/kg bw/d**	**1000 mg/kg bw/day** *Mortality and clinical observations:* 1 male found dead day 11 with hunched posture and/or piloerection days 8-11. *Body weight gain & food consumption:* BW gain males 31.3% ↓ overall (days 1-28). Food consumption 24.3% ↓ week 1 males, 32.7% ↓ week 2 males and 18.7% ↓ week 1 females. *Water consumption*: 76.0% ↑ weeks 1-4 males, 53.4% ↑ weeks 1-4 females. *Haematology:* 64.9% ↑ leukocytes in males. No effects in females. *Urinalysis:* 141.1% ↑ males and 96.7% ↑ females volume, 1.0% ↓ males and 0.9% ↓ females relative density, 588.3% ↑ males and 2400% ↑ females ketones. *Organ weights:* 21.3% ↑ males and 31.6% ↑ females relative liver weight, 50.9% ↑ males and 37.2%↑ females relative kidney weight. *Histopathology:* Kidney – tubular atrophy (5/5 males, 5/5 females); tubular casts (1/5 males, 4/5 females); tubular dilatation 5/5 males, 5/5 females); polymorphic infiltration (0/5 males, 3/5 females); single cell necrosis 3/5 males). Liver – glycogen deposition (4/5 males. 5/5 females); lymphohistiocytic infiltration (2/5 males, 4/5 females). **600 mg/kg bw/day** *Water consumption*: 40.6% ↑ overall (weeks 1-4) males, 22.1% ↑ overall (weeks 1-4) females. *Urinalysis:* 123.1% ↑ males and 88.5% ↑ females volume, 1.0% ↓ males and 0.9% ↓ females relative density, 383.3% ↑ males ketones. *Organ weights:* 27.1% ↑ females relative liver weight, 28.4% ↑ males and 17.2% ↑ females relative kidney weight. *Histopathology:* Kidney – tubular atrophy (5/5 males, 5/5 females); tubular casts (2/5 males); tubular dilatation 4/5 males, 5/5 females); polymorphic infiltration (2/5 males).	2001a DAR B.6.3.1(a)

Method	Results	Reference
	Liver – glycogen deposition (5/5 males. 5/5 females); lymphohistiocytic infiltration (1/5 females). **300 mg/kg bw/day** No adverse effects noted. NOAEL[$] 300 mg/kg bw/day males and females	
OECD 408 GLP Oral, gavage 90 day with 28 day recovery Rat/Wistar 10/sex/dose (main study): 0, 3, 10, 30, 100 and 300 mg/kg bw/day) plus 3 groups of 10/sex/dose: 0, 100 and 300 mg/kg bw/day for 4 week recovery. Pinoxaden technical - Batch No. 1192-PH-10 (95.7% purity) Vehicle: 0.5% aqueous carboxymethylcellulose and 0.1% Tween 80 **Relevant guidance value for 90-day rat study = 100 mg/kg bw/d**	**300 mg/kg bw/day** *Water consumption:* 19.2% ↑ males and 28% ↑ females (weeks 1-13). No effects at recovery. *Urinalysis:* 52.2% ↑ males and 52.5% ↑ females volume (wk 14); 217% ↑ males and 1160% ↑ females ketones (wk 14); 8.4% ↓ males and 2.5% ↓ females pH (wk 14). No effects at recovery. *Clinical chemistry*: 20.9% ↑ males and 15.1% ↑ females urea (wk 14); 13.0% ↑ males and 18.0% ↑ females creatinine (wk 14); 16.3% ↓ males glucose (wk 14); 3.8% ↓ females protein (wk 14); 4.7% ↓ females albumin (wk 14). No effects at recovery. *Organ weights:* 12.6% ↑ males and 6.6% ↑ females relative liver weight; 14.6% ↑ females absolute liver weight. No effects at recovery. **100 mg/kg bw/day** *Urinalysis*: 540% ↑ females ketones; 8.3% ↓ females pH. No effects at recovery. The effects seen at 100 mg/kg bw/day were minor and not considered adverse; **30, 10 and 3 mg/kg bw/day** No adverse effects noted. NOAEL[$] 100 mg/kg bw/day	2001b DAR B.6.3.1(b)
OECD 408 GLP Oral, dietary, 90 day with 28 day interim kill and FOB Rat/Wistar 12/sex/dose (main study 90 days), 5/sex/dose (interim sacrifice 28 days) 0, 150, 1000, 5000, 10000	**10000 ppm (890/965 mg/kg bw/day in males/females)** *Body weight*: 15.3%, 13.1%, 11.7%, 11.4% ↓ males, 15.3%, 9.5%, 6.4%, 4.3% ↓ females on day 2, wks, 3, 6, 14 respectively *Food consumption*: 56.3%, 9.9%, 6.7%, 7.7% ↓ males, 57.5%, 6.4%, 8.1%, 5.2% ↓ females on day 2, wks 3, 6, 13 respectively (main study). 61% ↓ males, 54.1% ↓ females on day 2 (28 day kill). *Water consumption*: 15.7%, 12.6%, 9.4%, 17.4% ↑ males weeks 1, 2, 4, 7, 20.5%,	2003 DAR B.6.3.1(c)

Method	Results	Reference
ppm corresponding to 0/0, 14.9/16.0, 97.5/110.5, 465.6/526.8, 899.5/964.9 mg/kg bw/day for males/females Pinoxaden technical - Batch No. EZ005006 (purity 97.2%). **Relevant guidance value for 90-day rat study = 100 mg/kg bw/d**	15.7% ↑ females weeks 1, 2 respectively (main study). *Haematology:* 4.0% ↓ females haemoglobin, 4.3% ↓ females haematocrit, 4.1% ↓ females RBC (main study); *Clinical chemistry:* 27.7% ↓ males, 14.7% ↓ females cholesterol (main study). 19.7% ↓ males 19.3% ↓ females cholesterol (28 day kill). 19.8% ↓ males, 23.6% ↓ females AST (main study). *Urinalysis*: 54.4% ↑ volume females (main study). *Histopathology:* Kidney - Cortical tubular basophilia/dilatation/atrophy (8/10 males, 6/10 females main study) and (3/5 males, 1/5 females 28 day kill). Renal cysts (10/10 males, 7/10 females main study). **5000 ppm (466/527 mg/kg bw/day in males/females)** *Bodyweight*: 6.0%, 3.2% ↓ males, 3.6%, 3.8% ↓ females on wks, 3, 6, respectively (main study). 6.4% ↓ males, 5.1% ↓ females on wk 3 (28 day kill). *Clinical chemistry:* 18.6% ↓ males, 15.5% ↓ females cholesterol (main study). 21.9% ↓ females AST (main study) The effects seen at 5000 ppm were minor and not considered adverse; **1000 ppm (98/111 mg/kg bw/day in males/females)** No adverse effects noted. **150 ppm (15/16 mg/kg bw/day in males/females)** No adverse effects noted. NOAEL[$] 5000 ppm (equivalent to 466/527 mg/kg bw/day in males and females).	
2 year chronic toxicity/ carcinogenicity OECD 453 (1981), GLP Oral, Gavage Rat, Wistar Hanlbm:WIST (SPF) 0, 1, 10, 100, 250, or 500 mg/kg bw/day Dosed for 24 months but 500 mg/kg bw/day group terminated week 61 Total of 90	***Generalised toxicity and non-neoplastic findings only after treatment for 12 months: organ weight and histopathology data are presented for 12 month interim animals. Other data are presented for all animals on test up to 12 months*** **500 mg/kg bw/day** *Survival*: 24/90 (males) died by week 53 (3/90 control). Group terminated at wk 61. *Clinical signs*: (males) 17/90 hunched posture; 14/90 piloerection weeks 1-53 (control 2/90 hunched, 3/90 piloerection). Usually noted for the first time within a week of death/moribund sacrifice. *Bodyweight gain*: ↓ 15% (males) 16.0% (females) weeks 1-52	2003 DAR B.6.5.1(a)

Method	Results	Reference
animals/sex/group 60/sex/group: main 2-yr study 10/sex/group: interim 12-month sacrifice 20/sex/group: haematology and clinical-chemistry investigations (24 months): vehicle 0.5% CMC, 0.1% Tween 80 Pinoxaden technical; Batch No. EZ005006 (97.2 % purity)	*Water intake*: ↑ 78.3% (males) 58.4% (females) weeks 1-52 *Haematology*: ↓ 10.2 – 4.0 red blood cell (males and females), ↓ approx. 7% haemoglobin concentration and haematocrit (males and females) ↑ 3.7% MCV (males) ↓ 3.3% MCV (females) week 53, ↓ approx. 14% reticulocyte counts (males) weeks 27 and 53 ↑ 16.2 to 22.7 % platelet counts (females) *Clinical Chemistry*: ↑ urea 42.4% (males),78.7% (females) week 27 ↑ creatinine 217% (males),72.5% (females) week 53. *Urinalysis*: ↑ volume approx. 2-fold (males and females) week 27. ↑ ketones (males and females) not statistically significant at most intervals ↓ pH (males) at some time points ↑ epithelial cells and casts in urinary sediment (females) *Organs: 12 months:* ↑ 14/13% (males) absolute/ relative liver weights ↑ 30%/34% (females) absolute/relative liver weights in females ↑ 22.0-20.1% (males) absolute/ relative kidney weights ↑ 6.9/10.3% (females) absolute/ relative kidney weights *Histopathology: 12 months* Kidney - ↑ chronic progressive nephropathy (males) 7/10 grade 3.9 (control 1/10 grade 2.0); 3/10 (females, 0/10 control); ↑ renal tubular atrophy (females) 6/10 grade 1.8 (control 2/10, 1.0) ; ↑ severity renal pelvic dilatation (males) 9/10 mean grade 2.3 (control 8/10 grade 1.3) Epididymides - ↑ incidence and grading of mineralization of "clear cells" in the tail area of epididymides 9/10 grade 2.0 (control 6/10, 1.0) **250 mg/kg bw/day** *Survival*: Reduced survival in males; *Body weight gain*: ↓ 6.4 % (males) weeks 1-52 *Water intake*: ↑ 28.0 % (males) 31.5% (females) weeks 1-52 *Haematology*: ↓ 4-5% haemoglobin concentration (males and females), haematocrit (males and females), red blood cell count (females) week 53. *Urinalysis*: ↑ volume approx. 50% (males and females) week 53 *Histopathology: 12 months* Kidney - ↑ chronic progressive nephropathy (males) 6/10 grade 3.5 (control 1/10 grade 2.0); ↑ renal tubular atrophy (females) 5/10 grade 1.6 (control 2/10, 1.0)	

Method	Results	Reference
	100 mg/kg bw/day No treatment-related effects up to week 53. **10 mg/kg bw/day** No treatment-related effects.	
302/EEC B.26 (1987) GLP Oral, gavage, 90 day Mouse/CD 1 Range finding study for chronic study – no clinical chemistry investigations performed 10/sex/dose 0, 10, 100, 400, 700, 1000 mg/kg bw/day Pinoxaden technical - Batch No. EZ005006 (purity 97.2%) Vehicle: 0.5% carboxymethylcellulose, 0.1% Tween 80% **Relevant guidance value for 90-day rat study = 100 mg/kg bw/d**	**1000 mg/kg bw/day** *Clinical signs:* Piloerection (8/10 males, 5/10 females) *Bodyweight gain:* 66.6% ↓ males, 60.2% ↓ females (days 1-92). *Water consumption:* 19.6% ↑ males (weeks 1-13). *Haematology*: 7.1% ↓ females haemoglobin, 5.2% ↓ females RBC, 3.8% ↓ females haematocrit, 25.3% ↑ platelets., *Organ weights:* 26.9% ↑ males, 16.7% ↑ females (liver wt), 26.9% ↑ males, 23.9%↑ females (relative liver wt). *Histopathology:* Kidney - Renal tubular basophilia (4/10 males, 2/10 females). **700 mg/kg bw/day** *Clinical signs:* Piloerection (3/10 females) *Haematology:* 5.0% ↓ females haemoglobin, 5.7% ↓ females RBC, 2.8% ↓ females haematocrit. *Organ weights:* 17.0% ↑ males, 14.7% ↑ females (abs liver wt), 16.2% ↑ males, 17.3%↑ females (relative liver wt). *Histopathology:* Kidney - Renal tubular basophilia (4/10 males, 1/10 females). **400 mg/kg bw/day** *Clinical signs:* Piloerection (6/10 females) *Haematology:* 6.1% ↓ females haemoglobin, 4.7% ↓ females RBC, 3.3% ↓ females haematocrit. **100 and 10 mg/kg bw/day** No adverse effects noted. NOAEL[$] 100 mg/kg bw/day	DAR B.6.3.2(a)

Method	Results	Reference
No applicable guideline (Range-finding study) GLP Oral, gavage (capsule), 28 day with assessment of toxicokinetic parameters Dog/Beagle 1/sex/dose 250, 500, 1000 mg/kg bw/day Pinoxaden technical - Batch No. EZ0050006 (purity 97.2%) **Relevant guidance value for 28-day rat study = 300 mg/kg bw/d**	**1000 mg/kg bw/day** *Clinical signs:* salivation/resistance to dosing (male and female); dehydration, cold to touch, pale, thin appearance (female). *Food consumption:* Slightly ↓ compared to pre-treatment (males and females). *Haematology:* ↑ WBC, ↑ neutrophil (d 15 & 18) compared to pre-treatment (males and females). ↓ activated partial thromboplastin time (d 28) compared to pre-treatment (females). *Clinical chemistry:* ↑ ALP compared to pre-treatment (females). Small ↓ cholesterol, albumin, total protein, GGT compared to pre-treatment (males and females). *Histopathology:* lymphoid hyperplasia in mesenteric lymph node (1/1 males, 1/1 females), in Peyer's patches (1/1 males). **500 mg/kg bw/day** *Clinical signs:* salivation/resistance to dosing, thin appearance (male and female), decreased activity, salivation, pale (male), pale coloured gums and tongue (female). *Food consumption:* Slightly ↓ compared to pre-treatment (males). *Haematology:* ↑ WBC, ↑ neutrophil (d 15 & 18) compared to pre-treatment (males and females). ↓ activated partial thromboplastin time (d 28) compared to pre-treatment (males and females). *Clinical chemistry:* ↑ ALP compared to pre-treatment (males and females). Small ↓ cholesterol, albumin, total protein, GGT compared to pre-treatment (males and females). *Histopathology:* Lymphoid hyperplasia in mesenteric lymph node (1/1 males 1/1 female), in Peyer's patches (1/1 male, 1/1 female), in cervical lymph nodes (1/1 male, 1/1 female). **250 mg/kg bw/day** *Food consumption:* Slightly ↓ compared to pre-treatment (males). *Haematology:* ↑ WBC, ↑ neutrophil (d 15 & 18) compared to pre-treatment (males and females). ↓ activated partial thromboplastin time (d 28) compared to pre-treatment (males and females). *Clinical chemistry:* ↑ ALP compared to pre-treatment (males and females). Small ↓ cholesterol, albumin, total protein, GGT compared to pre-treatment (males and females). *Histopathology:*	2003a DAR B.6.3.3(a)

Method	Results	Reference
	lymphoid hyperplasia in mesenteric lymph node (1/1 male, 1/1 female), in Peyer's patches (1/1 male, 1/1 female), in cervical lymph nodes (1/1 male, 1/1 female).	
OECD 409 GLP Oral, gavage (capsule), 90 day Dog/Beagle 4/sex/dose 0, 25, 100, 250, 500 mg/kg bw/day Pinoxaden technical - Batch No.EZ005006 (purity 97.2%) **Relevant guidance value for 90-day rat study = 100 mg/kg bw/d**	**500 mg/kg bw/day** *Mortality:* 1/4 male (killed wk 13), 4/4 female (killed wk 5) due to reduced food consumption and body weight loss. *Clinical signs:* Gastro-intestinal effects: salivation at dosing (3males, 3females), retching (2 males, 4females), fluid faeces (4 males, 4 females), vomit (4males, 2females), mucus in faeces (3males), regurgitation (4males, 3females), Other effects: pale/cold ears/mouth/tongue (3females), cold to touch (1 male), dehydrated (1male), activity decreased (1male, 1female), thin appearance (3males, 2females). *Bodyweights:* 4.3, 6.2% ↓ wks 4, 9 (males), 4.9% ↓ wk 4 (females). *Food consumption:* 14.9, 11.7% ↓ wks 1, 4 (males), 9.1, 52% ↓ wks 1, 4 (females). *Clinical chemistry:* Albumin 11.7, 11.9, 21.1% ↓ wks 4, 8, 13 (males); 18.4% ↓ wk 4 (females). Total protein 7.2, 9.9, 15.5% ↓ wks 4, 8, 13 (males), 8.6% ↓ wk 4 (females). Cholesterol 27.9% ↓ wk 4 (females). Triglycerides 46.5% ↑ wk 13 (males). ALP 26.1, 75.9, 127.2% ↑ wks 4, 8, 13 (males), 180.9% ↑ wk 4 (females). ALT 66.1% ↑ wk 4 (females). *Organ weights:* Liver 18.8% ↑ (males). *Histopathology:* Liver glycogen reduced (1/4 male, 2/4 female), increased apoptosis of liver (0/4 males, 1/4 females) thymic atrophy (0/4 male,1/4 female). **250 mg/kg bw/day** *Clinical signs:* Gastro-intestinal effects: fluid faeces (4males, 4females), mucus in faeces (2 males), regurgitation (3males, 1female), vomit (4males, 3females), salivation at dosing (1male, 2females), retching (1male), Other effects: pale/cold ears/mouth/tongue (1female), cold to touch (2males, 3females), dehydrated (2males, 1female), activity decreased (1male, 1female), thin appearance (1male, 1female). *Bodyweights:* 4.0, 4.9, 11.0% ↓ wks 4, 9, 14 (males, 1.6, 4.2% ↓ wks 9, 14 (females).	2003b DAR B.6.3.3(b)

Method	Results	Reference
	Food consumption: 5.4, 8.6% ↓ wks 1, 4 (males), 5.4, 13.0% ↓ wks 1, 4 (females). *Clinical chemistry:* Albumin 11.0, 13.2, 14.4% ↓ wks 4, 8, 13 (males); 10.2, 15.9, 19.8% ↓ wks 4, 8, 13 (female). Total protein 11.5% ↓ wk 13 (female). ALP 25.7, 84.3, 67.7% ↑ wks 4, 8, 13 (male), 47.6, 98.5, 125.6% ↑ wks 4, 8, 13 (female). **100 mg/kg bw/day** *Clinical signs:* Gastro-intestinal effects: fluid faeces (4males, 4females), mucus in faeces (2males), vomit (2males, 3females), salivation at dosing (4males, 1female), retching (2males), *Clinical chemistry*: Albumin 15.7, 8.4, 8.0% ↓ wks 4, 8, 13 (male). ALP 5.2, 52.4, 70.1% ↑ wks 4, 8, 13 (male), 57.3, 61.3, 82.8% ↑ wks 4, 8, 13 (female). **25 mg/kg bw/day** *Clinical chemistry:* Albumin 51.7, 93.9, 87.2% ↑ wks 4, 8, 13 (female). In the absence of any other effects and histopathological findings, this slight increase in albumin in females only was not considered adverse. NOAEL[$] is 25 mg/kg bw/day;	
OECD 452 GLP Oral, gavage (capsule) 1 year Dog/Beagle 4/sex/dose 0, 5, 25, 125 mg/kg bw/day Pinoxaden technical - Batch No.EZ005006 (purity 97.2%)	**125 mg/kg bw/day** *Clinical sign:* Gastro-intestinal effects: ↑salivation at dosing (42 observations in males, 47 observations in females), ↑vomit (23 observations in males, 13 observations in females), ↑mucus in faeces (10 observations in males, 6 observations in females), ↑fluid faeces 206 observations in males, 203 observations in females). *Clinical chemistry:* Albumin 6.3, 4.5, 6.1% ↓ wks 13, 26, 52 (males), 7.6, 11.6, 6.2% ↓ wks 13, 26, 52 (females). Total protein 5.1, 3.9, 2.0% ↓ wks 13, 26, 52 (males), 4.5, 5.1, 5.3% ↓ wks 13, 26, 52 (females). Bilirubin 19.9% ↓ wk 52 (males). ALP 18.4, 34.1, 75% ↑ wks 13, 26, 52 (males), 55.0, 58.7, 111.6% ↑ wks 13, 26, 52 (females). GGT 8.8% ↑ wk 52 (females). **25 mg/kg bw/day** *Clinical signs:* Gastro-intestinal effects - ↑salivation at dosing (4 observations in males, 11 observations in females), ↑ vomit (4 observations in males, 5 observations in females), ↑ fluid	2003c DAR B.6.3.3(c)

Method	Results	Reference
	faeces (33 observations in males, 3 observations in females) *Clinical chemistry:* Albumin 3.4, 2.6, 4.0%↓ wks 13, 26, 52 (males), 3.4, 2.6, 4.0% ↓ wks 13, 26, 52 (females). As at this dose level the incidences of the gastro-intestinal effects were low and often comparable to those observed in controls and the isolated decreases in albumin were within or close to the historical control ranges and not accompanied by organ weight changes and histopathology findings, these effects were not considered to be adverse. **5 mg/kg bw/day** No adverse effects noted. NOAEL[$] is 25 mg/kg bw/day.	

[$] = As given in the DAR

4.7.1 Non-human information

4.7.1.1 Repeated dose toxicity: oral

Rat

28 day gavage (2001a)

In a guideline 28-day gavage study in the rat, treatment with pinoxaden resulted in reduced body weight gain and slight leukocytosis in high dose males (1000 mg/kg bw/day). Urinalysis findings (increased volume and ketones), increased water intake and histopathology investigations revealed the kidney as a target organ at high dose levels (600 and 1000 mg/kg bw/day) in males and females. The kidney toxicity was characterized by increased relative kidney weights together with renal tubular dilatation and proximal tubular atrophy accompanied by single cell necrosis of tubular epithelial cells.

There were toxicologically relevant changes to liver weights from 600 mg/kg bw/day. The only histopathological change noted in the liver (increased glycogen deposition) was considered not to be adverse. Decreased plasma albumin seen in females at all treated levels was an isolated finding and of no toxicological relevance.

The NOAEL was considered to be 300 mg/kg bw/day in males and females based on clear histopathology damage in the kidneys and increased liver weights.

90 day gavage with 28 day recovery (2001b)

In a guideline 90-day gavage study in the rat, the only effects noted were slightly higher water intake levels and changes in a limited number of clinical chemistry (increased urea and creatinine, decreased glucose, protein and albumin) and urinalysis parameters (increased volume and ketones, decreased pH) at the top dose of 300 mg/kg bw/day in both sexes. Urinalysis parameters (increased ketones and decreased pH) were also slightly affected in females at 100 mg/kg bw/d. These effects appeared reversible after a recovery period, and there were no corresponding histopathological findings noted.

Slightly increased liver weights were noted at 300 mg/kg bw/day in males and females. Liver weight increases at doses lower than 300 mg/kg bw/day were less than 110% of controls and were therefore considered not to be adverse.

As the changes in urine parameters seen at 100 mg/kg bw/day were minor and specific to females, they were not considered to be adverse. Hence the NOAEL in this study was considered to be 100 mg/kg bw/day.

90 day dietary study with a 28 day interim kill and FOB (2003a)

In a guideline 90-day dietary study in the rat, administration of pinoxaden at the top dose of 10000 ppm (equivalent to 900 and 965 mg/kg bw/day in males and females, respectively) resulted in lower body weights, reduced food consumption, increased water intake and microscopic changes of the kidney (cortical tubular basophilia/dilatation/atrophy and renal cysts) in both sexes. At this dose, there were also some slight but statistically significant changes in haematological parameters in females (reductions in mean haemoglobin level, haematocrit and red blood cell count) and urinary volume was significantly higher in females. In addition, significant decreases in cholesterol and AST activities were noted in both sexes.

Animals fed diets containing 5000 ppm pinoxaden showed lower body weights during the first few weeks of treatment; however, the differences from controls were slight (approx. 95% of control body weights). There were also statistically significant decreases in cholesterol in both sexes and a reduction in plasma AST in females.

As the changes observed at 5000 ppm pinoxaden were minor and not associated with any histopathological changes, the NOAEL was considered to be 5000 ppm, equivalent to 466/527 mg/kg bw/day in males and females respectively.

2-year rat chronic study: general toxicity and non-neoplastic findings following treatment for 12 months (2003)

A guideline 2-year gavage chronic toxicity study in the rat is available. Only the general toxicity and non-neoplastic findings observed following treatment with pinoxaden for 12 months are presented here. In this study, severe generalised toxicity was seen at 250 and 500 mg/kg bw/day. Survival was significantly reduced in males at 500 mg/kg bw/day; therefore, this group was terminated at week 61. Significantly reduced bodyweight gains and increased water intake were noted in both sexes at 250 and 500 mg/kg bw/day. Clinical signs of toxicity (hunched posture and piloerection) were seen in most males at 500 mg/kg bw/day.

At the interim kill, histopathology showed chronic progressive nephropathy in animals treated at 250 and 500 mg/kg bw/day, renal tubular atrophy in females only at 250 and 500 mg/kg bw/day and renal tubular dilatation in males and females at 500 mg/kg bw/day. Kidney toxicity was accompanied by changes in urinalysis (increased volume and ketones and decreased pH) and clinical-chemistry (increased urea and creatinine in plasma) parameters and by increased kidney weight, mostly occurring at the top dose of 500 mg/kg bw/day.

Other findings included haematological findings indicative of anaemia at 250 and 500 mg/kg bw/day in both sexes and increased incidence and grading of mineralization of “clear cells” in the tail area of the epididymides in the 500 mg/kg bw/day males.

Mouse

90 day gavage study (range-finding) (2002)

In a 90-day gavage range-finding study in CD-1 mice, animals dosed at 1000 mg/kg bw/day pinoxaden showed clinical signs of toxicity (piloerection), depressed bodyweight gain, higher water intake (in males) and kidney effects (minimal renal tubular basophilia). Kidney findings were also seen at 700 mg/kg bw/day and clinical signs of toxicity occurred from 400 mg/kg bw/day. For females dosed with 400 mg/kg bw/day and above, the haematological profile was altered (lower haemoglobin concentration, erythrocyte count, haematocrit and, at 1000 mg/kg bw/day, higher platelet count). Higher liver weights (> 110% of controls) were present at 700 and 1000 mg/kg bw/day.

Dog

28 day capsule dosing study (range finding with toxicokinetics) (2003a)

In a 28 day range finding study in the dog, capsule administration of pinoxaden at 500 and 1000 mg/kg bw/day resulted in salivation at dosing, resistance to dosing and an increased incidence of vomit and regurgitation; based on toxicokinetic investigations included in the study (see Part B Section 4.1.1) the very high incidence of vomiting at 1000 mg/kg bw/day appeared to limit systemic exposure, and would not give meaningful dose response data. At these two dose levels, there were also clinical signs of toxicity (dehydration, pale and thin appearance, decreased activity), effects on food consumption, on haematology and clinical-chemistry parameters and histopathological findings (lymphoid hyperplasia) of the lymph nodes. It was considered that 1000 mg/kg bw/day would not be tolerated in studies of longer duration.

At 250 mg/kg bw/day pinoxaden, effects were limited to reduced food consumption in the male dog during the last few days of the study, changes in haematology and clinical-chemistry parameters and histopathological findings (lymphoid hyperplasia) of the lymph nodes.

90 day capsule dosing study (2003b)

In a guideline 90 day study in the dog, capsule administration of pinoxaden at the top dose of 500 mg/kg bw/day was highly toxic to the animals, causing lethalities, reductions in body weight and food consumption, gastro-intestinal effects (salivation, vomit, fluid faeces, regurgitation), clinical signs of toxicity (thin appearance, decreased activity, dehydration), changes in clinical-chemistry parameters and histopathological findings of the liver (reduced glycogen and increased apoptosis) and thymus (atrophy in 1 female). With the exception of mortalities and histopathological findings of the liver and thymus, similar effects were also seen at 250 mg/kg bw/day.

At 100 mg/kg bw/day, effects were limited to gastro-intestinal symptoms and slight changes in two clinical-chemistry parameters (decreased albumin and increased ALP).

On the basis of these effects, a NOAEL of 25 mg/kg bw/day was established from this study.

1 year dog capsule dosing study (2003c)

In a guideline 1-year study in the dog, capsule administration of pinoxaden at the top dose of 125 mg/kg bw/day caused gastro-intestinal effects (salivation, fluid faeces, vomit, mucous in faeces) and changes in a number of clinical pathology parameters (decreased albumin, total protein and bilirubin, increased ALP and GGT).

At 25 mg/kg bw/day, effects were limited to low incidences of gastro-intestinal effects (often comparable to those seen in controls) and isolated decreases in albumin (within or close to historical

control ranges and not accompanied by any organ weight changes and histopathological findings). These effects were not considered adverse.

On this basis, a NOAEL of 25 mg/kg bw/day was established from this study.

4.7.1.2 Repeated dose toxicity: inhalation

No data available

4.7.1.3 Repeated dose toxicity: dermal

Table 15: Summary table of relevant dermal repeated dose toxicity studies

Method	Results	Reference
OECD 410 GLP Dermal, 28 day Rat HanBrl:WIST (SPF) albino 10 sex/dose 0, 10, 100 or 1000 mg/kg bw/day, 6 hours/day on days 1-4, 7-11, 14-18 and 21-28 for males and on days 1-3, 6-10, 13-17 and 20-28 for females. Pinoxaden technical - Batch No.EZ005006 (purity 97.2%)	**1000, 100, 10 mg/kg bw/day** No adverse effects noted. NOAEL[$] 1000 mg/kg bw/day.	2001 DAR B.6.3.4

[$] = As given in the DAR

In a guideline 28 day dermal toxicity study (2001) there was no treatment-related mortality and there were no signs of overt toxicity. Slight erythema formation was observed at the application site in 2 males and 3 females at 100 mg/kg bw/day and also in 2 females at 10 mg/kg bw/day but not at 1000 mg/kg bw/day. Given the lack of a dose-response relationship, these dermal effects were not considered treatment-related. A NOAEL of 1000 mg/kg bw/day was established from this study.

4.7.1.4 Human information

No data available.

4.7.1.5 Other relevant information

No data available.

4.7.1.6 Summary and discussion of repeated dose toxicity

The repeated dose toxicity of pinoxaden has been investigated via the oral route in standard studies in the rat (by gavage and dietary administration), mouse (by gavage) and dog (by capsule administration). A 28-day study via the dermal route in the rat is also available.

In the rat, the kidney is the main and most sensitive target organ of toxicity following oral administration of pinoxaden. Kidney effects (increased organ weight, increased water intake, tubular dilatation/atrophy and related changes in some urinalysis parameters) occurred from a gavage dose of 600 mg/kg bw/day for 28 days.

Preliminary signs of kidney toxicity (increased water intake and changes in some urinalysis and clinical-chemistry parameters) were also seen from a gavage dose of 300 mg/kg bw/day for 90 days.

By dietary administration, kidney effects (increased water intake, tubular basophilia/dilatation/atrophy, renal cysts and increased urine volume) were noted at the higher dose of 900/965 (m/f) mg/kg bw/day for 90 days. These changes were accompanied by effects on body weight, food consumption, clinical-chemistry and haematology parameters.

Kidney toxicity (increased water intake, tubular dilatation/atrophy, chronic progressive nephropathy and changes in related urinalysis and clinical-chemistry parameters) was also observed from a dose of 250 mg/kg bw/day after 12 months of gavage administration. Associated with these effects, there were changes in haematological parameters (in females) and decreases in body weight. In addition, lethality occurred at 500 mg/kg bw/day.

No systemic toxicity was seen by the dermal route up to the limit dose of 1000 mg/kg bw/day for 28 days.

In the mouse, the kidney is also one of the main target organs of toxicity following gavage administration of pinoxaden for 90 days. Kidney effects (increased water intake and tubular basophilia) were seen from a dose of 700 mg/kg bw/day. There were also haematological changes, indicative of anaemia in females from a dose of 400 mg/kg bw/day.

In the dog, severe generalised toxicity (gastro-intestinal effects, clinical signs of toxicity and decreases in food consumption) was noted at the high doses of 500 and 1000 mg/kg bw/day following capsule administration of pinoxaden for 28 days. In addition, changes in haematological and clinical-chemistry parameters and lymphoid hyperplasia of the mesenteric lymph nodes occurred from a dose of 250 mg/kg bw/day for 28 days.

Similar effects (gastro-intestinal effects, clinical signs of toxicity, decreases in food consumption and body weight and changes in clinical-chemistry parameters) were seen in the 90 day study from a dose of 250 mg/kg bw/day. In addition, mortality and effects on the liver (increased weight and a low incidence of histopathological findings) and thymus (low incidence of atrophy) occurred at the top dose of 500 mg/kg bw/day. However, at the lower dose of 100 mg/kg bw/day, effects were limited to gastro-intestinal symptoms and slight changes in clinical chemistry parameters.

Gastro-intestinal effects and minor changes in clinical chemistry parameters were also noted in the 1-year study at the top dose of 125 mg/kg bw/day.

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

The repeated dose toxicity of pinoxaden has been investigated via the oral route in standard studies in the rat (by gavage and dietary administration), mouse (by gavage) and dog (by capsule administration). A 28-day study via the dermal route in the rat is also available.

Classification with STOT- RE is triggered by the occurrence of *significant* (and/or *severe* for Category 1) toxic effects at doses below specified guidance values. For STOT-RE Category 2, the

relevant guidance values for oral exposure are 100 mg/kg bw/day (rat 90-day study) and 300 mg/kg bw/day (rat 28-day study).

As described in section 4.7.1.6 above, in the rat, the kidney is the main and most sensitive target organ of toxicity following oral administration of pinoxaden. Kidney effects (increased organ weight, histopathological findings, increased water intake and related changes in urinalysis and clinical-chemistry parameters) occurred from a gavage dose of 600 mg/kg bw/day for 28 days, from a gavage dose of 300 mg/kg bw/day for 90 days, at the top dietary dose of 900/965 (m/f) mg/kg bw/day for 90 days and from a gavage dose of 250 mg/kg bw/day for 12 months. Therefore, in the rat, significant toxic effects on the kidney are seen, but these occur at dose levels well in excess of the specified guidance values.

In the mouse, the kidney is also one of the main target organs of toxicity following gavage administration of pinoxaden for 90 days. Kidney effects (increased water intake and tubular basophilia) were seen from a dose of 700 mg/kg bw/day. In addition, there were haematological changes, indicative of anaemia, in females from a dose of 400 mg/kg bw/day. Therefore, in the mouse, significant toxic effects on the kidney and blood are seen, but these occur at dose levels well in excess of the specified guidance values.

In the dog, severe generalised toxicity (gastro-intestinal effects, clinical signs of toxicity and decreases in food consumption) was noted at the high doses of 500 and 1000 mg/kg bw/day following capsule administration of pinoxaden for 28 days. In addition, changes in haematological and clinical-chemistry parameters and lymphoid hyperplasia of the mesenteric lymph nodes occurred from a dose of 250 mg/kg bw/day for 28 days.

Similar effects (gastro-intestinal effects, clinical signs of toxicity, decreases in food consumption and body weight and changes in clinical-chemistry parameters) were seen in the 90 day study from a dose of 250 mg/kg bw/day. In addition, mortality and effects on the liver (increased weight and a low incidence of histopathological findings) and thymus (low incidence of atrophy) occurred at the top dose of 500 mg/kg bw/day. However, at the lower dose of 100 mg/kg bw/day, effects were limited to gastro-intestinal symptoms and slight changes in clinical chemistry parameters.

Gastro-intestinal effects and minor changes in clinical chemistry parameters were also noted in the 1-year study at the top dose of 125 mg/kg bw/day.

Therefore, in the dog, severe generalised toxicity, including mortality, clinical signs of toxicity and effects on liver pathology and thymus, is seen at the high dose levels of 500 and 1000 mg/kg bw/day for 28 and/or 90 days. Significant toxic effects on haematological and clinical-chemistry parameters and lymphoid hyperplasia of the mesenteric lymph nodes are also seen from a dose of 250 mg/kg bw/day for 28 and/or 90 days. Hence, severe and/or significant toxic effects occur in the dog at dose levels well in excess of the specified (rat) guidance values.

However, gastro-intestinal effects (episodes of salivation at dosing, vomit, fluid faeces, and mucus in faeces) and minor changes in clinical chemistry parameters were seen from a dose of 100 mg/kg bw/day for 90 days and at the top dose of 125 mg/kg bw/day for 1 year. Although these effects appear to occur at dose levels close to the specified (rat) 90-day guidance value of 100 mg/kg bw/day, in the absence of associated body weight reductions and histopathology findings in any organ, they are not regarded as significant toxic effects.

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

As described in section 4.7.2 above, in the rat, significant toxic effects on the kidney occur at dose levels well in excess of the specified guidance values. In the mouse, significant toxic effects on the kidney and blood also occur at dose levels well in excess of the specified guidance values.

In the dog, gastro-intestinal effects and minor changes in clinical chemistry parameters occur at dose levels close to the specified (rat) 90-day guidance value of 100 mg/kg bw/day. However, in the absence of associated body weight reductions and histopathology findings in any organ, these effects are not regarded as *significant* toxic effects in the context of STOT-RE classification.

On this basis, classification of pinoxaden with STOT-RE is not warranted.

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

No classification – conclusive but not sufficient for classification

4.8 Germ cell mutagenicity (Mutagenicity)

The genotoxicity of pinoxaden was investigated *in vitro* in one unscheduled DNA synthesis (UDS) assay, one bacterial reverse mutation (Ames) assay, one cell mutation assay (Tk +/- mouse lymphoma L5178Y cells) and two chromosome aberration assays (Chinese hamster V79 cells), and *in vivo* in a mouse micronucleus study and in a rat UDS assay.

Table 16: Summary table of relevant *in vitro* and *in vivo* mutagenicity studies

Method	Results	Remarks	Reference
Unscheduled DNA Synthesis *In vitro* OECD 482 GLP Primary hepatocytes (from male rats) 9.38 - 300 ug/mL Pinoxaden technical - Batch No. EZ005006 (purity 97.2%).	Negative	Positive controls included; Cytotoxicity observed at higher concentrations;	2001 DAR B.6.4.1(a)
Bacterial reverse mutation *In vitro* OECD 471 GLP *Salmonella typhimurium* (TA 1535, TA 1537, TA 98, TA 100, TA 102) and *Escherichia coli* WP2 uvrA. 33 – 5000 µg/plate Pinoxaden technical - Batch No. EZ005006 (purity 97.2%).	+ S9: Negative - S9: Negative	Positive controls included; Tested up to the limit concentration;	2001 DAR B.6.4.1(b)

Method	Results	Remarks	Reference
Cell mutation *In vitro* OECD 476 GLP Thymidine Kinase Locus (Tk +/-) mouse lymphoma L5178Y cells 6.3 – 400 µg/mL (-S9) 6.3 – 150 µg/mL (+S9) Pinoxaden technical - Batch No. EZ005006 (purity 97.2%).	+ S9: Negative - S9: Negative	Concentration limited by cytotoxicity. Expt I: +/- S9, 4h Expt II: -S9, 24h Expt III: +S9, 4h Positive controls included	2003 DAR B.6.4.1(c)
Chromosome aberration *In vitro* OECD 473 GLP Chinese hamster V79 cells 20 – 125 µg/mL Pinoxaden technical - Batch No. EZ005006 (purity 97.2%).	+ S9: Positive - S9: Positive	Concentration limited by cytotoxicity. Expt I: +/- S9 4h exposure, 18h harvest Expt II: -S9 18h exposure, 18h harvest, and also 28h exposure, 28h harvest; +S9 4h exposure, 28h harvest Expt III: -S9 18h exposure, 18h harvest; +S9 4h exposure, 28h harvest Positive controls included.	2001 DAR B.6.4.1(d)
Chromosome aberration OECD 473 GLP *In vitro* Chinese hamster V79 cells 15 – 100 µg/mL Pinoxaden technical - Batch No. AMS 1055/2 (purity 99.5%).	+ S9: Positive - S9: Positive	Concentration limited by cytotoxicity. Expt I: +/-S9 4h exposure, 18h harvest; Expt II: +S9 4h exposure, 28h harvest; -S9 18h exposure & harvest, also 28h exposure, 28h harvest. Positive controls included.	2002 DAR B.6.4.1(e)
Micronucleus *In vivo* OECD 474 GLP Oral gavage Mouse/NMRI 5/sex/group 0, 500, 1000, 2000 mg/kg bw Vehicle: 40% ethanol in PEG. Pinoxaden technical - Batch No. EZ005006 (purity 97.2%).	Negative	Sampling time 24, 48h. Positive controls included. P/N ratio decreased at 2000 mg/kg bw, indicating bone marrow cytotoxicity.	2001 DAR B.6.4.2(a)

Method	Results	Remarks	Reference
Unscheduled DNA synthesis *In vivo* OECD 486 GLP Oral gavage Rat/Alpk:APfSD (3 males test, 1 male vehicle control, 1 male positive control) 0, 2000 mg/kg bw Vehicle: 0.5% carboxymethyl cellulose Pinoxaden technical - Batch No. EZ005006 (purity 97.2%).	Negative	Positive controls included; Negative up to the limit dose of 2000 mg/kg bw.	2002 DAR B.6.4.2(b)

4.8.1 Non-human information

4.8.1.1 *In vitro* data

The *in vitro* genotoxicity of pinoxaden was investigated in one unscheduled DNA synthesis assay, one bacterial reverse mutation (Ames) assay, one cell mutation assay (Tk +/- mouse lymphoma L5178Y cells) and two chromosome aberration assays (Chinese hamster V79 cells). Positive controls were included in all assays and behaved as expected in all assays.

Unscheduled DNA Synthesis (2001)

In a guideline unscheduled DNA synthesis (UDS) assay, a concentration dependent decrease in the number of nuclear and cytoplasm grain counts was observed up to the highest concentration tested, due to cytotoxicity. The calculation of net nuclear grain counts was consistently negative and there was no substantial shift to higher values in the percentage distribution of nuclear grain counts. It was concluded that under the test conditions, pinoxaden did not induce increased DNA repair synthesis in rat hepatocytes, up to cytotoxic concentrations.

Bacterial Reverse Mutation (2001)

In a guideline Bacterial Reverse Mutation assay, cytotoxic effects (evident as a reduction in the number of revertants) were observed with and without metabolic activation in strain TA 100 and in strain TA 98 with metabolic activation in experiment 1, and in strains TA 1537 and TA 100 with and without metabolic activation, and in strain TA 102 without metabolic activation in experiment II. The plates incubated with the test item showed normal background growth up to 5000 µg/plate with and without metabolic activation in both independent experiments. No substantial increase in revertant colony numbers of any of the six tester strains was observed following treatment with pinoxaden at any concentration, either in the presence or absence of metabolic activation (S9 mix). It was concluded that under the test conditions, pinoxaden was non-mutagenic up to the limit concentration of this assay.

Mammalian Cell Mutation (2003)

A guideline Cell Mutation Assay using the thymidine kinase Locus (Tk +/-) in mouse lymphoma L5178Y cells was conducted to assess pinoxaden's ability to induce gene mutations or clastogenic effects in mammalian cells. The concentration range of the main experiments was limited by cytotoxicity of the test item.

No substantial and reproducible dose dependent increase in mutant frequency exceeding the historical range of negative and solvent controls was observed after 4 h of treatment in the presence and absence of metabolic activation. The threshold of twice the colony count of the corresponding solvent control was reached in the first culture at 400 µg/ml and exceeded in the second culture at 100 µg/ml. Since in both cultures the observed effects were weak, occurred at single concentrations only, could not be reproduced and did not show a dose-dependency, the increased mutant frequencies were considered to be attributable to spontaneous events rather than to mutagenic activity of the test item itself.

It was concluded that under the experimental conditions, pinoxaden was non-mutagenic up to cytotoxic concentrations.

Chromosome Aberration Tests (2001;2002)

Two *in vitro* guideline cytogenetic assays using Chinese hamster V79 cells were conducted, one using technical samples and the other analytical samples of pinoxaden.

In the first assay, technical pinoxaden (purity 97.2%.) was evaluated for clastogenic potential in a series of independent *in vitro* cytogenetic experiments, using Chinese hamster V79 cells, treated in the presence and absence of a rat liver-derived metabolic activation system (S9) (2001). The cells were exposed to pinoxaden over the concentration range 20 – 125 µg/ml, the highest concentration being limited by the cytotoxicity of the test material.

Statistically significant and biologically relevant increases in the number of cells carrying structural chromosomal aberrations were observed after treatment with the test item with and without S9 treatment. It was concluded that pinoxaden was clastogenic in this test in the absence and presence of S9.

In the second assay, analytically pure pinoxaden (99.5% pure) was evaluated for clastogenic potential in a series of independent *in vitro* cytogenetic experiments, using Chinese hamster V79 cells, treated in the presence and absence of a rat liver-derived metabolic activation system (S9) (2002). The cells were exposed to pinoxaden over the concentration range 15 – 100 µg/ml, the highest concentration being limited by the cytotoxicity of the test material.

In the absence and the presence of S9, statistically significant and biologically relevant increases in the number of cells carrying structural chromosomal aberrations were observed after treatment with the test item. In conclusion, pinoxaden was considered to be clastogenic in this test in the absence and presence of S9.

4.8.1.2 *In vivo* data

The *in vivo* genotoxicity of pinoxaden was investigated in one micronucleus study in the mouse and one rat liver unscheduled DNA synthesis (UDS) assay.

Mouse Micronucleus (2001)

In a guideline study, technical pinoxaden (97.2% pure) was evaluated for its ability to induce micronuclei in bone marrow polychromatic erythrocytes in orally dosed NMRI mice.

A small but statistically significant ($p<0.05$) increase (double the control value) in the incidence of micronucleated polychromatic erythrocytes was observed at the lowest (500 mg/kg bw) dose level at the 24 hour sampling time. As the value obtained was within the historical control range for the laboratory, and there was no increase over controls at either the 1000 or 2000 mg/kg bw dose levels, the small increase observed at 500 mg/kg was considered not to be biologically significant. A reduction in the P/N ratio was observed at the top dose, indicating bone marrow cytoxicity.

It was concluded that under the experimental conditions reported, the test item did not induce micronuclei up to a dose causing bone marrow cytotoxicity.

In Vivo Rat Liver Unscheduled DNA Synthesis Assay (2002)

In a guideline study, technical pinoxaden (97.2% pure) was tested for the ability to induce unscheduled DNA synthesis (UDS) in the liver of Alpk:APfSD rats, using an autoradiographic technique. The dose level used, 2000 mg/kg bw, was administered by oral gavage.

No adverse reactions to treatment were observed for animals dosed with pinoxaden. Evaluation of the mean net nuclear grain count and percentage of cells in repair showed that pinoxaden did not induce DNA repair, as measured by UDS, at a limit dose of 2000 mg/kg bw. It was concluded that under the test conditions, pinoxaden did not induce DNA repair in the rat liver *in vivo*.

4.8.2 Human information

No information available.

4.8.3 Other relevant information

No further relevant information.

4.8.4 Summary and discussion of mutagenicity

The mutagenic potential of pinoxaden has been examined in a range of guideline *in vitro* and *in vivo* assays.

In *in vitro* assays for gene mutations, pinoxaden was negative in both bacterial and mammalian cells (L5178Y mouse lymphoma). Pinoxaden was also negative for DNA damage/repair when assessed in isolated rat hepatocytes.

Two *in vitro* cytogenetic assays using Chinese hamster V79 cells were conducted, one using technical and the other analytical grade of pinoxaden. Both studies were positive with increased incidences of chromosomal aberrations in both the absence and presence of metabolic activation. These increases were associated with cytotoxicity. There was no evidence of significant clastogenic activity in the mammalian cell gene mutation assay from the analysis of small colonies.

In vivo, pinoxaden was non-clastogenic in the mouse bone marrow micronucleus assay up to a dose (2000 mg/kg bw) causing bone marrow cytotoxicity. There was no evidence of DNA damage in rat liver in a UDS assay conducted at the limit dose of 2000 mg/kg bw.

Overall, it can be concluded that although pinoxaden was clastogenic *in vitro*, this activity was not expressed *in vivo.*

4.8.5 Comparison with criteria

Substances can be classified in Category 1A, 1B or 2 for germ cell mutagenicity. For Category 1 A and B, the substance should be known to induce heritable changes or be regarded as if it will induce heritable changes in germ cells of humans. This is based on human data or positive results from *in vivo* studies in animals. There are no human data or positive results *in vivo* to suggest that pinoxaden causes heritable mutations and therefore is not a Category 1A or Cat 1B mutagen.

For Category 2, the substance is regarded to cause concern for humans owing to the possibility that it may induce heritable mutations in the germ cells of humans. Classification is based on positive results in mammals and /or, in some cases, in *in vitro* experiments with supporting information from other in vivo studies or chemical structure activity relationship to known germ cell mutagens. For pinoxaden, although a positive result for clastogenicity was obtained *in vitro*, this activity was not expressed *in vivo.* Therefore, the criteria for classification are not met and it is proposed not to classify pinoxaden as a germ cell mutagen.

4.8.6 Conclusions on classification and labelling

No classification – conclusive but not sufficient for classification

4.9 Carcinogenicity

4.9.1 Non-human information

4.9.1.1 Carcinogenicity: oral

The carcinogenic potential of pinoxaden has been investigated by the oral route in one rat study and 2 mouse bioassays (one by gavage and the other by dietary administration). There are also 2 mechanistic investigations conducted in the mouse to address the lung effects seen in the first mouse gavage bioassay.

Table 17: Summary table of relevant carcinogenicity studies

<table>
<tr><th>Method</th><th>Results
Remarks</th><th>References</th></tr>
<tr>
<td>2 year chronic toxicity/ carcinogenicity
OECD 453 (1981)
GLP
Oral, Gavage/ vehicle 0.5% CMC, 0.1% Tween 80

<u>Rat,</u> Wistar Hanlbm:WIST (SPF)

Doses: 0, 1, 10, 100, 250, or 500 mg/kg bw/day

Total of 90 animals/sex/group

60/sex/group: main 2-yr study

10/sex/group: interim 12-month sacrifice

20/sex/group: haematology and clinical-chemistry investigations (24 months)

pinoxaden technical; Batch No. EZ005006 (97.2 % purity)

500 mg/kg bw/day male group terminated at week 61

Increased mortality in males at 500 and 250 mg/kg bw/day. BUT number of survivors at 1, 10 or 100 mg/kg bw/day (3 dose levels) was similar to control group and therefore the study is acceptable.</td>
<td>
Neoplastic findings:
Leiomyosarcoma of the stomach in males: 2/60 (3.3%) at 250 mg/kg bw/day vs 0/59 in controls.
Hepatocellular adenoma was present in females: 5/59 (8%) at 500 mg/kg bw/day vs 2/60 (3.3%) in controls
Endometrial adenocarcinoma was noted in the uterus: 2/59 (3.3%), 3/60 (5%) and 4/59 (7%) at 100, 250 and 500 mg/kg bw/day respectively vs 1/60 (1.6%) in controls
Tumour incidences
<table>
<tr><th rowspan="2">Tumours</th><th colspan="6">Males (mg/kg bw/day)</th></tr>
<tr><th>0</th><th>1</th><th>10</th><th>100</th><th>250</th><th>500</th></tr>
<tr><td>Stomach No examined</td><td>59</td><td>60</td><td>60</td><td>59</td><td>60</td><td>-</td></tr>
<tr><td>Leiomyosarcoma</td><td>0</td><td>0</td><td>0</td><td>0</td><td>2 (3.3%)</td><td>-</td></tr>
<tr><td>Lab Historical control</td><td colspan="6">0.0 % (range 0.0 to 0.0 %) from 5 studies</td></tr>
<tr><th rowspan="2">Tumours</th><th colspan="6">Females (mg/kg/day)</th></tr>
<tr><th>0</th><th>1</th><th>10</th><th>100</th><th>250</th><th>500</th></tr>
<tr><td>Stomach No examined</td><td>60</td><td>60</td><td>59</td><td>60</td><td>60</td><td>59</td></tr>
<tr><td>Leiomyosarcoma</td><td>0</td><td>0</td><td>1</td><td>0</td><td>0</td><td>0</td></tr>
<tr><td colspan="7"></td></tr>
<tr><td>Liver No. examined</td><td>60</td><td>60</td><td>60</td><td>59</td><td>60</td><td>-</td></tr>
<tr><td>Hepatocellular adenoma</td><td>3</td><td>0</td><td>2</td><td>2</td><td>1</td><td>-</td></tr>
</table>
<table>
<tr><th rowspan="2">Tumours</th><th colspan="6">Females (mg/kg bw/day)</th></tr>
<tr><th>0</th><th>1</th><th>10</th><th>100</th><th>250</th><th>500</th></tr>
<tr><td>Stomach No examined</td><td>60</td><td>60</td><td>59</td><td>60</td><td>60</td><td>59</td></tr>
<tr><td>Leiomyosarcoma</td><td>0</td><td>0</td><td>1</td><td>0</td><td>0</td><td>0</td></tr>
<tr><td colspan="7"></td></tr>
<tr><td>Liver No. examined</td><td>60</td><td>60</td><td>59</td><td>60</td><td>60</td><td>59</td></tr>
<tr><td>Hepatocellular adenoma</td><td>2(3.3%)</td><td>0</td><td>0</td><td>0</td><td>2</td><td>5(8%)</td></tr>
<tr><td>Lab Historical control</td><td colspan="6">3.2 % (range 0.0 to 8.0 %) from 5 studies</td></tr>
<tr><td colspan="7"></td></tr>
<tr><td>Uterus No. examined</td><td>60</td><td>59</td><td>60</td><td>59</td><td>60</td><td>59</td></tr>
<tr><td>Endometrial adenocarcinoma</td><td>1 (1.6%)</td><td>0</td><td>0</td><td>2 (3.3%)</td><td>3 (5%)</td><td>4 (7%)</td></tr>
<tr><td>Lab Historical control</td><td colspan="6">4.5 % (range 0.0 to 8.2 %) from 5 studies</td></tr>
</table>
</td>
<td>2003
DAR B.6.5.1(a)</td>
</tr>
</table>

Method	Results Remarks	References
	See section 4.7.1.1 for details of generalised toxicity and non-neoplastic findings at 12 months ***Generalised toxicity and non-neoplastic findings at 24 months:*** **500 mg/kg bw/day** *Survival*: 24/90 (males) died by week 53 (3/90 control). Terminated week 61. Survival rate: 58.33% (females, control 71.67%) week 104. *Clinical signs*: ↑ hunched posture and piloerection (males), usually noted for the first time within a week of death/moribund sacrifice. *Bodyweight*: ↓ 23% gain (females) weeks 1-104 – males terminated *Water intake*: ↑ 63% (females) weeks 1-104 – males terminated *Haematology*: ↓ 7% haemoglobin (females) week 105 – males terminated ↓ 7% haematocrit (females) week 105 – males terminated ↓ 3.5% MCV (females) week 105 – males terminated ↑ 23 % platelet counts (females) week 105 – males terminated *Urinalysis*: ↑ volume 77% (females) week 105 – males terminated. ↑ ketones 450% (females) week 105 –males terminated. *Organs:* ↑ 17/33% (females) absolute/ relative liver weights, week 104 – males terminated ↓15/6% (females) absolute/ relative kidney weights, week 104 – males terminated *Histopathology: 24 months* Kidney - ↑ chronic progressive nephropathy (females) – males terminated ↑ renal tubular atrophy (females) – males terminated ↑ severity renal pelvic dilatation (females) – males terminated ↑ renal cysts (females) – males terminated **250 mg/kg bw/day** *Survival* Survival rate: 38.33% (males, control 71.67%) week 104. *Clinical signs*: ↑ hunched posture and piloerection (males), usually noted for the first time within a week of death/moribund sacrifice. *Bodyweight*: ↓ 13% gain (females), 13% (males) wk 1-104 *Water intake:* ↓ 37.5% (females), 42% (males) wk 1-104 *Urinalysis*: ↑ volume 67% (males), 46% (females) week 105 *Organs:* ↑ 8/18% (females) absolute/ relative liver weights, week 104 ↓15/9% (females) absolute/ relative kidney weights, week 104	

<table>
<tr><th>Method</th><th>Results
Remarks</th><th>References</th></tr>
<tr><td></td><td>
Histopathology: 24 months
Kidney - ↑ chronic progressive nephropathy (males and females)
↑ renal tubular atrophy (females)
↑ severity renal pelvic dilatation (females)

100 mg/kg bw/day
Histopathology: 24 months
Kidney - ↑ renal tubular dilatation (males and females)
↑ renal tubular vacuolation (females)

10 mg/kg bw/day
No treatment-related effects.

NOAEL$ (toxicity) 10 mg/kg bw/day
NOAEL$ (carcinogenicity) 500 mg/kg bw/day
</td><td></td></tr>
<tr><td>18 month carcinogenicity

OECD 451 (1981), (Acceptable. Deviations from OECD 451 (1981): at scheduled terminal sacrifice, male survival rates were 47% and 47% in the 300 and 40 mg/kg bw/day groups respectively, compared to 83% in the control group. These survival rates were considered sufficient to evaluate the carcinogenic potential of pinoxaden).

Ophthalmology and clinical chemistry investigations not conducted

GLP

Oral, Gavage/ vehicle 0.5% CMC, 0.1% Tween 80

Mouse Crl:CD-1(ICR) BR albino

70/sex/group</td><td>
Neoplastic findings
<table>
<tr><th rowspan="2">Tumour type</th><th colspan="5">Males (mg/kg bw/day)</th></tr>
<tr><th>0</th><th>5</th><th>40</th><th>300</th><th>750</th></tr>
<tr><td>Lungs examined</td><td>70</td><td>70</td><td>69</td><td>70</td><td>69</td></tr>
<tr><td>Lung adenomas</td><td>8
(11.4%)</td><td>4
(5.7%)</td><td>4
(5.7%)</td><td>11
(15.7%)</td><td>10↑
(14.3%)</td></tr>
<tr><td>Lab Historical control</td><td colspan="5">10.0% (range 6.0 to 14.0%) from 5 dietary studies</td></tr>
<tr><td>Lung carcinomas</td><td>3
(4.3%)</td><td>5
(7.1%)</td><td>8↑*
(11.4%)</td><td>9↑*
(12.9%)</td><td>5↑
(7.1%)</td></tr>
<tr><td>Lab Historical control</td><td colspan="5">8.8 % (range 2.0 to 12.0%) from 5 dietary studies</td></tr>
<tr><td>Combined</td><td>11
(16%)</td><td>9
(13%)</td><td>11
(16%)</td><td>18↑*
(26%)</td><td>12↑
(17%)</td></tr>
<tr><th rowspan="2">Tumour type</th><th colspan="5"></th></tr>
<tr><th colspan="5">Females (mg/kg bw/day)</th></tr>
<tr><th></th><th>0</th><th>5</th><th>40</th><th>300</th><th>750</th></tr>
<tr><td>Lungs examined</td><td>70</td><td>70</td><td>70</td><td>70</td><td>70</td></tr>
<tr><td>Lung adenomas</td><td>5
(7.1%)</td><td>5
(7.1%)</td><td>1
(1.4%)</td><td>10
(14.3%)</td><td>4
(5.7%)</td></tr>
<tr><td>Lab Historical control</td><td colspan="5">4.8% (range 2.0 to 8.0%) from 5 dietary studies</td></tr>
<tr><td>Lung carcinomas</td><td>5
(7.1%)</td><td>4
(5.7%)</td><td>8
(11.4%)</td><td>0
(0%)</td><td>6
(8.6%)</td></tr>
<tr><td>Lab Historical control</td><td colspan="5">3.6% (range 0.0 to 6.0 %) from 5 dietary studies</td></tr>
<tr><td>Combined</td><td>10
(14%)</td><td>8
(11%)</td><td>9
(13%)</td><td>10
(14%)</td><td>10
(14%)</td></tr>
</table>
All statistical analyses were conducted with correction for survival:
↑= Statistically significant positive trend (2p ≤ 0.05, Peto test)
*= Statistically significant pairwise comparison to control (2p ≤ 0.05, Peto test)
</td><td>2003
DAR
B.6.5.2(a)</td></tr>
</table>

Method	Results Remarks	References
Doses: 0, 5, 40, 300 and 750 mg/kg bw/day pinoxaden technical; Batch No. EZ005006 (97.2 % purity)	***Generalised toxicity and non-neoplastic findings*** **750 mg/kg bw/day** *Survival:* Survival rate: 47% (males) 63% (females) at 18 months (vs 83% males, 76% females in controls) due to deaths – most of them were accidental (see below) ↑ incidence of 'accidental deaths' in the study. Majority of accidental deaths were later confirmed by macro- and histopathology to be associated with effects on the respiratory tract. *Clinical signs* Tonic convulsion (males); piloerection (males and females); hunched posture (females). *Bodyweight*: ↓ 11% (males), 20% (females) at 18 months. ↓ bodyweight gain 51% (males), 84% (females) weeks 1-78 *Food efficiency:* ↓ in males and females *Water intake*: ↑ 24% (males); 31% (females); *Haematology* ↑ 28% platelet count (males) *Organ weights:* ↑ 23% liver abs and 43% rel (males); ↑ 14% liver abs and 42% rel (females); ↑ 22% kidney rel (females); *Gross pathology* Lung - ↑ incidence of foamy outflow from the bronchi (males and females) in animals that died or were sacrificed intercurrently *Microscopic findings* Lung - ↑ hyalinosis 20/70 (males); 19/70 (females); (controls: 2 males, 5 females) ; ↓ phagocytic cells 6/70 (males); 12/70 (females) (controls: 23 males, 22 females); Combined incidence of hyalinosis and phagocyte cells: 37% (males) 44% (females) (controls: 36% males, 39% females). These findings were considered to reflect direct exposure of the lungs to the test material through gavage dosing/mis-dosing (see mechanistic investigations). Liver - ↑ glycogen deposition 51/70 males (33/70 control), 57/70 females, severity 2.3 (control 50/70, severity 1.5) ***300 mg/kg bw/day*** *Survival*: Survival rate: 47% (males), 66% (females) (vs 83% males, 76% females in controls) due to deaths – most of them were accidental (see below) ↑ incidence of 'accidental deaths'. Majority later confirmed by macro- and histopathology to be associated with effects on the respiratory tract. *Body weight*: ↓ (7%) (females) at 18 months ↓ 38% (females) body weight gain (week 1 to 78) *Haematology* ↑ 20% platelet count (males) *Organ weights:*	

<table>
<tr><th>Method</th><th>Results
Remarks</th><th>References</th></tr>
<tr><td></td><td>↑ 10% liver abs and 14% rel (males); ↑ 6% liver abs and 14% rel (females);
Gross pathology
Lung - ↑ incidence of foamy outflow from the bronchi (males and females) in animals that died or were sacrificed intercurrently
Microscopic findings
Lung - ↑ hyalinosis 8/70 (males); 6/70 (females); (controls: 2 males, 5 females); ↓ phagocytic cells 20/70 (males); 15/70 (females) (controls: 23 males, 22 females); Combined incidence of hyalinosis and phagocytic cells: 29% (males) 30% (females) (vs control 36% males, 39% females).
These findings were considered to reflect direct exposure of the lungs to the test material through gavage dosing/mis-dosing (see mechanistic investigations).
Liver - ↑ glycogen deposition 46/70 males (vs 33/70 controls), 55/70 (females), severity 1.8 (vs control 50/70, severity 1.5)

40 mg/kg bw/day
Survival: Survival rate: 57% (males), 69% (females) 18 months (vs 83% males; 76% female control group) due to deaths – most of them were considered accidental (see below)
↑ incidence of ‘accidental deaths’. Majority later confirmed by macro- and histopathology to be associated with effects on the respiratory tract.

5 mg/kg bw/day
No treatment-related findings.

NOAEL$ (toxicity) = 5 mg/kg bw/day
NOEL$ (carcinogenicity) = 40 mg/kg bw/day</td><td></td></tr>
<tr><td>18 month carcinogenicity

OECD 451 (1981), GLP

Oral, Dietary vehicle 0.5% CMC, 0.1% Tween 80

Mouse Crl:CD-1(ICR) BR albino

70/sex/group

Doses: 0, 150, 500, 1500 and 4000 ppm

Equivalent to: 0, 16.3, 60.7, 181.2 and 573.7 mg/kg bw/day in males; and 0, 20.2,</td><td>Neoplastic findings
There was no evidence for a carcinogenic effect.
Lung tumour data provided for comparison with earlier study.

<table>
<tr><th colspan="6">Incidence of lung tumours overall incidence (premature decedents +terminal sacrifice)</th></tr>
<tr><th rowspan="2">Finding</th><th colspan="5">Males (mg/kg bw/day)</th></tr>
<tr><th>0</th><th>150</th><th>500</th><th>1500</th><th>4000</th></tr>
<tr><td>Examined</td><td>50</td><td>50</td><td>50</td><td>50</td><td>1</td></tr>
<tr><td>Adenoma (benign)</td><td>1</td><td>0</td><td>0</td><td>0</td><td>0</td></tr>
<tr><td>adenocarcinoma</td><td>0</td><td>0</td><td>0</td><td>1</td><td>0</td></tr>
<tr><td></td><td colspan="5">Females (mg/kg bw/day)</td></tr>
<tr><td></td><td>0</td><td>150</td><td>500</td><td>1500</td><td>4000</td></tr>
<tr><td>Examined</td><td>50</td><td>50</td><td>50</td><td>50</td><td>0</td></tr>
<tr><td>Adenoma (benign)</td><td>0</td><td>1</td><td>0</td><td>1</td><td>0</td></tr>
<tr><td>Adenocarcinoma</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td></tr>
</table>

Generalised toxicity and non-neoplastic findings</td><td>2005
DAR
B.6.5.2(d)</td></tr>
</table>

Method	Results Remarks	References
75.7, 216.5 and 706.4 mg/kg bw/day in females pinoxaden technical; Batch No. EZ005006 (97.2 % purity)	**4000 ppm (574 mg/kg bw/day in males, 706 mg/kg bw/day in females)** Sacrificed at week 40 - dose exceeded MTD. *Survival:* There were no effects on survival rates. *Body weight* ↓ 13% (males) 14% (females) week 39 ↓ 31% (males), 33% (females) weight gain, week 1-40 *Food utilisation efficiency*: ↓ 20.7% (males), ↓ 27.4% (females) week 1-13. **1500 ppm (181 mg/kg bw/day in males, 217 mg/kg bw/day in females)** *Body weight* : ↓ approx. 9% both sexes week 91 ↓ 19% (males), 20.4% (females) weight gain, week 1-91 *Food utilisation efficiency*: ↓ 13.3% (males) week 1-13. **500 ppm (61 mg/kg bw/day in males, 76 mg/kg bw/day in females)** *Body weight:* ↓ 6.2% (females) week 91 ↓ 12% (females) weight gain, week 1-91. **150 ppm (16 mg/kg bw/day in males, 20 mg/kg bw/day in females)** No treatment-related effects NOAEL[$] (toxicity) = 500 ppm (61 mg/kg bw/day) in males and 150 ppm (20 mg/kg bw/day) in females NOAEL[$] (carcinogenicity) = 4000 ppm (574/706 mg/kg bw/day in males/females)	

[$] = As given in the DAR

Table 18: Supplemental studies to investigate mouse lung effects

Method	Results Remarks	References
Investigation of effects of direct application of test material on mouse lung parenchyma *Ex-vivo* study on mouse (strain not reported) excised lung Investigative study - no guideline	**75 mg/mL pinoxaden (volume 250 µL)** After 1 minute, there was reduced eosinophilic staining of the alveoli. After 10 minutes, similar changes to the 1-minute, but also lysis of the intravascular red blood cells were noted. **Vehicle** Changes seen with vehicle alone were similar to those seen in lungs treated with pinoxaden (in vehicle).	2004 DAR B.6.5.2(b)

Method	Results Remarks	References
GLP Single dose applied to the lungs by a cannula for 1 or 10 minutes: 0 (control), 250µL vehicle or 250 µL of 75 mg/mL pinoxaden No of animals: 1/ group/time point. Vehicle 0.5% CMC, 0.1% Tween 80 in distilled water Pinoxaden technical; Batch No. EZ005006 (97.2 % purity)	**Controls** No changes in untreated lungs.	
Investigation of effects of gavage administration of vehicle into the oesophagus of the mouse at a high position *In vivo* study in the mouse/CD-1 5/treatment Investigative study - no guideline GLP Single oral gavage dose of vehicle (0.5% CMC, 0.1% Tween 80 in distilled water) Treatments: untreated (control), normal (catheter in the stomach) and high oesophagus Animals sacrificed at 72 hr post-dosing; lungs (+trachea and bronchi) prepared for histopathology investigations.	**Vehicle gavaged in the oesophagus at a high position:** The vehicle enters the lungs; Slight haemorrhage and minimal hyaline changes observed. **Vehicle gavaged in the stomach (normal):** The vehicle does not enter the lungs; No histopathological changes seen in the lungs. **Untreated animals:** No effects.	2004 DAR B.6.5.2(c)

Rat (2003)

In a guideline gavage carcinogenicity study in rats, at the top dose of 500 mg/kg bw/day excessive toxicity (including clinical signs of toxicity) in males resulted in the early termination of the group at week 61. Survival rate was significantly reduced in males at 250 mg/kg bw/day. Significantly reduced bodyweight gains and increased water intake were noted in both sexes at 250 mg/kg bw/day and in females at 500 mg/kg bw/day.

Histopathology performed at the end of the study revealed chronic progressive nephropathy in animals treated at 250 mg/kg bw/day and above, renal tubular atrophy in females at 250 mg/kg bw/day and above, and renal tubular dilatation in males and females at 100 mg/kg bw/day and above.

Kidney histopathology was accompanied by decreased kidney weights and changes in related urinalysis (increased volume and ketones) and clinical chemistry (increased urea and creatinine) parameters from a dose of 250 mg/kg bw/day. Other findings included a tendency towards lower haemoglobin concentrations in males and females at 500 mg/kg bw/day.

There was an increased incidence of liver adenoma in females at 500 mg/kg bw/day (8% vs 3.3% in controls – lab HCD range: 0 – 8%) and of endometrial adenocarcinoma from 100 mg/kg bw/day (3.3%, 5% and 7% at 100, 250 and 500 mg/kg bw/day vs 1.6% in controls – Lab HCD range 0 – 8.2%). However, as these increased incidences were within the laboratory historical control ranges, these findings were considered to be incidental.

A slightly increased incidence of leiomyosarcoma (malignant tumour of the smooth muscle tissue) of the non-glandular stomach was noted in males at 250 mg/kg bw/day (2/60 - 3.3% vs 0% in controls – Lab HCD range: 0 – 0% from 5 studies). This increase was considered not to be a specific, treatment-related effect of pinoxaden based on i) the occurrence of one tumour in females at the low dose group of 10 mg/kg bw/day but not at higher dose levels, which is indicative of the potential spontaneous nature of this tumour; ii) the lack of any pre-neoplastic lesions in the stomach; and iii) the presence of significant generalised toxicity (reduced survival (38.3% vs 71.7% in controls), clinical signs of toxicity and effects on body weight (↓ 13% in males) and water intake).

Overall, in this guideline 2-year gavage chronic toxicity/carcinogenicity study in the rat, there were no carcinogenicity effects up to a dose (250-500 mg/kg bw/day) which exceeded the MTD in males and females. The main target organ of toxicity was the kidney, with effects occurring from a dose of 100 mg/kg bw/day.

Mouse (2003)

In the first guideline 18-month gavage carcinogenicity study in the mouse, increased mortality was noted in both sexes at doses ≥40 mg/kg bw/day. This observed trend of increased mortality was considered to be the result of unintended exposure of the lungs (due to gavage dosing/mis-dosing) to the test material/vehicle rather than a systemic effect of pinoxaden, as evidence of lung lesions (hyalinosis – see below) was a major factor in the unscheduled deaths observed in this study. Two subsequent investigative studies confirmed this hypothesis (see below).

Chronic administration of pinoxaden produced treatment-related toxicity, including clinical signs of toxicity at 750 mg/kg bw/day, decreased bodyweight at 750 mg/kg bw/day and 300 mg/kg bw/day (females only), increased water intake at 750 mg/kg bw/day, haematology findings (increased platelet counts) in males at 300 and 750 mg/kg bw/day, increased liver weight accompanied by glycogen deposition at 300 and 750 mg/kg bw/day and a slight increased incidence of

histopathological findings of the lung (hyalinosis) at 300 and 750 mg/kg bw/day. The lung findings were considered to be the result of unintended exposure of the lungs to the test material/vehicle through gavage dosing/mis-dosing. Other than increased mortality, there were no other treatment-related effects at 40 mg/kg bw/day.

In male animals, there was a statistically increased trend in the incidence of lung adenoma at 750 mg/kg bw/day (14.3% vs 11.4% in controls – Lab HCD range 6-14%) and of lung carcinoma at 40, 300 and 750 mg/kg bw/day (11.4%, 12.9% and 7.1% respectively vs 4.3% in controls – Lab HCD range: 2-12%). However, the combined incidence of adenoma and carcinoma was statistically significantly increased only at 300 and 750 mg/kg bw/day (26% and 17% respectively vs 16% in controls). In female animals, despite an isolated increase in adenoma at 300 mg/kg bw/day (14.3% vs 7.1% in controls), statistical analysis indicated no significant positive trend for the combined incidence of adenoma and carcinoma.

Overall, there was a slight increase in the incidence of lung adenoma and carcinoma in male mice at 300 and 750 mg/kg bw/day. However, when considering that the increase was small and just above the laboratory historical control range; showed no clear dose response relationship; occurred at doses causing lethality and poor survival; and might have been related to the unintended direct ingress of material/vehicle into the lung through gavage dosing/mis-dosing (see investigative studies), it was concluded that these tumours were not related to oral exposure to pinoxaden. This conclusion was confirmed by the absence of lung tumours in a second mouse carcinogenicity study conducted by dietary administration up to doses (574/706 mg/kg bw/day) exceeding the MTD (2005 – see below).

Overall, in this gavage carcinogenicity study in the mouse, there were no clear carcinogenicity effects up to a dose (750 mg/kg bw/day) which caused lethality and poor survival. It was deemed that the observed reduction in survival was the result of the unintended exposure of the lungs to the test material/vehicle through gavage dosing/mis-dosing.

Mouse (2005)

To confirm the hypothesis that the lung effects and the consequent poor survival observed in the Geerspach (2003) study were the result of the unintended exposure of the lungs to the test material/vehicle through gavage dosing/mis-dosing, a second guideline mouse carcinogenicity study was conducted in which pinoxaden was administered in the diet (2005). Animals were treated with diets containing 0, 150, 500, 1500 or 4000 ppm pinoxaden (equivalent to 0, 16/20, 61/76, 181/217 or 574/706 mg/kg bw/day in males/females).

In this study, there were no treatment related effects on survival or on clinical signs of toxicity. Significant reductions on body weights and body weight gains and on food utilization efficiency were seen at the top dose of 4000 ppm in both sexes. These animals were therefore terminated at week 40. There was also a reduction in bodyweight gain of 19% in both sexes at 1500 ppm. At 500 ppm, bodyweight was 6% lower than in control females. There were no other changes at this dose levels that were considered to be of toxicological significance.

Pinoxaden had no effect on the number of tumour bearing animals or on the incidence or type of tumours.

Overall, in this dietary carcinogenicity in the mouse, there were no carcinogenicity effects up to a dose (574/706 mg/kg bw/day) which exceeded the MTD.

4.9.1.2 Carcinogenicity: inhalation

No information available.

4.9.1.3 Carcinogenicity: dermal

No information available.

4.9.2 Human information

No information available.

4.9.3 Other relevant information

Two investigative studies were conducted to test the hypothesis that the lung effects (hyalinosis) seen in the gavage mouse carcinogenicity study predominantly at 300 and 750 mg/kg bw/day (2003) were the result of the unintended exposure of the lungs to the test material/vehicle through gavage dosing/mis-dosing.

In the first study, excised mouse lungs were treated with 0 (control), 250 µL of pinoxaden solution (75 mg/mL) in vehicle (0.5% CMC and 0.1% Tween 80 in water) or 250 µL of vehicle alone for 1 or 10 minutes (2004). After treatment, the lungs were fixed and sections examined. The vehicle used in this study was the same as that used in the gavage mouse carcinogenicity study.

Microscopic changes (eosinophilic staining of the alveoli and lysis of intravascular red blood cells) were seen in the lungs dosed with pinoxaden (in vehicle) or with vehicle alone. The findings were considered to be qualitatively similar to those (hyalinosis) seen in the gavage mouse carcinogenicity study, but less severe.

On the basis of these findings, the study authors concluded that the most likely cause of the lung lesions seen in the (2003) study was the direct exposure of the lungs to the vehicle through gavage mis-dosing/accidental dosing. The lung lesions were more severe in the (2003) study compared to those seen in this study because of repeated application for longer periods of time. In addition, the incidence of the lesions was higher at higher dose levels (300 and 750 mg/kg bw/day) compared to the lower doses (5 and 40 mg/kg bw/day) because the dosing solution was thicker at these dose levels, making expulsion from the lungs by natural physiological means more difficult.

In the second investigative study (2004), groups of 5 CD-1 mice were dosed by gavage with the same vehicle (0.5% CMC and 0.1% Tween 80 in water) used in the gavage mouse carcinogenicity study. One group remained untreated and served as control. One group received a single dose of the vehicle through a catheter inserted in the stomach (“normal” group) and a third group received a single dose of the vehicle through a catheter positioned relatively high in the oesophagus. Animals were terminated 72 hours after dosing and the lungs removed and prepared for histopathology.

The vehicle did not enter the lungs in the “normal” group and no effects were observed in the lungs. In the high oesophagus group, the vehicle entered the lungs and histopathological findings (slight haemorrhage and minimal hyaline changes) were observed 72 hours post dosing. The microscopic findings noted in this group were consistent with the lung lesions (hyalinosis) observed in the gavage mouse carcinogenicity study (2003). On the basis of these results, the study authors concluded that the most likely cause of the lung lesions seen in the (2003) study was the direct exposure of the lungs to the vehicle through gavage mis-dosing, possibly as a consequence of the cannula being mis-positioned relatively high in the oesophagus.

4.9.4 Summary and discussion of carcinogenicity

The carcinogenic potential of pinoxaden has been investigated by the oral route in one guideline rat study (by gavage) and two guideline mouse bioassays (one by gavage and the other by dietary administration). There are also two mechanistic investigations conducted in the mouse to address the lung effects seen in the first mouse gavage bioassay.

In the rat study, at the top dose of 500 mg/kg bw/day excessive toxicity in males resulted in the early termination of the group at week 61. Histopathology revealed the presence of kidney toxicity, with chronic progressive nephropathy occurring in animals treated at 250 mg/kg bw/day and above, renal tubular atrophy occurring in females at 250 mg/kg bw/day and above, and renal tubular dilatation occurring in males and females at 100 mg/kg bw/day and above.

A slightly increased incidence of leiomyosarcoma of the non-glandular stomach was noted in males at the top dose of 250 mg/kg bw/day (2/60 - 3.3% vs 0% in controls – Lab HCD range: 0 – 0%). This increase was considered not to be a specific, treatment-related effect of pinoxaden based on i) the occurrence of one tumour in females at the low dose group of 10 mg/kg bw/day but not at higher dose levels, which is indicative of the potential spontaneous nature of this tumour; ii) the lack of any pre-neoplastic lesions in the stomach; and iii) the presence of significant generalised toxicity (reduced survival (38.3% vs 71.7% in controls), clinical signs of toxicity and effects on body weight (↓13% in males) and water intake).

Overall, in the rat, there were no treatment-related carcinogenicity effects up to a dose (250-500 mg/kg bw/day) which exceeded the MTD in males and caused significant toxicity in females. The main target organ of toxicity was the kidney, with effects occurring from a dose of 100 mg/kg bw/day.

In the mouse gavage study, increased mortality was noted in both sexes at doses ≥40 mg/kg bw/day. This observed trend of increased mortality was considered to be the result of unintended exposure of the lungs (due to gavage dosing/mis-dosing) to the test material/vehicle rather than a systemic effect of pinoxaden, as evidence of lung lesions (hyalinosis) at 300 and 750 mg/kg bw/day was a major factor in the unscheduled deaths observed.

In this study, there was a slight increase in the incidence of lung adenoma and carcinoma in male mice at 300 and 750 mg/kg bw/day (combined: 26% and 17% respectively vs 16% in controls). However, when considering that the increase was small and just above the laboratory historical control range; showed no clear dose response relationship; occurred at doses causing lethality and poor survival; and might have been related to the unintended direct ingress of material/vehicle into the lung through gavage dosing/mis-dosing (see investigative studies), it was concluded that these tumours were not related to oral exposure to pinoxaden. This conclusion was confirmed by the absence of lung tumours (or any other tumours) in a second mouse carcinogenicity study conducted by dietary administration up to doses (574/706 mg/kg bw/day) exceeding the MTD.

Overall, in the mouse, there were no clear carcinogenicity effects in the first study up to a gavage dose (750 mg/kg bw/day) which caused lethality and poor survival, and in the second study up to a dietary dose (574/706 mg/kg bw/day) which exceeded the MTD.

Two investigative studies were conducted to test the hypothesis that the lung effects (hyalinosis) seen in the gavage mouse carcinogenicity study predominantly at 300 and 750 mg/kg bw/day were the result of the unintended exposure of the lungs to the test material/vehicle through gavage dosing/mis-dosing.

These studies showed that the most likely cause of the lung lesions seen in the (2003) study was the direct exposure of the lungs to the vehicle through gavage mis-dosing, possibly as a consequence of the gavage cannula being mis-positioned relatively high in the oesophagus. The lung lesions were more severe in the (2003) study compared to those seen in these mechanistic investigations because of repeated application for longer periods of time. In addition, the incidence of the lesions was higher at higher dose levels (300 and 750 mg/kg bw/day) compared to the lower doses (5 and 40 mg/kg bw/day) because the dosing solution was thicker at these dose levels, making expulsion from the lungs by natural physiological means more difficult.

4.9.5 Comparison with criteria

Classification in Category 1A for carcinogenicity is not justified as there is no evidence of pinoxaden having caused cancer in humans.

Substances should be classified in Category 1B where there is sufficient evidence of carcinogenicity in experimental animals and in Category 2 where there is limited evidence of carcinogenicity in experimental animals. The carcinogenic potential of pinoxaden has been investigated by the oral route in one guideline rat study (by gavage) and two guideline mouse bioassays (one by gavage and the other by dietary administration). In rats, a slightly increased incidence of leiomyosarcoma of the non-glandular stomach was noted in males at the top dose of 250 mg/kg bw/day (2/60 - 3.3% vs 0% in controls – Lab HCD range: 0 – 0%). This increase was considered not to be a specific, treatment-related effect of pinoxaden based on i) the occurrence of one tumour in females at the low dose group of 10 mg/kg bw/day but not at higher dose levels, which is indicative of the potential spontaneous nature of this tumour; ii) the lack of any pre-neoplastic lesions in the stomach; and iii) the presence of significant generalised toxicity (reduced survival, clinical signs of toxicity and effects on body weight and water intake).

Overall, in the rat, it is considered that there were no specific, treatment-related carcinogenicity effects up to a dose (250-500 mg/kg bw/day) which exceeded the MTD in males and females.

In the mouse gavage study, there was a slight increase in the incidence of lung adenoma and carcinoma in male mice at 300 and 750 mg/kg bw/day. However, when considering that the increase was small and just above the laboratory historical control range; showed no clear dose response relationship; occurred at doses causing lethality and poor survival; and might have been related to the unintended direct ingress of material/vehicle into the lung through gavage dosing/mis-dosing, it was concluded that these tumours were not related to oral exposure to pinoxaden. This conclusion was confirmed by the absence of lung tumours (or any other tumours) in a second mouse carcinogenicity study conducted by dietary administration up to doses (574/706 mg/kg bw/day) exceeding the MTD.

Overall, in the mouse, there were no clear carcinogenicity effects in the first study up to a gavage dose (750 mg/kg bw/day) which caused lethality and poor survival, and in the second study up to a dietary dose (574/706 mg/kg bw/day) which exceeded the MTD.

In conclusion, it is concluded that the available evidence shows that pinoxaden is not carcinogenic in rats and mice by the oral route. Therefore, it is not proposed to classify pinoxaden as a carcinogen.

4.9.6 Conclusions on classification and labelling

No Classification – conclusive but not sufficient for classification

4.10 Toxicity for reproduction

The reproductive toxicity of pinoxaden has been investigated in a two generation reproduction study in the rat and in five prenatal developmental toxicity studies, one in rats and four in rabbits.

4.10.1 Effects on fertility

4.10.1.1 Non-human information

Table 19: Summary table of relevant reproductive toxicity studies

Method	Results	Reference
Two Generation Oral (gavage) OECD 416 (1983) GLP Rat, Hanlbm:WIST (SPF) 30/sex/group 0, 10, 50 250 or 500 mg/kg bw/day Vehicle: 0.5% (w/v) carboxymethylcellulose in 0.1% (w/v) aqueous polysorbate 80 Batch: EZ005006 (purity 97.2%)	***Parental toxicity*** <u>500 mg/kg bw/day</u> *F0*: ↓ body weight gain 8% days 1-71 males only ↑ water consumption 44% males*, 25% females* week 10 ↑ relative kidney weight 21% males*; ↑ relative liver weight 18% males*, 27% females* Chronic nephropathy and tubular atrophy males & females ; Pelvic dilatation in males; Liver glycogen deposition in females *F1*: ↑ water consumption 52% males*, 33% females* week 10 ↑ relative kidney weight 16% males*; ↑ relative liver weight 18% males*, 29% females* Chronic nephropathy and tubular atrophy males & females ; Liver glycogen deposition in females <u>250 mg/kg bw/day</u> *F0:* ↑ relative kidney weight 13% males*; ↑ relative liver weight 12%* males, 18% females* *F1*: ↑ water consumption 26% males*, 29% females* week 10 ↑ relative kidney weight 10% males*; ↑ relative liver weight 8% males, 19% females <u>50 mg/kg bw/day</u> *F0:* ↑ relative liver weight 7% females*; *F1:* ↑ relative kidney weight 6% males; ↑ relative liver weight 10.5% females* <u>10 mg/kg bw/day</u> No adverse effects NOAEL[$] 10 mg/kg bw/day on the basis of increased liver weight in F1 females at 50 mg/kg bw/day	2003a DAR B.6.6.1, 5.6.1(a)

Method	Results	Reference
	Reproductive toxicity No effects at any dose level NOAEL$ 500 mg/kg bw /day ***Offspring toxicity*** <u>500 mg/kg bw /day</u> F1: ↓ body weight 7% males* and females* day 21 F2: ↓ body weight 5% males*, 4% females day 21 <u>250 mg/kg bw/day</u> No adverse effects NOAEL$ 250 mg/kg bw/day based on body weight effects at 500 mg/kg bw/day in F1 and F2 pups.	

* Statistically significant; $ As given in the DAR

In an OECD- and GLP- compliant two-generation reproduction study in the rat, the effects of daily oral (gavage) administration of pinoxaden on reproduction were investigated (2003a).

For the F0 generation, young adult male and female rats were dosed once daily by oral gavage with 0, 10, 50, 250 or 500 mg/kg bw/day. After 10 weeks of dosing, the animals (30/sex/dose) were paired 1:1 within each dose group until there was evidence of positive mating or for 14 days, whichever occurred first. The mated females were allowed to litter. Litters (F1) were culled to four male and four female pups, where possible, on day 4 post partum. After weaning of the last litter, selected F1 offspring (30 animals/sex/dose) were dosed once daily for 10 weeks. The F1 animals were allowed to mate and rear their offspring (F2) to weaning as for the F0 generation.

At 500 mg/kg bw/day, there was no significant effect on the body weight gain or food intake of the F0 or F1 males and females. Overall (days 1-71), body weight gain of the F0 males was lower than controls by 8% although not statistically significantly different. Water consumption was significantly increased for F0 & F1 males and females at 500 mg/kg bw/day and in F1 males and females at 250 mg/kg bw/day.

At necropsy, absolute and relative kidney weights were increased in F0 and F1 males and F0 females at 500 mg/kg bw/d. These increased weights were accompanied by an increased incidence and/or severity of chronic nephropathy and tubular atrophy. At 250 mg/kg bw/day, increased kidney weight in F0 and F1 males was not accompanied by histopathological changes.

Absolute and relative liver weights were increased in F0 and F1 males at 250 and 500 mg/kg bw/day and in F0 and F1 females at 50 mg/kg bw/day and above. The increased liver weight in females at 50 mg/kg bw/day was not accompanied by any histopathological change in the liver and was therefore considered not to be of toxicological significance. At 250 and 500 mg/kg the increased liver weight was accompanied by increased glycogen deposition.

There were no treatment-related effects on the number of animals mating, the number of females becoming pregnant or on the mean pre-coital time. Oestrous cycles were not affected by treatment. Sperm parameters showed no treatment-related effects.

There were no treatment-related effects on litter size at birth, pup viability to day 4 or to day 21. Mean pup weights at birth were similar in all groups. At 500 mg/kg bw/day, mean pup body

weights were lower than controls from day 4 for males and females in the F1 and F2 generations. Statistically significant differences from controls occurred on day 4 (F1 females), days 7, 14 and 21 (F1 males and females) and days 7 and 14 (F2 males).

There were no treatment-related effects on the developmental landmarks i.e. the time of balano-preputial separation or vaginal opening in F1 pups.

Minor changes in pup organ weights were considered not to be adverse in the absence of treatment-related findings from histologic examination.

In conclusion, in this study, no effects on fertility and reproductive performance were seen up to a dose (500 mg/kg bw/day) causing parental toxicity (body weight effects, increased water consumption, liver and kidney effects). Offspring toxicity (effects on pup body weight during lactation) was seen at 500 mg/kg bw/day.

4.10.1.2 Human information

No information available.

4.10.2 Developmental toxicity

The developmental toxicity of pinoxaden has been investigated in the rat (one full study) and rabbit (one preliminary study, two full studies and two investigative studies).

4.10.2.1 Non-human information

Table 26: Summary table of relevant developmental toxicity studies

Method	Results	Reference
Developmental toxicity Oral (gavage) OECD 414 (1981) GLP Rat, Hanlbm:WIST (SPF) 24 mated females/group 0, 3, 30 300 or 800 mg/kg bw/day on days 6-20 of gestation Vehicle: aqueous solution of carboxymethylcellulose (0.5% w/w) & Tween 80 (0.1% w/w) Batch: EZ005006 (purity 97.2%)	*Maternal toxicity* 800 mg/kg bw/day: piloerection for 2-7 days in most animals; ↓ body weight gain (33% lower than controls days 6 to 21, net weight loss after adjustment for gravid uterus weight)*; ↓ food consumption (max. 28% lower than controls, days 16-21)*; ↓ gravid uterus weight* 300 mg/kg bw/day: ↓ body weight gain (8% lower than controls days 6 to 21)*; ↓food consumption (max. 10% lower than controls days 16-21)*. 3 & 30 mg/kg bw/day No effects *Developmental toxicity* 800 mg/kg bw/day: ↓ foetal weight (8% lower than controls)*; reduced ossification of cranial bones and digits (variations)*. 300 mg/kg bw/day: reduced ossification of 3 structures only (variations). 3 & 30 mg/kg bw/day No effects Maternal and developmental NOAEL[$] 30 mg/kg bw/day	2003b DAR B.6.6.1, IIA5.6 (a)

Method	Results	Reference
Preliminary/Dose-ranging developmental toxicity Oral (gavage) GLP Russian rabbits, Chbb:HM 8 mated females/group 0, 30, 150, 300, 700 or 1000 mg/kg bw/day on days 7-28 of gestation Vehicle: aqueous solution of carboxymethylcellulose (0.5% w/w) & Tween 80 (0.1% w/w) Batch: EZ005006 (purity 97.2%)	*Maternal toxicity* 1000 mg/kg bw/day: 2/8 animals found dead after 1 or 2 doses. Hunched posture, reduced activity and body weight loss in all animals. Group terminated. 700 mg/kg bw/day: 2/8 animals found dead after 5 or 6 doses. Hunched posture, reduced activity and body weight loss in all animals. Group terminated. 300 mg/kg bw/day: 1/8 animals found dead after 12 doses. Hunched posture, reduced activity and body weight loss in all animals. Group terminated. 150 mg/kg bw/day: 1/8 animals moribund after 8 doses; terminated. 1/8 hunched posture and reduced activity. Initial weight loss and ↓ (26%) weight gain GD 7-29 for females with viable foetuses, 87%* for all pregnant females; ↓ (62%)* food consumption GD 7-12. 4/7 pregnancies with no live foetuses. 30 mg/kg bw/day: No clinical signs. Small initial weight loss, ↓ (20%) weight gain GD 7-29, ↓ (19%) food consumption GD 7-12; *Developmental toxicity* 150 mg/kg bw/day: 4/7 total resorption (early deaths)*, ↓ (12%) foetal weight; 30 mg/kg bw/day: No significant effects. Dose levels of 3, 10, 30 and 100 mg/kg bw/day selected for developmental toxicity study.	2003a DAR B.6.6.3, IIA 5.6.1 (a)
Developmental toxicity Oral (gavage) OECD 414 (1981) GLP Russian rabbits, Chbb:HM 24 mated females/group 0, 3, 10, 30 or 100 mg/kg bw/day on days 7-28 of gestation Vehicle: aqueous solution of carboxymethylcellulose (0.5% w/w) & Tween 80 (0.1% w/w) Batch: EZ005006 (purity 97.2%)	*Maternal toxicity* 100 mg/kg bw/day: ↓ body weight gain (68%)* GD 7-29; ↓ (36%)* food consumption GD 7-12. 3, 10 & 30 mg/kg bw/day: No significant effects. *Developmental toxicity* 100 mg/kg bw/day: ↓ (11%)* foetal weight; 3 foetuses from different litters with diaphragmatic hernia (2 foetuses) or fissure (1 foetus). 30 mg/kg bw/day: 1 foetus with diaphragmatic hernia. 3 & 10 mg/kg/bw/day: No significant effects NOAEL$^{\$}$ maternal toxicity = 30 mg/kg bw/day; NOAEL$^{\$}$ dev toxicity = 10 mg/kg bw/day based on diaphragmatic malformations	2003b DAR B.6.6.3, IIA 5.6.1 (b)

Method	Results	Reference
Non-standard developmental toxicity Oral (gavage) OECD 414 (1981) GLP Russian rabbits, Chbb:HM 24 mated females/group 0 or 100 mg/kg bw/day on days 7-28 of gestation Vehicle: aqueous solution of carboxymethylcellulose (0.5% w/w) & Tween 80 (0.1% w/w) Batch: EZ005006 (purity 97.2%)	*Maternal toxicity* 100 mg/kg bw/day: ↓ body weight gain (44%)* GD 7-29; ↓ food consumption (29%)* GD 7-12. One female with abortion, one with total resorption. *Developmental toxicity* 100 mg/kg bw/day: No abnormalities of the diaphragm observed.	2003c DAR B.6.6.3, IIA 5.6.1 (c.(i))
Non-standard developmental toxicity Oral (gavage) OECD 414 (1981) GLP Russian rabbits, Chbb:HM (24 mated females/group) 0 or 100 mg/kg bw/day on days 7-28 of gestation Vehicle: aqueous sln of carboxymethylcellulose (0.5% w/w) & Tween 80 (0.1% w/w) Batch: EZ005006 (purity 97.2%)	*Maternal toxicity* 100 mg/kg bw/day: ↓ body weight gain (35%)* GD 7-29; ↓ food consumption (30%) GD 7-12. One female found dead on GD 23. One female killed on day 27 after abortion; two females with total resorption, one female with abortion at caesarean section. *Developmental toxicity* 100 mg/kg bw/day: ↑ post-implantation loss (54% vs 34% in controls)* No abnormalities of the diaphragm observed.	2003d DAR B.6.6.3, IIA 5.6.1 (c.(ii)
Developmental toxicity Oral (gavage) OECD 414 (1981) GLP Russian rabbits, Chbb:HM (24 mated females/group) 0, 3, 10, 30 or 100 mg/kg bw/day on days 7-28 of gestation Vehicle: aqueous sln of carboxymethylcellulose (0.5% w/w) & Tween 80 (0.1% w/w) Batch: EZ005006 (purity 97.2%)	*Maternal toxicity* 100 mg/kg bw/day: ↓ body weight gain (63%)* GD 7-29; ↓ (42%)* food consumption GD 7-12. One female killed moribund; two females aborted. 30 mg/kg bw/day: ↓ body weight gain; ↓ food consumption; 3 & 10 mg/kg bw/day: No significant effects. *Developmental toxicity* 100 mg/kg bw/day: ↑ post-implantation loss (38% vs 0.8% in controls)* 3, 10 and 30 mg/kg bw/day: No significant effects. NOAEL$ maternal toxicity = 10 mg/kg bw/day; NOAEL$ dev toxicity = 30 mg/kg bw/day;	2003c DAR B.6.6.3, IIA 5.6.1 (d)

* Statistically significant; $ As given in the DAR

Prenatal developmental toxicity in the rat (2003b)

In an OECD- and GLP-compliant prenatal developmental toxicity study, groups of 24 time-mated female Wistar rats were dosed by oral gavage with 0, 3, 30, 300 or 800 mg/kg bw/day on gestation days 6 through to 20 and terminated on day 21 for evaluation of maternal and developmental effects. At 800 mg/kg bw/day, one female (not pregnant) was terminated on day 17 due to respiratory sounds, dyspnoea, hunched posture, reduced activity and piloerection; the lungs were observed to be red & mottled. [The cause of death is not considered in the study report. On the basis of the respiratory observations and the necropsy findings in the lungs, this single mortality is not clearly treatment-related and may have been the result of a dosing incident.] Piloerection was seen for 2-7 days in most animals given 800 mg/kg bw/day. At 300 and 800 mg/kg bw/day there was a dose-related reduction in body weight and food consumption. This was marked at 800 mg/kg bw/day, resulting in an overall net loss of body weight and a reduced gravid uterus weight, and minimal at 300 mg/kg bw/day.

There were no treatment-related effects on the number of implantations, pre- or post-implantation loss or the number of viable foetuses. Mean foetal bodyweights were significantly lower than those of controls at 800 mg/kg bw/day only.

There were no treatment-related malformations. The incidence of visceral variations was low and there were no clear effects of treatment. No skeletal malformations were observed and there was no effect of treatment on the incidence of skeletal anomalies. Skeletal variations occurred in almost all foetuses including controls. At 800 mg/kg bw/day, there was a statistically significant increase in the incidence of incomplete ossification of cranial bones (parietal, interparietal, frontal and occipital) and the paws (metatarsal-1 and distal phalanges of posterior digits) and unossified calcaneus. At 300 mg/kg bw/day, there was a statistically significant increased incidence of incomplete ossification of interparietal bone, metatarsal -1 and distal phalanges of posterior digits.

Overall, in this developmental toxicity study in the rat, delayed ossification and reduced foetal weights were seen from a dose of 300 mg/kg bw/day in the presence of maternal toxicity (effects on body weight and food consumption). The developmental effects were considered to be the secondary, unspecific consequence of the observed maternal toxicity. The NOAEL for maternal and developmental toxicity was therefore established at 30 mg/kg bw/day. There was no indication of teratogenic potential.

Developmental toxicity in the rabbit

A preliminary dose-range finding study in pregnant rabbits was followed by four prenatal developmental toxicity studies. The results from the first prenatal developmental toxicity study suggested a possible association between prenatal exposure to pinoxaden and a low incidence of foetal diaphragmatic malformations. In-depth analysis of the findings suggested that the study outcome could have been due to a genetic influence (familial relationship). As a consequence, two non-standard, investigative developmental toxicity studies were conducted. No foetuses were found to have malformations of the diaphragm in these studies. In addition, as the validity of the results from the first study was brought into question, a fourth full prenatal developmental toxicity study was undertaken, in which the parentage of the females was known and controlled and where semen from male donors was used to inseminate females evenly across the groups.

1) **Preliminary/dose-range finding study in the pregnant rabbit (2003a)**

For the preliminary rabbit prenatal developmental toxicity study, groups of 8 time-mated female Russian rabbits were dosed by oral gavage with 0, 30, 150, 300, 700 or 1000 mg/kg bw/day on

gestation days 7 through to 28 and terminated on day 29. Dose levels of ≥ 300 mg/kg bw/day were in excess of the maximum tolerated dose and these groups were terminated prematurely.

At 150 mg/kg bw/day, one animal was in a moribund condition on treatment day 8 and therefore terminated on GD 15. Another animal showed reduced activity and hunched posture on days 15-19. There were no treatment-related clinical signs of toxicity at 30 mg/kg bw/day.

Slight body weight loss was seen after treatment start at 30 and 150 mg/kg bw/day. Body weight gain for both groups remained lower than control values throughout the treatment period.

Food consumption was reduced at 150 mg/kg bw/day during the treatment period and only for the first week of treatment at 30 mg/kg bw/day.

Four animals at 150 mg/kg bw/day had total resorptions. This was reflected in a high incidence of early resorptions and increased post-implantation loss and a reduced number of live foetuses for the group. Three litters at 150 mg/kg bw/day were not affected by increased post-implantation loss and had comparable numbers of live foetuses, with the control and 30 mg/kg bw/day groups.

Foetal body weight was lower than the control at 30 and 150 mg/kg bw/day but was not statistically significantly different. External and visceral examination of the foetuses revealed no remarkable findings.

On the basis of these data, dose levels of 0, 3, 10, 30 and 100 mg/kg bw/day were evaluated in a prenatal developmental toxicity study.

2) Prenatal developmental toxicity in the rabbit (2003b)

In an OECD- and GLP-compliant prenatal developmental toxicity study, groups of 24 pregnant female Russian rabbits were given gavage doses of 0, 3, 10, 30 or 100 mg/kg bw/day pinoxaden from day 7 to 28 (inclusive) of gestation (the day of insemination was designated gestation day 0).

Maternal body weight gain at 100 mg/kg bw/day was reduced during the treatment period (↓ 68% days 7-29) but there was no effect on gravid uterus weight. Lower maternal body weight gain seen at 30 mg/kg bw/day following the onset of dosing on day 7, was not statistically significant.

Food consumption at 100 mg/kg bw/day was significantly reduced throughout the treatment period.

At 100 mg/kg bw/day, mean foetal body weight was significantly reduced (11%) in comparison with the control. There were no total resorptions at this dose and no increases in pre or post-implantation loss. Litter size was comparable for all groups.

There were no treatment-related foetal external findings. At visceral examination, malformation of the diaphragm was seen in three foetuses from different litters at 100 mg/kg bw/day (diaphragmatic hernia in two and fissure of diaphragm in one). One foetus at 30 mg/kg bw/day had diaphragmatic hernia. There were no treatment-related foetal skeletal malformations, anomalies or variations.

Overall, in this developmental toxicity study in the rabbit, developmental toxicity (reduced foetal weight) was seen at the top dose of 100 mg/kg bw/day in the presence of maternal toxicity (effects on body weight and food consumption). These foetal effects were considered to be the secondary, unspecific consequence of the observed maternal toxicity. However, a low incidence of malformations of the diaphragm was seen from a dose of 30 mg/kg bw/day. No reliable or suitable historical control data are available for this finding in this strain of rabbits.

Whilst investigating possible reasons for the occurrence of diaphragmatic malformations in the top and mid dose groups, it was noted that information on the parentage and sibling status of the

animals was not obtained or utilised in the allocation of the females and male semen donors to the treatment groups, casting a shadow over the reliability/validity of the study. In addition, it was established that the foetuses with malformations of the diaphragm all had the same father (male number 119). In order to further investigate the possible role of genetic influences on the occurrence of the diaphragmatic lesions, two non-standard prenatal developmental toxicity studies were therefore undertaken.

3) **Prenatal developmental toxicity in the rabbit: Single buck (2003c)**

The purpose of this study was to investigate the potential role of genetic influences on the occurrence of diaphragmatic effects seen in the previous study by testing whether malformations of the diaphragm (hernia/fissure) could be repeated or not when using male semen donor no. 119. A dose level of 100 mg/kg bw/day was chosen for use in this study as the effect on the diaphragm had been seen at this dose level in the previous study. Groups of 24 female Russian rabbits were used.

In this study, one female in the 100 mg/kg bw/day group aborted on GD 26. One control female and one in the 100 mg/kg bw/day group had total resorption of the litter at term.

Body weight gain at 100 mg/kg bw/day was reduced during the treatment period (↓ 44% GD 7-29) but there was no effect on gravid uterus weight.

Food consumption at 100 mg/kg bw/day was significantly reduced at GD 7-12, 12-16 and 16-20.

At 100 mg/kg bw/day, there was one total resorption but no statistically significant increase in pre or post-implantation loss in the other litters. Litter size was comparable for both groups. Mean foetal body weight was not lower than control at 100 mg/kg bw/day. A single occurrence of total resorption was observed in the control group.

There were no treatment-related foetal external findings. At visceral examination, there were no occurrences of diaphragmatic hernia or fissure and no treatment-related findings. No skeletal examination was conducted.

Malformation of the foetal diaphragm was not repeated in this study when using the same male parent and the same dose of pinoxaden associated with the finding in the first study (2003b in (2) above). This study shows that it is unlikely the malformation of the foetal diaphragm originated as a consequence of the genetic make-up of male no. 119.

4) **Prenatal developmental toxicity in the rabbit: Multiple bucks (2003d)**

The purpose of the second non-standard prenatal developmental toxicity study was to further investigate the potential role of the sibling status on the occurrence of diaphragmatic effects seen in the previous study by testing whether or not malformation of the diaphragm (hernia/fissure) could be repeated when excluding male no. 119 and matings between siblings. A dose level of 100 mg/kg bw/day was chosen for use in this study as the effect on the diaphragm had been seen at this dose level in the first study. Groups of 24 female Russian rabbits were used.

One female in the 100 mg/kg bw/day group was found dead on GD 23. Although no remarkable clinical signs were observed prior to death and the macroscopic findings at necropsy were considered to be autolytic, the death was presumed to be treatment-related. The death of a second female given this dose was attributed to injury and therefore incidental to treatment. In addition, a third female aborted on GD 27 and another female was found to have aborted at examination post mortem. A further two females were found to have totally resorbed their litters. As five control females and eight in the 100 mg/kg bw/day group were not pregnant the number of females with viable foetuses at term was 19 in the control group and 11 in the 100 mg/kg bw/day group.

Body weight gain at 100 mg/kg bw/day was reduced during the treatment period (35%) but there was no effect on gravid uterus weight.

Food consumption at 100 mg/kg bw/day was significantly reduced throughout the early treatment period.

At 100 mg/kg bw/day, there were two total resorptions, with a statistically significant increase in post-implantation loss. Mean foetal body weight was not significantly reduced at 100 mg/kg bw/day.

There were no treatment-related foetal external findings. At visceral examination, there were no occurrences of diaphragmatic hernia or fissure and no treatment-related findings. No skeletal examination was conducted.

Malformation of the foetal diaphragm was not repeated in this study which excluded male parent no. 119 and sibling matings but used the same dose of pinoxaden as in the first study (2003b in (2) above). This study shows that when the relationship between the experimental animals is known and the allocation of females and males used for insemination is controlled, malformations of the foetal diaphragm are no longer detected.

5) Prenatal developmental toxicity in the rabbit (2003c)

To complete a weight of evidence assessment, a fourth prenatal developmental toxicity study was undertaken. In this study, the potential genetic and familial influences of sibling matings and non-randomised male donors on the results were removed. This full guideline study was a repeat of the first study (2003b), utilising the same dose levels of pinoxaden and the same strain of rabbits.

One female at 100 mg/kg bw/day was terminated on GD26 due to its moribund condition (emaciated due to severe loss of body weight and recumbent, having showed reduced activity for the previous two days). Two females at 100 mg/kg bw/day aborted on GD 27. These premature terminations were considered treatment-related.

Maternal body weight gain at 100 mg/kg bw/day was reduced during the treatment period (63%) but there was no effect on gravid uterus weight. Lower maternal body weight gain seen at 30 mg/kg bw/day following the onset of dosing on day 7, was not statistically significant.

Food consumption at 100 mg/kg bw/day was reduced throughout the treatment period. Food consumption at 30 mg/kg bw/day was lower, but not statistically significantly different from the control group during the early treatment period.

There were statistically significant effects on post-implantation loss and number of live foetuses due to early resorptions at the top dose of 100 mg/kg bw/day.

There were no treatment-related foetal external or visceral findings. There were no treatment-related foetal skeletal malformations, anomalies or variations.

It has been argued that the resorptions observed at the top dose of 100 mg/kg bw/day in this study might have masked a possible effect of pinoxaden on the diaphragm. This is highly unlikely because the diaphragmatic malformations (hernia and fissure) seen with pinoxaden in the first study are not fatal in utero, and thus, if they had occurred, they would have been unrelated to the resoprtions observed in this study and would have been detected.

Overall, in this developmental toxicity study in the rabbit, developmental toxicity (resorptions and post-implantation loss) was seen at the top dose of 100 mg/kg bw/day in the presence of maternal toxicity (clinical signs of toxicity, abortions and effects on body weight and food consumption).

These foetal effects were considered to be the secondary, unspecific consequence of the observed maternal toxicity. Maternal effects (reduced body weight and food consumption) were also seen at a dose of 30 mg/kg bw/day. On this basis, the NOAEL for maternal toxicity was set at 10 mg/kg bw/day and the NOAEL for developmental toxicity was established at 30 mg/kg bw/day. No malformations of the foetal diaphragm were observed up to the maternally toxic dose of 100 mg/kg bw/day.

4.10.2.2 Human information

No information available

4.10.3 Other relevant information

No further data available

4.10.4 Summary and discussion of reproductive toxicity

The reproductive toxicity of pinoxaden has been investigated in a two generation reproduction study in the rat and in five prenatal developmental toxicity studies, one in rats and four in rabbits.

Fertility

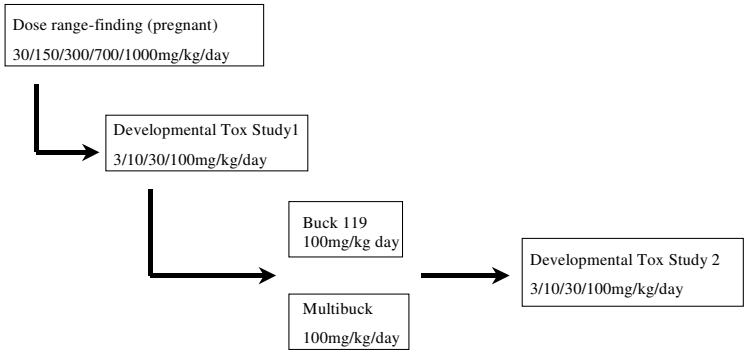
An OECD- and GLP-compliant two-generation rat reproduction toxicity study was conducted using dose levels of 0, 10, 50, 250 or 500 mg/kg bw/day. In this study, no effects on fertility and reproductive performance were seen up to a dose (500 mg/kg bw/day) causing parental toxicity (body weight effects, increased water consumption, liver and kidney effects). Offspring toxicity (effects on pup body weight during lactation) was seen at 500 mg/kg bw/day.

Development

An OECD- and GLP-compliant rat prenatal developmental toxicity study was conducted using dose levels of 0, 3, 30, 300 or 800 mg/kg bw/day. In this study, delayed ossification and reduced foetal weights were seen from a dose of 300 mg/kg bw/day in the presence of maternal toxicity (effects on body weight and food consumption). The developmental effects were considered to be the secondary, unspecific consequence of the observed maternal toxicity. The NOAEL for maternal and developmental toxicity was therefore established at 30 mg/kg bw/day. There was no indication of teratogenic potential.

The effects of pinoxaden on prenatal development in the rabbit have been assessed in 5 studies (dose range-finding study, full guideline study no.1, single-buck investigative study, multi-buck investigative study and full guideline study no.2).

Figure 4.1.4-1: Rabbit developmental toxicity studies conducted with pinoxaden



In a dose range-finding study (2003a), dose levels of ≥ 300 mg/kg bw/day were in excess of the maximum tolerated dose and the rabbits were terminated prematurely. The dose level of 150 mg/kg bw/day also induced severe toxicity resulting in the death of 1/8 rabbits, body weight loss and reduced food intake. Four of the 7 pregnant rabbits given this dose had no live foetuses at termination with all implantations being resorbed early in pregnancy (coincident with the onset of dosing). No diaphragmatic malformations were observed up to the top dose level of 150 mg/kg bw/day. A dose of 100 mg/kg bw/day was selected as the highest dose for evaluation in the full study. A lower dose level of 30 mg/kg bw/day was also selected, having shown only minimal differences from control in maternal body weight gain, food intake and foetal body weight.

In the first full OECD- and GLP-compliant developmental toxicity study (2003b – see point (2) under Developmental toxicity in the rabbit in section 4.10.2.1 above), developmental toxicity (reduced foetal weight) was seen at the top dose of 100 mg/kg bw/day in the presence of maternal toxicity (effects on body weight and food consumption). These foetal effects were considered to be the secondary, unspecific consequence of the observed maternal toxicity. However, a low incidence of malformations of the diaphragm was seen from a dose of 30 mg/kg bw/day (1 foetus in 1 litter at 30 mg/kg bw/day and 3 foetuses in 3 litters at 100 mg/kg bw).

Whilst investigating possible reasons for the occurrence of diaphragmatic malformations in the top and mid dose groups, it was noted that information on the parentage and sibling status of the animals was not obtained or utilised in the allocation of the females and male semen donors to the treatment groups, casting a shadow over the reliability/validity of the study. In addition, it was established that the foetuses with malformations of the diaphragm all had the same father (male number 119). In order to further investigate the possible role of genetic influences on the occurrence of the diaphragmatic lesions, two non-standard prenatal developmental toxicity studies (a single-buck study and a multi-buck study) were therefore undertaken.

The purpose of the first study was to investigate the potential role of genetic influences on the occurrence of diaphragmatic effects seen in the previous study by testing whether malformations of the diaphragm (hernia/fissure) could be repeated when using male semen donor no. 119 (2003c – see point (3) under Developmental toxicity in the rabbit in section 4.10.2.1 above). A dose level of 100 mg/kg bw/day was chosen for use in this study as the effect on the diaphragm had been seen at this dose level in the previous study. Groups of 24 female Russian rabbits were used.

Malformations of the foetal diaphragm were not repeated in this study when using the same male parent and the same dose of pinoxaden associated with the finding in the first study. This study

shows that it is unlikely the malformation of the foetal diaphragm originated as a consequence of the genetic make-up of male no. 119.

The purpose of the second study was to further investigate the potential role of the sibling status on the occurrence of diaphragmatic effects seen in the previous study by testing whether or not malformation of the diaphragm (hernia/fissure) could be repeated when excluding male no. 119 and matings between siblings (2003d – see point (4) under Developmental toxicity in the rabbit in section 4.10.2.1 above). A dose level of 100 mg/kg bw/day was chosen again and groups of 24 female Russian rabbits were used.

Malformations of the foetal diaphragm were not repeated in this study which excluded male parent no. 119 and sibling matings but used the same dose of pinoxaden as in the first study. This study shows that when the relationship between the experimental animals is known and the allocation of females and males used for insemination is controlled, malformations of the foetal diaphragm are no longer detected.

In view of these results and considering that the validity of the results from the first full study had been brought into question, a second full prenatal developmental toxicity study was undertaken, in which the potential genetic and familial influences of sibling matings and non-randomised male donors on the results were removed (2003c – see point (5) under Developmental toxicity in the rabbit in section 4.10.2.1 above). This study was a repeat of the first study (2003b – see point (2) under Developmental toxicity in the rabbit in section 4.10.2.1 above), utilising the same dose levels of pinoxaden.

Developmental toxicity (resorptions and post-implantation loss) was seen in this study at the top dose of 100 mg/kg bw/day in the presence of maternal toxicity (clinical signs of toxicity, abortions and effects on body weight and food consumption). These foetal effects were considered to be the secondary, unspecific consequence of the observed maternal toxicity. Maternal effects (reduced body weight and food consumption) were also seen at a dose of 30 mg/kg bw/day. On this basis, the NOAEL for maternal toxicity was set at 10 mg/kg bw/day and the NOAEL for developmental toxicity was established at 30 mg/kg bw/day. No malformations of the foetal diaphragm were observed up to the maternally toxic dose of 100 mg/kg bw/day.

Overall, in the rabbit, the weight of evidence from four prenatal developmental toxicity studies indicates that unspecific developmental toxicity (resorptions, post-implantation loss and reduced foetal weight) occurs at around 100 mg/kg bw/day pinoxaden in the presence of maternal toxicity. These foetal effects are considered to be the secondary, unspecific consequence of the observed maternal toxicity.

A low incidence of malformations of the diaphragm was seen from a dose of 30 mg/kg bw/day (1 foetus in 1 litter at 30 mg/kg bw/day and 3 foetuses in 3 litters at 100 mg/kg bw) in the first study. However, this was not repeated in three subsequent studies (using groups of 24 pregnant females and the relevant dose of 100 mg/kg bw/day) in which genetic and familial influences of sibling matings and non-randomised male donors were removed. Overall, the available evidence suggests that the diaphragmatic malformations seen in the first study might have arisen from matings between siblings or other related individuals. Failure to control for these factors in the first study brings into question the reliability of such findings. Overall, it is considered that pinoxaden has no teratogenic potential or specific developmental effects in the rabbit. An independent review (provided in Annex 1) reaches the same conclusion.

4.10.5 Comparison with criteria

Fertility

The potential effects of pinoxaden on fertility and reproductive performance have been investigated in a guideline multigeneration study in the rat. In this study, no effects on fertility and reproductive performance were seen up to a dose (500 mg/kg bw/day) causing parental toxicity (body weight effects, increased water consumption, liver and kidney effects).

When comparing these findings with the criteria, the following conclusions can be drawn:

Category 1A (known human reproductive toxicant) is not appropriate as *there is no human evidence establishing a causal relationship* between exposure to pinoxaden and an adverse effect on fertility.

Category 1B (presumed human reproductive toxicant) is also not appropriate as *there is no clear evidence of an adverse effect on fertility in experimental animals that is considered not to be the secondary, non-specific consequence of other toxic effects*. No effects on fertility and reproductive performance were seen in a guideline study up to a dose (500 mg/kg bw/day) causing parental toxicity.

Category 2 (suspected human reproductive toxicant) is also not appropriate because *there is no evidence of an adverse effect on fertility in experimental animals that is considered not to be the secondary, non-specific consequence of other toxic effects*. No effects on fertility and reproductive performance were seen in a guideline study up to a dose (500 mg/kg bw/day) causing parental toxicity.

Overall, therefore, classification of pinoxaden for fertility is not warranted.

Development

The developmental toxicity potential of pinoxaden has been investigated in five guideline prenatal developmental toxicity studies, one in rats and four in rabbits.

In the rat, unspecific developmental toxicity (delayed ossification and reduced foetal weights) were seen from a dose of 300 mg/kg bw/day in the presence of maternal toxicity (effects on body weight and food consumption). The developmental effects were considered to be the secondary, unspecific consequence of the observed maternal toxicity.

In the rabbit, the weight of evidence from four prenatal developmental toxicity studies indicates that unspecific developmental toxicity (resorptions, post-implantation loss and reduced foetal weight) occurs at around 100 mg/kg bw/day pinoxaden in the presence of maternal toxicity. These foetal effects are considered to be the secondary, unspecific consequence of the observed maternal toxicity.

A low incidence of malformations of the diaphragm was seen from a dose of 30 mg/kg bw/day (1 foetus in 1 litter at 30 mg/kg bw/day and 3 foetuses in 3 litters at 100 mg/kg bw) in the first study. However, this was not repeated in three subsequent studies (using groups of 24 pregnant females and the relevant dose of 100 mg/kg bw/day) in which genetic and familial influences of sibling matings and non-randomised male donors were removed. Overall, the available evidence suggests that the diaphragmatic malformations seen in the first study might have arisen from matings between siblings or other related individuals. Failure to control for these factors in the first study

brings into question the reliability of such findings. Overall, it is considered that pinoxaden has no teratogenic potential or specific developmental effects in the rabbit.

When comparing these findings with the criteria, the following conclusions can be drawn:

Category 1A (known human reproductive toxicant) is not appropriate as *there is no human evidence establishing a causal relationship* between exposure to pinoxaden and an adverse effect on development.

Category 1B (presumed human reproductive toxicant) is also not appropriate as *there is no clear evidence of an adverse effect on development in experimental animals that is considered not to be the secondary, non-specific consequence of other toxic effects*. Delayed ossification and reduced foetal weights in the rat and resorptions, post-implantation loss and reduced foetal weights in the rabbit were considered to be the secondary, unspecific consequence of the observed maternal toxicity. The diaphragmatic malformations seen in one study in the rabbit are considered to be unrelated to treatment with pinoxaden and are likely to have arisen from matings between siblings or other related individuals.

Category 2 (suspected human reproductive toxicant) is also not appropriate because *there is no evidence of an adverse effect on development in experimental animals that is considered not to be the secondary, non-specific consequence of other toxic effects*. Delayed ossification and reduced foetal weights in the rat and resorptions, post-implantation loss and reduced foetal weights in the rabbit were considered to be the secondary, unspecific consequence of the observed maternal toxicity. The diaphragmatic malformations seen in one study in the rabbit are considered to be unrelated to treatment with pinoxaden and are likely to have arisen from matings between siblings or other related individuals.

4.10.6 Conclusions on classification and labelling

Not classified for fertility or development – conclusive but not sufficient for classification

4.11 Other effects

4.11.1 Non-human information

4.11.1.1 Neurotoxicity

Table 50: Summary of relevant neurotoxicity studies

Method	Results / Remarks	References
Acute neurotoxicity OECD 424 (1997) Oral, Gavage/ vehicle 0.5% CMC, 0.1% Tween 80 in distilled water Rat, Alpk:APfSD Wistar derived Single dose of 0, 100, 500 or 2000 mg/kg bw 10/sex/group NOA407855 technical; Batch No. EZ005006 (97.2 % purity)	**2000 mg/kg bw** No treatment-related effects. NOAEL > 2000 mg/kg bw	2003d DAR B.6.8.1(a)
Subchronic neurotoxicity OECD 424 (1997), Oral, Gavage/ vehicle 0.5% CMC, 0.1% Tween 80 in distilled water Rat, Alpk:APfSD Wistar derived 90 consecutive daily doses, 0, 10, 100 or 500 mg/kg bw/day 12/sex/group NOA407855 technical; Batch No. EZ005006 (97.2 % purity)	**500 mg/kg bw/day** *Clinical observations* ↑ salivation 148 incidences in 12 males, 224 incidences in 12 females (control 0), ↑ signs of salivation 16 incidences in 6 males, 25 incidences in 9 females (control 0) **100 mg/kg bw/day** *Clinical observations* ↑ salivation 62 incidences in 9 males, 44 incidences in 11 females (0 control) Salivation considered a behavioural or anticipatory response to dosing and of no neurotoxicological significance. NOAEL at least 500 mg/kg bw/day	2003e DAR 6.8.1(b)

In an acute neurotoxicity study there were no potential neurotoxic effects at any dose, including the limit dose of 2000 mg/kg/bw. In a subchronic study, oral administration of dose levels up to 500 mg/kg bw/day for at least 90 consecutive days was well tolerated and there were no effects of treatment at doses of up to 500 mg/kg bw/day. The subchronic neuropathological and neurotoxicological NOAEL was considered to be greater than 500 mg/kg bw/day for male and female rats.

4.11.1.2 Immunotoxicity

No information available.

4.11.1.3 Specific investigations: other studies

4.11.2 Human information

No information available.

4.11.3 Summary and discussion

Pinoxaden was examined for evidence of neurotoxic potential in an acute and a subchronic neurotoxicity rat study. There was no evidence for neurotoxic potential in either study, nor was there any evidence of neurotoxic potential from the rest of the animal database.

4.11.4 Comparison with criteria

As there was no evidence for neurotoxic potential, no classification is required.

4.11.5 Conclusions on classification and labelling

CLP: No classification required under STOT-SE or STOT-RE for neurotoxicity

5 ENVIRONMENTAL HAZARD ASSESSMENT

Available environmental fate and ecotoxicology studies have been considered and summarised in the Draft Assessment Report, July 2006 (Volume 3, Annex B8, parts 1-3: Environmental Fate and Behaviour and Volume 3, Annex B9, parts 1-2; Ecotoxicology). The key information pertinent to determining the environmental hazard classification for pinoxaden is presented below. Reference to the DAR is provided.

5.1 Degradation

Table 51: Summary of relevant information on degradation

Method	Results	Remarks	Reference
Hydrolysis OECD 111 / US EPA Subd. N, 161-1 GLP	Hydrolysis pH and temperature dependent: At 15°C, 1st order half-life was 0.6 days at pH9 and 23.3 days at pH 7 At 20°C, 1st order half-life was 0.3 days at pH 9, 14.9 day at pH 7 and 25.3 days at pH 5	Unlabelled: Purity: 97% [phenyl-1-^{14}C]pinoxaden (NOA-407855): Radiochemical purity: 97.5%; 97.4% Specific activity: 1.94 & 1.98 MBq/mg	Phaff, 2003a DAR B.2.1.15
Aqueous photolysis US EPA Subd. N, 161-2 GLP	DT50 : 22.3 days Natural summer sunlight at 30-50°N. Corrected for dark control, irradiated DT50 : 10.1 days	Unlabelled NOA-407855: Purity: 97.0% [phenyl-1-^{14}C] pinoxaden (NOA-407855): Purity: 98.8% Specific activity: 30.0 MBq/mg. Conducted at 25°C	Reischmann, 2001 DAR B.2.1.16
Quantum yield for direct phototransformation Aqueous photolysis OECD 101 and EPA guidelines OPPTS 835.2210 GLP	ϕ = 0.0117±0.0005 Half-life range from 82.2 days in summer at 30°N to 954 days in winter at 50°N	Purity 97.00 ± 2.0%	Schmidt, 2003 DAR B.2.1.17
Ready biodegradation OECD Guideline No.301B GLP	12% degradation by day 29, therefore 'not readily biodegradable'	Purity 95.7%. Conducted at 20°C	Grade, 2000 DAR B.8.4.3

<table>
<tr><th>Method</th><th>Results</th><th>Remarks</th><th>Reference</th></tr>
<tr>
<td>Water/sediment study
OECD draft (2000) and BBA (1990)
GLP</td>
<td>
<table>
<tr><td></td><td>Water kw DT50 (days)</td><td>Sediment ks DT50 (days)</td></tr>
<tr><td colspan="3"><u>River</u></td></tr>
<tr><td>pinoxaden (NOA 407855)</td><td>0.26</td><td>0.77</td></tr>
<tr><td>NOA 407854 (M2)</td><td>No degradation*</td><td>64.5</td></tr>
<tr><td colspan="3"><u>Pond</u></td></tr>
<tr><td>pinoxaden (NOA 407855)</td><td>0.28</td><td>2.0</td></tr>
<tr><td>NOA 407854 (M2)</td><td>No degradation*</td><td>64.8</td></tr>
</table>
* Rate constant was 0
Pinoxaden whole system DT50 < 1 day in both systems
</td>
<td>Purity 97.00 ± 2.0%
[phenyl-1-^{14}C]pinoxaden (NOA-407855):
Radiochemical purity: 96.2%
Specific activity: 1.98 MBq/mg.
Conducted at 20°C and water pH 8.1-8.3</td>
<td>Adam, 2003a
DAR B.8.2.1</td>
</tr>
<tr>
<td>Water/sediment study
OECD draft (2000) and BBA (1990)
GLP</td>
<td>Pinoxaden whole system DT50 ≤0.7 days in river and pond systems, in the dark or in sunlight equivalent to 30°-50°N
(Takes into account the combined effects of biodegradation and photolysis in a water / sediment system.)</td>
<td>Unlabelled pinoxaden (NOA-407855):
Purity 97.00 ± 2.0%
Oxadiazepine 3,6-14C pinoxaden (NOA-407855):
Radiochemical purity: 98.9%
Specific activity: 2.0 MBq/mg.
Conducted at 20°C and water pH 7.2-7.4</td>
<td>Adam, 2003b
DAR B.8.4.4.1</td>
</tr>
</table>

5.1.1 Stability

Hydrolysis

One sterile aqueous hydrolysis study is available showing that pinoxaden would be expected to hydrolyse very rapidly only when surface water pH was relatively high. At neutral and lower pH values the hydrolysis rate would be more moderate.

Study 1 (Phaff, R 2003a)

Using phenyl ^{14}C radiolabelled pinoxaden (NOA 407855) and in accordance with the principals of GLP, following guidelines OECD (OECD 111, 1981) and EPA (161-1, 1982) hydrolysis was

conducted at various pH and at temperatures of 15°C (pH 7 and 9), 25°C and 50°C (pH 4, 5, 7, and 9), and 60°C (pH 4 and 5) in sterile buffer solutions in the dark for 30 days.

Pinoxaden was found to hydrolyse at all four pH values and all temperatures, but hydrolysis was found to be pH dependent and was greatly accelerated under alkaline conditions with (calculated), 1st order half-life being 0.2 days at pH 9, but up to 25.3 days at pH 5 at 20°C.

At pH 7, pinoxaden had a calculated half-life at 20°C of 14.9 days. Consequently, under environmental conditions, pinoxaden would be expected to hydrolyse very rapidly only when surface water pH was relatively high. Under most environmental conditions the hydrolysis rate would be more moderate. The mass balance was > 94.8 % AR throughout, indicating that there was no major production of volatiles.

Table 52: Hydrolytic half-lives (days) of pinoxaden under various laboratory conditions

Temperature	pH 4	pH 5	pH 7	pH 9
15°C	n.p.	n.p.	23.3	0.6
20°C (calculated)	24.1	25.3	14.9	0.3
25°C	17.2	17.5	9.9	0.2
50°C	3.9	3.5	1.2	< 0.2
60°C	2.2	1.9	n.p.	n.p.

n.p. = not performed

Photolysis

Two aquatic photolysis studies (one aqueous photolysis study and one quantum yield of direct photochemical degradation study) are available showing that pinoxaden undergoes limited photodegradation and is considered photolytically stable under environmentally relevant conditions for the purposes of classification. Photolysis is not an issue for interpretation of the algal toxicity tests.

Study 1 (Reischmann, 2001)

Following GLP and to EPA (161-2, 1982) guidelines, the photolysis of phenyl ^{14}C radiolabelled pinoxaden was assessed at 25°C in sterile buffer solution at pH 4.3. This is considered an appropriate pH because pinoxaden (NOA 407855) was relatively stable to hydrolysis at pH 4-5. A xenon arc lamp, filtered for wavelengths <290 nm, was used to irradiate the samples using 12 hour light/dark cycles for a period considered to be equivalent to 29.5 days natural summer sunlight at latitudes of 30-50°N.

Pinoxaden degraded with a DT50 of 10.1 days (summer sunlight) in irradiated samples, compared with a DT_{50} of 18.4 days in the dark control. The net photolysis rate was 22.3 days and thus it can be concluded that pinoxaden (NOA 407855) undergoes photolysis at a moderate rate. Hydrolysis is more likely to be a more significant component of degradation (particularly at higher pH).

The major photolytic metabolite of pinoxaden was NOA 407854 (M2), which reached a maximum concentration of 35.2 % AR in the irradiated samples. A number of minor metabolites were also detected including NOA 447204 (M3) (maximum 1.6 % AR), NOA 440626 (maximum 3.1 % AR), and an unknown fraction (maximum 4.9 % AR). None of the minor metabolites exceeded 4.9 % AR.

Study 2 (Schmidt, 2003)

Following OECD (101) and EPA (OPPTS 835.2210) guidelines (to GLP), the quantum yield of the direct photochemical degradation of pinoxaden (NOA 407855) was investigated in buffered aqueous solution at a pH of 7.3 to 7.4 containing 10% acetonitrile, added as co-solvent.

The theoretical aquatic photolytic half-life of pinoxaden (NOA 407855) was calculated using the computer program GCSolar. In shallow water bodies, for various seasons and latitudes, this ranged from 82.2 days in summer at 30°N to 954 days in winter at 50°N.

The quantum yield for direct phototransformation was determined to be $\phi = 0.0117 \pm 0.0005$.

Table 53: Photolytic half-life times of pinoxaden (NOA 407855) for different latitudes and seasons (days) in shallow waters.

	30°N	40°N	50°N
Spring	97.0	115	145
Summer	82.2	88.2	98.5
Autumn	114	207	355
Winter	219	399	954

5.1.2 Biodegradation

5.1.2.1 Biodegradation estimation

Estimation not applicable as studies are available

5.1.2.2 Screening tests

Study 1 (Grade, 2000)

This ready biodegradation test was conducted to GLP in activated sludge at 20°C following OECD Guideline 301B. It resulted in 12% degradation (based on theoretical carbon dioxide) at day 29. Substances are only considered to be readily biodegradable in this test if CO_2 production is > 60 % of the theoretical level within 10 days of achieving the 10% level. On this basis, it is concluded that pinoxaden is not 'readily biodegradable'. Pinoxaden is, however, not expected to be persistent in aquatic environments due to its rapid degradation as demonstrated by DT50 values of <1 day in water/sediment systems - see below.

5.1.2.3 Simulation tests

Study 1 (Adam, 2003a)

In a GLP, water/sediment study following OECD (2000 draft) and BBA (1990) guidelines (equivalent to OECD 308), phenyl ^{14}C-radiolabelled pinoxaden was applied to a river system (Rhein) with a loam / sandy loam sediment and a pond system (Rotenfluh) with a silty clay loam sediment over a period of 147 days. The water pH at sampling was 8.3 for the river sample and 8.1

for the pond sample. The study was performed under both aerobic and artificially induced anaerobic conditions both at 20°C. Results from the anaerobic incubation are not considered further here.

Pinoxaden (NOA 407855) degraded rapidly, forming NOA 407854 (M2), in both the river and the pond systems, with DT50 and DT90 values in the water and in the total system of less than 1 day. The relatively high pH of these systems may have encouraged hydrolysis. The DT50 in the sediment was a maximum of 2 days in the pond system. The majority of pinoxaden (NOA 407855) remained in the water phase, where it degraded. Partitioning to sediment was weak, as would be expected given the low K_{OC} of the active substance. The maximum concentration of pinoxaden (NOA 407855) in the sediment was only 1.7 % AR in the river system and 0.2 % AR in the pond system.

Given that the water DT50 and DT90 values for NOA 407854 (M2) did not correspond to simple first order kinetics, the pesticide Rapporteur under Reg 1107/2009 calculated simple first order dissipation values for the water phase from peak formation at day 7 in each aerobic system. Half-lives are 294.4 days (DT90 = 984.7 days, r^2 = 0.851) for the River system and 128.8 days (DT_{90} = 427.9 days, r^2 = 0.864) for the Pond system. The first order DT50 values for degradation in water (kw) and sediment (ks) are given below.

Table 54: True degradation rates in aerobic water/sediment system for NOA 407855 and NOA 407854 (M2)

	Water k_w DT50 (days)	**Sediment k_s DT50 (days)**
River		
pinoxaden (NOA 407855)	0.268	0.774
NOA 407854 (M2)	No degradation*	64.474
Pond		
pinoxaden (NOA 407855)	0.276	2.000
NOA 407854 (M2)	No degradation*	64.793

* Rate constant was 0

Mineralisation was only a minor element of dissipation. At the end of the study, 147 days after application, a maximum of 4.1 % AR CO_2 was trapped from the pond system. Organic volatiles were below the limit of detection in both systems. Incorporation into non-extracted sediment residues is a further route of dissipation, with up to 14.1 % AR being present in sediment organic matter after 147 days of incubation in the pond system.

Study 2 (Adam, 2003b)

A GLP, aerobic aquatic/sediment study using oxadiazepine-ring radiolabelled ^{14}C-pinoxaden following OECD (2000 draft) and BBA (1990) guidelines is available (equivalent to OECD 308). This further was conducted in the dark and also under both artificial and natural light conditions, in the same river and pond systems as described above over a period of 100 days at 20°C. The River system used a loam sediment and the water pH was 7.4. The pond system had a silty, clay loam sediment with water pH of 7.2. In both systems the water and the sediment phase were aerobic.

Under these conditions, pinoxaden (NOA 407855) degraded following largely by the same route as for the previous aquatic/sediment study. In all conditions, pinoxaden (NOA 407855) degraded rapidly with a DT50 of <1 day in all compartments. NOA 407854 (M2) degraded slowly in the dark incubations; similar to the previous study. The DT50 for NOA 407854 (M2) was >1 year in the water compartment, 183 days in the sediment, and > 1 year for the total system for the river. For

the pond system, the DT50 was again slightly shorter, with values of 154 days (water), 97 days (sediment) and 270 days (total system).

The results of the illuminated incubations indicate that under suitable conditons photolysis contributes significantly to the degradation of NOA 407854 (M2) in water/sediment systems. In this study, the DT50 of NOA 407854 (M2) was reduced to 24.6 days (natural summer sunlight at 30 – 50 ºN) for the river system, and 25.7 days (natural summer sunlight at 30 – 50 ºN) for the pond system. Since pinoxaden (NOA 407855) is not applied in summer, it was suggested for pesticide registration that an average DT50 for NOA 407854 (M2) of 43 days is appropriate. This degradation rate would take into account the combined effects of biodegradation and photolysis in a water/sediment system.

Table 55: Dissipation rates of pinoxaden (NOA 407855) and NOA 407854 (M2) in the water/sediment study with pinoxaden (NOA 407855) (with and without irradiation).

	Compartment	Half-life pinoxaden (NOA 407855) (days)		Half-life NOA 407854 (M2) (days)	
		measured	30°-50°N	measured	30°-50°N
River (Dark)	Water	0.6	not applicable	> 1 year	not applicable
	Sediment	0.1		183.2	
	Total System	0.7		>1 year	
River (Artificial sunlight)	Water	0.7	0.2	111.1	33.3
	Sediment	0.2	0.05	84.4	25.3
	Total System	0.7	0.2	112.3	33.7
River (Natural sunlight)	Water	0.4	0.1	131.7	22.4
	Sediment	0.2	0.03	73.5	12.5
	Total System	0.4	0.1	144.7	24.6
Pond (Dark)	Water	0.4	not applicable	154.2	not applicable
	Sediment	0.2		96.7	
	Total System	0.4		269.7	
Pond (Artificial sunlight)	Water	0.6	0.2	63.6	22.3
	Sediment	0.1	0.04	44.3	15.5
	Total System	0.6	0.2	64.5	22.6
Pond (Natural sunlight)	Water	0.1	0.02	102.1	17.4
	Sediment	0.3	0.05	126.0	21.4
	Total System	0.2	0.03	151.3	25.7

5.1.3 Summary and discussion of degradation

Hydrolysis of pinoxaden was investigated in buffered sterile solutions at pHs between 4 and 9. Hydrolysis was pH and temperature dependent, being faster at alkaline pH values and higher temperatures. The main hydrolysis metabolite was M2 and this was stable to hydrolysis in all conditions tested.

Aqueous photolysis of ^{14}C phenyl labelled pinoxaden under simulated sunlight was not significant in relation to hydrolysis rate under the environmental conditions represented. However, photolysis may contribute to the environmental degradation of M2 in aqueous media.

Pinoxaden should be considered not readily biodegradable according to the available study, which showed 12% degradation by day 29.

Degradation of pinoxaden was investigated in two water/sediment systems under aerobic conditions. Degradation of pinoxaden was rapid in both systems (DT50 whole system < 1 day) with practically no partitioning to the sediment. Metabolite M2 is the only major metabolite identified in both the water and the sediment phases and it was shown to be persistent. It has been questioned whether the rapid degradation of pinoxaden seen in the water/sediment studies is representative, since one of them at least (Adam, 2003a) was conducted at a high pH and this was seen to significantly increase sterile hydrolysis, which might be a predominant route of degradation.

In Adam (2003a) the water pH was 8.3 for the river system and 8.1 for the pond system. Whole system DT50 values in this first study were 0.27-0.28 days (the recalculated 1st order water phase DT50s were 0.268-0.276 days). In the second simulation study Adam (2003b) conducted in the dark as well as under artificial and natural sunlight, the same river and pond systems as in the first study were used but the pH of the river system water was 7.4 and for the pond system it was 7.2. Under these conditions, pinoxaden degraded at a similar rapid rate as in the previous study. The degradation DT50 was <1 day in all compartments (actual values in water and whole system in the dark: 0.4-0.7 days; in the light: 0.1-0.7 days (0.02-0.2 recalculated at 30°-50°N).

Given that whole system DT50s were still less than 1 day and similar at pH 7.2-7.4 as at pH 8.1-8.3, pH doesn't appear to make such a difference to degradation in non-sterile whole sediment water/systems. This may be due to a combination of the influence of biotic degradation, the presence of sediment and photolysis in the illuminated systems, although in isolation these processes make less difference. This decreased relevance of pH in more natural biotic systems is borne out by a statement made in the Summary and assessment of water degradation studies at B.8.5 in the pinoxaden DAR (Vol.3 p583) where the influence of pH was considered as follows:

> 'Since marked pH dependence was observed for the active substance and its metabolites in hydrolysis studies, it might be expected that the dissipation in both of these systems would be influenced by hydrolysis, and that at lower pH values, dissipation would be much slower. However, laboratory soil route and rate of degradation studies conducted on the active substance using a wide range of soils with varying pH, both acidic and alkaline [pH 5-8], indicated that the active substance degraded rapidly with little or no pH influence. This would suggest that in environmentally relevant, microbially active systems, pH effects on hydrolysis would be unlikely to be pronounced. Given that aqueous systems in the environment would be microbially active, pinoxaden would be expected to degrade rapidly in aqueous systems regardless of pH. Therefore, the rapporteur suggests that [whilst one] water / sediment study was conducted at a pH at which pinoxaden was likely to hydrolyse more rapidly, the study is still relevant.'

This argument was accepted in the pesticide exposure modelling and risk assessment for pinoxaden, which is intended to cover a naturally occurring range of environmentally relevant pH. Therefore, it is proposed to be acceptable for hazard classification also.

Mineralisation was only a minor element of dissipation of pinoxaden in these water/sediment systems. On this basis alone pinoxaden would not be considered to undergo rapid ultimate degradation and would be considered 'not rapidly degradable'. However, pinoxaden is not expected to be persistent in aquatic environments due to its rapid degradation as demonstrated by DT50 values of <1 day in the water/sediment systems. Pinoxaden does not degrade directly to CO_2 but to other unclassified degradants (see Table 58 in 5.4), therefore production of <60% CO_2 does not mean that pinoxaden as a molecule will persist in aquatic environments. As a result it is

proposed that pinoxaden be considered 'rapidly degradable' for the purposes of hazard classification under CLP. This is discussed further in Section 5.5.

5.2 ENVIRONMENTAL DISTRIBUTION

5.2.1 Adsorption/Desorption

Table 56: Summary of relevant information on adsorption/desorption

Method	Results	Remarks	Reference
Absorption/desorption OECD Guideline (106, 2000) and EPA(Subdivision N, Series 163-1, 1982) GLP	K_{FOC}– 173 - 323(mL/g)	Unlabelled: Purity 97.00 ± 2.0% [phenyl-1-^{14}C]pinoxaden (NOA-407855): Purity 98.6% Specific activity: 1.98 MBq/mg	Adam, 2002 DAR B.8.2.1
EPA (Subdivision N, Series 163-1, 1982) GLP	K_{FOC}– 299 – 852 (ml/g)	[phenyl-1-^{14}C]pinoxaden (NOA-407855) Chemical purity >99.9% Radiochemical purity: 98.8% Specific activity: 48.8 µCi/mg	Spare, 2003 DAR B.8.2.1
EPA (Subdivision N, Series 163-1, 1982) GLP	desorption coefficient – 2.9 to 24	[phenyl-1-^{14}C]pinoxaden (NOA-407855) Chemical purity 98.3% Radiochemical purity: 99% Specific activity: 51.3 µCi/mg	Moore, 2003 DAR B.8.2.1

Study 1 (Adam, 2002)

The adsorption of ^{14}C-phenyl pinoxaden (NOA 407855) was assessed in four soils following OECD Guideline 106 (2000) and EPA Guideline Subdivision N, Series 163-1 (1982), to GLP.

Adsorption values were in the range 173 to 323 mL/g K_{FOC} (sandy loam with 1% organic carbon to sandy clay loam with 2.5% organic carbon).

Study 2 (Spare, 2003)

The adsorption of ^{14}C-phenyl pinoxaden (NOA 407855) was assessed in four soils following EPA Guideline Subdivision N, Series 163-1 (1982), to GLP.

Adsorption values were in the range 299 to 852 mL/g K_{FOC} (loamy sand with 1.2% organic carbon to silty clay loam with 1.0% organic carbon).

Study 3 (Moore, 2003)

The adsorption of ^{14}C-phenyl pinoxaden (NOA 407855) was assessed in four soils following EPA Guideline Subdivision N, Series 163-1 (1982), to GLP.

Due to rapid degradation, desorption coefficients could only be calculated up to the day six sampling point and these ranged from 2.9 to 24. From this study, it was concluded that there was no significant increase in desorption coefficient for pinoxaden (NOA 407855) after an ageing period of six days.

Summary

For the parent substance, nine soils in two separate studies were used with pH ranging from 5.1 to 7.5 and organic carbon contents between 0.35 % and 3.2 %. Adsorption K_{FOC} ranged from 121 mL/g to 852 mL/g (with a median value of 323 mL/g). 1/n values ranged from 0.93 to 1.12 (median 1.03). Since pinoxaden (NOA 407855) does not dissociate, a relationship between pH and K_{OC} was not expected, and none was observed. According these studies, pinoxaden may be classified to have high to medium mobility in soil and is unlikely to partition preferentially to sediment and soil.

5.2.2 Volatilisation

Three studies in the pesticides DAR(Nicollier, 2003a; DAR B.8.2.3, Nicollier, 2003b; DAR B.8.2.4 and Widmer, 2003; DAR B.8.7) indicate pinoxaden (NOA 407855) has a vapour pressure of 2.0×10^{-7} Pa at 20°C (Geoffroy, 2003a; DAR B.2.1.5) and Henry's Law Constant of 9.2×10^{-7} Pa.m^3/mol at 25°C (Stulz, 2003; DAR B.2.1.6). These values indicate that the active substance has a low propensity to volatilise from soil or water. This is confirmed by the results of the tests on volatilisation from soil and plant surfaces where there was virtually no loss of applied radioactivity by volatilisation over a 24 hour period.

The atmospheric half-lives calculated by the method of Atkinson (1.1 hours) suggest that even if the active substance were to volatilise, degradation would be rapid and the risk of long range transport would be low.

5.2.3 Distribution modelling

Not included in this Report.

5.3 AQUATIC BIOACCUMULATION

5.3.1 Aquatic bioaccumulation

The log Kow of pinoxaden (NOA 407855) is 3.2 (Part B Section 1.3 above), hence the potential for bioaccumulation was considered with respect to application for inclusion under Reg. 1107/2009. Since the DT50 values in water and sediment (water DT50 of 0.28 days, sediment DT50 of 2.0 days) are all ≤2 days there was considered to be a limited potential for exposure and hence bioaccumulation. Furthermore, the results from the mammalian adsorption, distribution, metabolism and excretion studies indicate a low potential for bioaccumulation and extensive metabolism and excretion within a short time period for pinoxaden (as confirmed by Part B Section 4.1.3).

The pesticides Rapporteur under Reg. 1107/2009 agreed that the bioaccumulation potential for pinoxaden (NOA 407855) is low and does not require further consideration.

5.3.1.1 Bioaccumulation estimation

The log Kow of pinoxaden (NOA 407855) is 3.2 (Part B Section 1.3). Under CLP no concern is highlighted as this value is <4. It is further noted from the pinoxaden DAR and EFSA Conclusion that the log Kow for degradants M2 and M3 are -1.1 and 1.8 respectively.

5.3.1.2 Measured bioaccumulation data

No studies available and does not require consideration.

5.3.2 Summary and discussion of aquatic bioaccumulation

Although the log Kow of pinoxaden (NOA 407855) being 3.2 required further consideration in relation to its pesticidal use, under CLP no concern is highlighted as the log Pow is <4.

Within the context of this submission, the bioaccumulation potential of pinoxaden and its degradants is concluded to be low and does not require further consideration.

5.4 AQUATIC TOXICITY

Unless otherwise stated, all of the ecotoxicological studies on pinoxaden were performed reliably and to GLP and are considered suitable for hazard classification purposes, these are summarised in Table 57 below. Data are also available in the pesticide DAR and EFSA Conclusion (Pinoxaden; EFSA Scientific Report 2013; 11 (8):3269) on the main degradants M2 (NOA 407854) and NOA447204 (M3), these are summarised in Table 58.

Table 57: Summary of relevant information on the aquatic toxicity of pinoxaden

Substance (purity)	Species	Test guidelines	Endpoint	Toxicity value	Conditions	Reference
Acute toxicity to fish						
Pinoxaden (97.2%)	*Oncorhynchus mykiss*	OECD 203	96 hr LC50	10.3 mg a.s./L (mean measured)	Flow-through	2000a DAR B.9.2.1.1 – fish (a)
Pinoxaden (97.2%)	*Pimephales promelas*	OECD 203	96 hr LC50	20 mg a.s./L (mean measured)	Flow-through	2003 DAR B.9.2.1.1 – fish (b)
Pinoxaden (97.7%)	*Cyprinodon variegatus*	US EPA OPPTS 850.1075	96 hr LC50	>16 mg a.s./L (mean measured)	Flow-through	2003a DAR B.9.2.1.1 – fish (c)
Prolonged toxicity to fish						
Pinoxaden (97.2%)	*Oncorhynchus mykiss*	OECD 215	28 d NOEC growth 28 d NOEC mortality	6.6 mg a.s./L 3.2 mg a.s./L (mean measured)	Flow-through	2000b DAR B.9.2.2.1 (a)
Acute toxicity to aquatic invertebrates						
Pinoxaden (97.2%)	*Daphnia magna*	OECD 202	48 hr EC50	52 mg a.s./L (mean measured)	Flow-through	Knauer, 2003 DAR B.9.2.1.1 – invertebrates (a)
Pinoxaden (97.7%)	*Americamysis bahia*	US EPA OPPTS 850.1035	96 hr LC50	8.3 mg a.s./L (mean measured)	Flow-through	Palmer *et al.*, 2003b DAR B.9.2.1.1 – invertebrates (b)
Pinoxaden (97.7%)	*Crassostrea virginica*	US EPA OPPTS 850.125	96 hr LC50 96 hr EC50 (shell deposition)	>0.88 mg a.s./L 0.40 mg a.s./L (mean measured)	Flow-through	Palmer *et al.*, 2003c DAR B.9.2.1.1 – invertebrates (c)

Substance (purity)	Species	Test guidelines	Endpoint	Toxicity value	Conditions	Reference
Toxicity to algae						
Pinoxaden (97.2%)	*Pseudo-kirchneriella subcapitata*	OECD 201	72 h E_rC50 72 h NOE_rC	41 mg/L 8.0 mg/L (nominal)	Static	Knauer, 2002a DAR B.9.2.1.1 –algae (a)
Pinoxaden (97.2%)	*Anabaena flos -aquae*	OECD Draft Guideline (1996)	96 h E_rC50 96 h NOE_rC	16.4 mg/L 1.25 mg/L (nominal)	Static	Grade, 2003a DAR B.9.2.1.1 –algae (b)
Pinoxaden (97.2%)	*Navicula pelliculosa*	US EPA OPPTS 850.5400	72 h E_rC50 96 h NOE_rC	14 mg/L 7.5 mg/L (nominal)	Static	Maynard, & Stewart, 2002 DAR B.9.2.1.1 –algae (d)
Pinoxaden (97.2%)	*Skeletonema costatum*	OECD 201	72 h E_rC50 72 h E_rC50 72 h NOE_rC 72 h NOE_rC	1.72 mg/L (nominal) 0.80 mg a.s./L (mean measured pinoxaden only) 0.94 mg/L (nominal) 0.52 mg a.s./L (mean measured pinoxaden only)	Static	Swarbrick & Maynard, 2002 DAR B.9.2.1.1 –algae (c)
Toxicity to higher aquatic plants						
Pinoxaden (97.2%)	*Lemna gibba*	Draft OECD 221	7 d E_rC50 (frond no.) 7 d NOE_rC (frond no. & dry weight)	13.9 mg/L (nominal) 9.7 mg a.s./L (initial measured) 0.625 mg/L (nominal) 0.438 mg a.s./L (initial measured)	Static	Grade, 2002 DAR B.9.2.1.1 – higher plants (a)
Pinoxaden (97.2%)	*Phragmytes australis*	Based on draft OECD 221	20 d E_rC50 (growth - plant height) 20 d NOE_rC (height, biomass and chlorosis)	8.5 mg/L 3.0 mg/L (nominal)	Static	Knauer, 2002b DAR B.9.2.1.1 – higher plants (b)

Table 58: Summary of relevant information on the aquatic toxicity of pinoxaden degradants

Substance (purity)	Species	Test guidelines	Endpoint	Toxicity value	Conditions	Reference
Acute toxicity to fish						
NOA 407854 (M2)	*Oncorhynchus mykiss*	OECD 203	96 h LC50	>100 mg/L (nominal)	Static	1999 DAR B.9.2.1.2 – fish (b)
NOA 447204 (M3)	*Oncorhynchus mykiss*	OECD 203	96 h LC50	>120 mg/L (nominal)	Static	2001a DAR B.9.2.1.2 – fish (b)
Chronic toxicity to fish						
NOA407854 (M2)	*Pimephales promelas*	US EPA OPPTS 850.1400	32 d NOEC	1.0 mg/L (highest nominal conc.n tested, i.e. ≥1.0 mg/L)	Flow-through	2003 DAR B.9.2.2.2 – fish (a)
Acute toxicity to aquatic invertebrates						
NOA 407854 (M2)	*Daphnia magna*	OECD 202	48 h EC50	>100 mg/L (nominal)	Static	Grade, R., 2000a DAR B.9.2.1.2 – invertebrates (a)
NOA 447204 (M3)	*Daphnia magna*	OECD 202	48 h EC50	>120 mg/L (nominal)	Static	Wallace, S., J., 2001b DAR B.9.2.1.2 – invertebrates (b)
Chronic toxicity to aquatic invertebrates						
NOA407854 (M2)	*Daphnia magna*	OECD 211	21 d NOEC	6.25 mg/L (nominal)	Semi-static	Bätscher R., 2003 DAR B.9.2.2.2 (b)

Toxicity to algae						
NOA 407854 (M2)	*Pseudo-kirchneriella subcapitata*	OECD 201	72 h E_rC50 72 h NOE_rC	>100 mg/L 100 mg/L (nominal)	Static	Grade R., 2000b DAR B.9.2.1.2 – algae (a)
NOA 447204 (M3)	*Pseudo-kirchneriella subcapitata*	OECD 201	96 h E_rC50 72 h NOE_rC	>120 mg/L 15 mg/L (nominal)	Static	Wallace, S. J., 2001c DAR B.9.2.1.2 – algae (b)
Toxicity to higher aquatic plants						
NOA 407854 (M2)	*Lemna gibba*	OECD 221	7 d E_rC50 (frond no.) 7 d NOE_rC (frond no. & dry weight)	14.6 mg/L 4.0 mg/L (nominal)	Static	Grade, R., 2000c DAR B.9.2.1.2 – higher plants (a)
NOA 447204 (M3)	*Lemna gibba*	OECD 221	7 d E_rC50 (frond no.) 7 d NOE_rC (frond no. & dry weight)	>100 mg/L 50 mg/L (nominal)	Static	Grade, R., 2003b DAR B.9.2.1.2 – higher plants (b)

5.4.1 Fish

5.4.1.1 Short-term toxicity to fish

Study 1 (2000a)

The acute toxicity to rainbow trout (*Oncorhynchus mykiss*) was determined under flow-through conditions following OECD Guideline 203. The nominal exposure concentrations of pinoxaden (purity 97.2 %) were 3.5, 5.7, 9.1, 15 and 23 mg/L. The mean measured concentrations of pinoxaden were 58-65% of nominal and were 2.28, 3.71, 5.80, 8.76 and 14.47 mg a.s./L. Based on mean measured concentrations, the 96 h LC50 was 10.3 mg a.s./L. Sub-lethal effects were observed at mean measured concentrations above 5.8 mg a.s./L and the 96 h NOEC was therefore 5.8 mg a.s./L.

Study 2 (2003)

The acute toxicity to the fathead minnow (*Pimephales promelas*) was determined under flow-through conditions following OECD Guideline 203. The nominal exposure concentrations of pinoxaden (purity 97.2 %) were 3.2, 5.6, 10, 18 and 32 mg /L. The mean measured concentrations of pinoxaden were 71.8 to 100% of nominal and were 3.0, 5.4, 9.2, 16 and 24 mg a.s./L. Based on mean measured concentrations, the 96 h LC50 was 20 mg a.s./L. Sub-lethal effects were observed at mean measured concentrations above 16 mg a.s./L and the 96 h NOEC was 16 mg a.s./L.

Study 3 (2003a)

The acute toxicity to the sheepshead minnow (*Cyprinodon variegatus*) was determined under flow-through conditions following the Guideline US EPA OPPTS 850.1075. The nominal exposure concentrations of pinoxaden (purity 97.7%) were 2.6, 4.3, 7.2, 12 and 20 mg/L. The measured concentrations of pinoxaden ranged from 78.1 to 94.0% of nominal and the mean measured test concentrations were 2.1, 3.5, 6.7, 9.9 and 16 mg a.s./L. No mortality or sub-lethal effects were observed in the controls or at any treatment level. Based on mean measured concentrations, the 96 h LC50 was >16 mg a.s./L. The 96 h NOEC was a measured 16 mg a.s./L. (Note - the EFSA Conclusion for pinoxaden lists the LC50 as 16 mg/L but this is a mistake).

5.4.1.2 Long-term toxicity to fish

Pinoxaden has a whole water/sediment system DT50 of < 1 day and therefore will not exist for long in the aquatic environment. The Rapporteur for the pesticide risk assessment under Reg. 1107/2009 considered it reasonable to assume that short-term exposure to pinoxaden (as possible in the environment prior to degradation) would not lead to any long-term sub-lethal effects in fish. This justification was accepted in the DAR and EFSA peer review as a reason for not requiring a long-term/chronic fish toxicity study on pinoxaden (a chronic fish study on the more persistent M2 degradant was available). Nevertheless, a valid GLP fish toxicity study has been made available since the pesticide DAR was produced (2000b). This is a prolonged juvenile fish growth test to OECD 215 rather than an early life stage or longer chronic study but for a substance with a relatively short aquatic DT50 and low bioaccumulation potential, this study is considered suitable for use in chronic classification.

Study 1 (2000b)

Juvenile trout (*Oncorhynchus mykiss*) were exposed to nominal levels of 0.44, 0.88, 1.8, 3.5, 7.0 and 14 mg /L pinoxaden (purity 97.2%) in a flow-through test for 28 days following OECD guideline 215. DMF was used to prepare the solutions. The concentrations of pinoxaden (NOA 407855) were maintained at 80-120% of nominals throughout the study but the study author has expressed biological endpoints in terms of mean measured concentrations, which were 4.12, 0.859, 1.74, 3.20, 6.58, 12.9 mg a.s./L.

No signs of sub-lethal effects were observed at any test concentration. After 28 days of exposure the average weight in the control group was reported to have increased by 256%. A statistically significant increase in mortality was reported at the two highest test concentrations. At the highest concentration with survival (6.58 mg a.s./L measured), no statistically significant effects were reported for individual specific growth rates (in terms of body weight). Thus, the 28-day NOEC based on growth or other sub-lethal effects was 6.58 mg a.s./L. The NOEC for mortality was however 3.2 mg a.s./L based on mean measured concentrations.

5.4.2 Aquatic invertebrates

5.4.2.1 Short-term toxicity to aquatic invertebrates

Three valid GLP studies assessing the short-term toxicity of pinoxaden to aquatic invertebrates are available.

Study 1 (Knauer, K 2003)

The acute toxicity of pinoxaden (purity 97.2%) to *Daphnia magna* was assessed following GLP and to OECD Guideline 202 in a flow-through test design for 48 hours. Exposure solutions of nominally 7.5, 15, 30, 60, and 120 mg a.s./L and an untreated control were included. The mean measured concentrations of pinoxaden were 75-80% of nominal and were 5.6, 11.6, 23.9, 45.4 and 92.9 mg a.s./L. There was no mortality (immobility) in the control group and no sub-lethal effects observed in either the control or the test groups. After 48 hours, immobility of 5 and 100% respectively was however observed in the top two test concentrations. The 48h EC50 of pinoxaden (NOA 407855) to *Daphnia magna* was 52 mg a.s./L based on immobility and mean measured concentrations. The 48h NOEC, also based on immobility, was a measured 23.9 mg a.s./L.

Study 2 (Palmer, S. J., Kendall, T. Z. and Krueger, H. O., (2003b) Knauer, 2003b)

The acute toxicity of pinoxaden (purity 97.7%) to mysids (*Americamysis bahia*) was assessed following GLP and US EPA OPPTS 850.1035 in a flow-through test design for 96 hours. Exposure solutions of nominally 2.6, 4.3, 7.2, 12 and 20 mg a.s./L and an untreated and solvent control (DMF) were included. The measured concentrations of pinoxaden ranged from 76.1 to 100% of nominal and overall mean measured test concentrations were 2.4, 3.9, 6.5, 9.7 and 18 mg a.s./L. Sub-lethal effects, as well as mortality, were reported at test concentrations at and above 6.5 mg pinoxaden/L and therefore the NOEC was a measured 3.9 mg pinoxaden/L. The 96h LC50 was 8.3 mg pinoxaden/L based on measured concentrations.

Study 3 (Palmer, S. J., Kendall, T. Z. and Krueger, H. O., (2003b) Knauer, 2003b)

The effects of pinoxaden (purity 97.7%) on shell deposition in Eastern oysters (*Crassostrea virginica*) was assessed following GLP and US EPA OPPTS 850.1025 in a flow-through test design for 96 hours. Exposure solutions of nominally 0.063, 0.13, 0.25, 0.50, 1.0 mg a.s./L and an untreated and solvent (DMF) control were included. The measured results of pinoxaden over 96 hours indicated that the actual concentrations ranged from 67.7 to 91.5% of nominal. The overall mean measured test concentrations were 0.046, 0.097, 0.18, 0.43 and 0.88 mg a.s./L.

Sub-lethal signs of toxicity on shell deposition were observed from 0.097 mg pinoxaden/L. Therefore the no-observed effect concentration was a measured 0.46 mg pinoxaden/L. No mortalities were seen in this test and so the 96h LC_{50} of pinoxaden (NOA 407855) in *Crassostrea virginica* is greater than 0.88 mg pinoxaden/L, the highest mean measured concentration tested. The 96h EC_{50} based on effects on shell growth of pinoxaden (NOA 407855) in *Crassostrea virginica* was 0.40 mg pinoxaden/L based on measured concentrations.

5.4.2.2 Long-term toxicity to aquatic invertebrates

No long-term/chronic toxicity studies are available for aquatic invertebrates.

Pinoxaden has a whole water/sediment system DT50 of < 1 day, and therefore will not exist for long in the aquatic environment. Due to this, the Rapporteur for the pesticide risk assessment under Reg. 1107/2009 considered it reasonable to assume that short-term exposure to pinoxaden (as possible in the environment prior to degradation) would not lead to any long-term sub-lethal effects in aquatic invertebrate populations. This justification was accepted in the pesticide DAR and EFSA peer review as a reason for not requiring a long-term/chronic toxicity study on pinoxaden (a chronic *Daphnia* study on the more persistent M2 degradant was available).

5.4.3 Algae and aquatic plants

Six valid studies assessing the toxicity of pinoxaden to various algae and aquatic plant species are available.

Study 1(Knauer, K 2002a)

A 72-hour, GLP, algal growth inhibition study following OECD guideline 201 using the unicellular green algae *Pseudokirchneriella subcapitata* (formerly *Selenastrum capricornutum*) using pinoxaden (purity 97.2%) is available.

Exposure solutions of nominally 1, 2, 4, 8, 16 and 32 mg a.s./L, a solvent (DMF) and an untreated control were included. Concentrations of the test substance, pinoxaden (NOA 407855), depleted significantly throughout the study, from 50-103.8% of nominals at 0 h to 2.5-5% of nominals after 72 h (with the lowest test concentration being <LOQ). Overall mean measured test concentrations of pinoxaden were 0.12, 0.24, 0.46, 1.43, 2.89 and 5.25 mg a.s./L. Concentrations of NOA 407854 (M2) were also measured and the sum of pinoxaden (NOA 407855) and NOA 407854 (M2) stoichiometrically calculated based on molecular weights. The sum of the two chemicals was calculated to be above 80% of the nominal; hence toxicity was assumed to be due to both pinoxaden (NOA 407855) and NOA 407854 (M2) and endpoints were subsequently expressed relative to nominal pinoxaden concentrations.

Algal cell density in control cultures increased 306.9-fold after 72 hours. The nominal 72 h E_rC50 was 41 mg/L and the nominal 72 h E_bC50 was 16 mg/L. The nominal 72 h no-observed effect concentration was 8.0 mg/L for growth rate and 4.0 mg/L for biomass.

Study 2 (Grade, R 2003a)

A 96 hour, GLP, algal growth inhibition study following OECD guideline 201 to the blue-green algae *Anabaena flos-aquae* using pinoxaden (purity 97.2%) is available. Exposure solutions of nominally 0.625, 1.25, 2.5, 5.0 and 10 mg a.s./L and an untreated control were included. Concentrations of the test substance, pinoxaden (NOA 407855), depleted significantly throughout the study, from 76-87% of nominals at 0 h to 48-63% of nominals after 96 h. Overall mean measured test concentrations of pinoxaden were 0.38, 0.75, 1.61, 3.39 and 7.40 mg a.s./L. Concentrations of NOA 407854 (M2) were also measured and the sum of pinoxaden (NOA 407855) and NOA 407854 (M2) stoichiometrically calculated. The sum of the two chemicals was calculated to be above 80% of the nominal; hence toxicity was assumed to be due to both pinoxaden (NOA 407855) and NOA 407854 (M2) and endpoints were subsequently expressed relative to nominal pinoxaden concentrations.

The multiplication factor of the algal cell density in the control after 96 hours was four. Except for effects on growth, no other symptoms of toxicity were observed. The 96 hour E_bC50 to the blue-green algae *Anabaena flos-aquae* was calculated to be 5.0 mg/L and the E_rC50 was 16.4 mg/L based on nominal concentrations. The nominal NOEC for growth rate was 1.25 mg/L and for biomass it was 0.625 mg/L. Note that neither biomass nor specific growth rate inhibition was calculated at 72 hours, so only 96 hour endpoints are available from the study report.

Study 3 (Swarbrick and Maynard, 2002)

A 96 hour, GLP, algal growth inhibition study following the 1996 OECD Draft guideline to the marine algae *Skeletonema costatum* using pinoxaden (purity 97.2%) is available.

Exposure solutions of nominally 0.12, 0.23, 0.47, 0.94, 1.9, 3.8, 7.5 and 15 mg a.s./L were prepared along with an untreated control. Concentrations of the test substance, pinoxaden (NOA 407855)

depleted significantly throughout the study, from 77-101% of nominals at 0 h to 18-30% of nominals after 96 h (with the lowest two test concentrations being <LOQ). Overall mean measured test concentrations of pinoxaden were 0.07, 0.10, 0.22, 0.52, 0.96, 1.45, 3.64 and 7.01 mg a.s./L. Concentrations of NOA 407854 (M2) were also measured and the sum of pinoxaden (NOA 407855) and NOA 407854 (M2) stoichiometrically calculated. The sum of the two chemicals was calculated to be above 80% of the nominal; hence toxicity was assumed to be due to both pinoxaden (NOA 407855) and NOA 407854 (M2) and endpoints were subsequently presented relative to nominal pinoxaden concentrations.

The 72 hour E_bC50 to *Skeletonema costatum* was calculated to be 1.18 mg/L and the 72 hour E_rC50 was 1.72 mg/L based on nominal concentrations. The nominal 72-96 hour NOEC for biomass was 0.23 mg/L and the NOEC for growth rate was 0.94 mg/L. **NOTE**: For this most sensitive algal species, due to the rapid degradation of pinoxaden to a relatively non-toxic degradant (M2), the 72 hour E_rC50 and overall NOE_rC for growth rate were recalculated based on mean measured concentrations of pinoxaden only. The resulting 72 hour E_rC50 was 0.80 mg pinoxaden/L and the 72 hour NOE_rC was 0.52 mg pinoxaden/L. This more precautionary E_rC50 is preferred to the higher initial measured value of 1.32 mg/L given in the EFSA Conclusion for pinoxaden.

Study 4 (Maynard, S.J. and Stewart K.M. 2002)

A 96 hour, GLP, algal growth inhibition study following US EPA OPPTS 850.5400 to unicellular freshwater diatom, *Navicula pelliculosa* (strain UTEX 667 maintained under axenic conditions), using pinoxaden (purity 97.2%) is available.

Exposure solutions of nominally 0.23, 0.47, 0.94, 1.9, 3.8, 7.5, 15 and 30 mg a.s./L were prepared along with an untreated control. Concentrations of the test substance, pinoxaden (NOA 407855) depleted significantly through the study, from 91-113% of nominals at 0 h to 53-61% of nominals after 96 h. Overall mean measured test concentrations of pinoxaden were 0.18, 0.33, 0.72, 1.45, 2.68, 5.87, 12.16 and 23 mg a.s./L. Concentrations of NOA 407854 (M2) were also measured and the sum of pinoxaden (NOA 407855) and NOA 407854 (M2) stoichiometrically calculated. The sum of the two chemicals was calculated to be above 80% of the nominal; hence toxicity was assumed to be due to both pinoxaden (NOA 407855) and NOA 407854 (M2) and endpoints were subsequently expressed relative to nominal pinoxaden concentrations.

The nominal 72 h E_bC50 and E_rC50 values were 10.5 and 14 mg/L, respectively. The overall nominal 72-96 hour NOEC for both biomass and growth rate was 7.5 mg/L. (Note - the nominal NOEC of 8.0 mg/L given in the pinoxaden DAR is incorrect (that was purportedly initial measured)).

Study 5 (Grade R 2002)

A 7 day, GLP study is available to determine the toxicity of pinoxaden (purity 97.2%) to the freshwater duckweed, *Lemna gibba* (G3). This test was performed according to ASTM guideline E 1415-91, US EPA/OPPTS 850.4400 and to OECD guideline No.221 (Oct. 2000 draft).

Exposure solutions of nominally 0.625, 1.25, 2.5, 5.0, 10, 20 and 40 mg a.s./L were prepared with solvent control(DMF) and an untreated control. Concentrations of the test substance, pinoxaden (NOA 407855) depleted significantly throughout this static study, from 70-98% of nominals at Day-0 to 0.75-18.4% of nominals at Day-7 (with the lowest test concentration being <LOQ). Overall mean measured test concentrations of pinoxaden were 0.23, 0.50, 0.77, 1.19, 1.31, 2.18 and 3.17 mg a.s./L. Concentrations of NOA 407854 (M2) were also measured and the sum of pinoxaden (NOA 407855) and NOA 407854 (M2) stoichiometrically calculated. The initial measured sum of the two chemicals was calculated to be 76-98% of nominals; hence the author justified the expression

of endpoints as nominal since, apart from one value at 76%, all initial measured levels of pinoxaden plus M2 were >80% of nominals. Toxicity was also assumed to be due to both pinoxaden and M2. By the end of the test (Day-7) the combined levels of pinoxaden plus M2 had however dropped to 12.4-93.4% of nominals, this was explained as being due to uptake and further metabolism by the *Lemna*.

The 7-day E_bC50 and E_rC50 values were 5.0 and 13.9 mg/L, respectively, based on nominal concentrations. The overall nominal 7-day NOEC for both biomass and growth rate was 0.625 mg/L. Due to the low recoveries of pinoxaden plus M2 even at Day-0, these endpoints were subsequently recalculated by the pesticide Rapporteur using the initial measured concentrations of pinoxaden. Based on these initial measured pinoxaden concentrations, the 7 day E_bC50 and E_rC50 values were 3.5 and 9.7 mg a.s./L, respectively, and the overall initial measured NOEC was 0.438 mg a.s./L.

Study 6 (Knauer K 2002b)

A 20 day, GLP study using the freshwater common reed, *Phragmytes australis*, which is an emergent, rooted, monocotyledonous species, using pinoxaden (purity 97.2%) is available. This was based on OECD guideline 221 (1996 draft) along with ASTM guideline E 1415-91, US-EPA Guideline Number 122-2 and 123-2. Exposure solutions of nominally 0.1, 0.3, 1.0, 3.0 and 10 mg pinoxaden/L were prepared with a solvent (DMF) and an untreated control. Concentrations of the test substance, pinoxaden (NOA 407855) depleted significantly through the study, from 50-100% of nominals at Day-0 to 0.7-10% of nominals at Day-20 (with the second lowest test concentration being <LOD). Overall mean measured test concentrations of pinoxaden were 0.03, 0.02, 0.08, 0.17 and 0.83 mg a.s./L. Concentrations of NOA 407854 (M2) were also measured and the sum of pinoxaden (NOA 407855) and NOA 407854 (M2) stoichiometrically calculated. The initial measured sum of the two chemicals was calculated to be above 80% of nominals; hence the author justified the expression of endpoints as nominals since all initial measured levels of pinoxaden plus M2 were >80% of nominals. Toxicity was also assumed to be due to both pinoxaden and M2. By the end of the test (Day-20) the combined levels of pinoxaden plus M2 had however dropped to 33.3-60% of nominals, this was explained as being due uptake and further metabolism by the reeds as well as into the soil used to root the plants.

Visual effects such as chlorosis, where leaves were partially yellow, were reported from the highest nominal treatment (10 mg pinoxaden/L). Although a statistically significant increase in plant height and weight was seen at the lower treatment levels, this was not considered adverse by the authors or in the DAR. At higher concentrations plant height appeared to be affected to the greatest extent with a 64.5% reduction at the highest concentration (compared with a 21% effect on dry weight). The 20-day EC50 of pinoxaden was calculated to be 8.5 mg/L (nominal) based on plant height and 11.0 mg/L based on biomass. The nominal NOEC based on both of these parameters and chlorosis was 3.0 mg/L. These results were not re-calculated based on initial or mean measured pinoxaden-only concentrations since combined initial measured levels were >80% of nominals and other algae/plants were considered to be more sensitive.

5.4.4 Other aquatic organisms (including sediment)

Water/sediment studies (Part B Section 5.1.2.3) identified that pinoxaden (NOA 407855) showed weak partitioning to the sediment, with a maximum 1.7% AR in the river system and 0.2% AR in the pond system. Furthermore the DT50 in the sediment was a maximum of 2 days. The need to consider toxicity of pinoxaden to sediment dwelling organisms was therefore not required according to SANCO/3268/2001 (the 'Aquatic Guidance Document' under pesticide regulations).

5.5 COMPARISON WITH CRITERIA FOR ENVIRONMENTAL HAZARDS (SECTIONS 5.1 – 5.4)

Degradation

Under sterile hydrolysis conditions, pinoxaden was shown to hydrolyse very rapidly but only at higher surface water pH. Under more neutral and acidic conditions the hydrolysis rate was more moderate.

Pinoxaden undergoes slightly enhanced but limited photodegradation and, for the purposes of classification, is considered photolytically stable under environmentally relevant conditions.

A ready biodegradation test resulted in 12% degradation (based on theoretical carbon dioxide production) at day 29. On this basis, it is concluded that pinoxaden is 'not readily biodegradable'.

Mineralisation was only a minor element of dissipation of pinoxaden in aquatic water/sediment systems. On the basis of this information alone, pinoxaden would also be considered 'not rapidly degradable', however, pinoxaden is not expected to be persistent in aquatic environments due to its 'rapid' degradation as demonstrated by DT50 values of <1 day in whole water/sediment systems. This was considered to be relatively independent of pH (see discussion at 5.1.3). Pinoxaden does not degrade directly to CO_2 but to other unclassified degradants (see below and Table 58), therefore production of <60% CO_2 alone does not mean that pinoxaden will persist as a hazardous substance in aquatic environments. Therefore, on this basis, it is proposed that pinoxaden can be considered 'rapidly degradable' for the purposes of hazard classification under CLP.

Bioaccumulation

The log Kow of pinoxaden is 3.2 and is lower than the trigger value of 4 under CLP, it is not therefore considered to have a high bioaccumulation potential. The degradants of pinoxaden also show a low potential for bioaccumulation (log Kow for M2 = -1.1, for M3 = 1.8).

Degradants

NOA 407854 (M2) is the only major (>10% Applied Radioactivity) degradant identified in both water and the sediment phases and it was shown to be persistent. NOA 447204 (M3) and other degradants were relatively minor in abiotic and biotic test systems. A full set of valid acute and some chronic fish, invertebrate and algae/aquatic plant data is available for M2 and M3 (see Table 58). It is noted that these degradants are at least an order of magnitude less acutely toxic than the parent pinoxaden. The data in Table 58 indicate that M2 and M3 would not themselves be acutely or chronically classified with regards to their aquatic hazard. All acute L/EC values are >>1 mg/L and the lowest NOEC for M2 is the 32-day value for fathead minnow of ≥1.0 mg/L which is an artefact of being the highest concentration tested.

Pinoxaden acute toxicity and hazard

A full set of valid acute fish, invertebrate and algae/aquatic plant data is available for pinoxaden (see Table 57). Pinoxaden is a herbicide and, as anticipated, algae and aquatic plants are the most sensitive trophic group. Acute L/EC50s for fish, daphnia and mysid shrimp are >1 mg/L indicating a low acute hazard to these groups. An acute LC50 is available for the oyster of >0.88 mg/L but this was the highest level tested and no mortality was seen at this concentration. A 96 hour EC50 for shell deposition of 0.4 mg/L was also reported from this oyster study, such an endpoint has previously been used for acute classification although the Notifier has argued that it is not relevant for such purposes as it based on growth rather than the usual mortality or immobilisation. It is, in any case, greater than the key algal acute endpoints discussed below.

All of the algal/plant studies are confounded by being static and showing rapid degradation of pinoxaden to M2. The study authors and Notifier have argued to express the endpoints based on nominal pinoxaden concentrations since, in most cases, the mean measured concentrations of pinoxaden plus M2 were >80% and toxicity was assumed to be due to both substances. In the case of the algal studies, M2 is however of relatively low toxicity compared with pinoxaden (E_rC50 >100 mg/L see Table 58) and so, whilst this argument may have been accepted for risk assessment, this combined approach does not reflect the true effects and thus hazard of pinoxaden alone. The test on *Skeletonema* showed some of the greatest losses of pinoxaden during the course of the study, it was also the most sensitive alga/diatom tested. The Notifier has therefore re-calculated the 72 hour E_rC50 and NOE_rC for this species to give mean measured endpoints for pinoxaden only of 0.80 and 0.52 mg a.s./L respectively.

Studies have also been submitted on the higher aquatic plants/macrophytes, *Lemna gibba* and *Phragmytes australis*, these were also static and affected by substantial losses of pinoxaden. As with the algal studies, these losses were balanced by an increase in the formation of degradant M2, although there was greater overall dissipation or further degradation seen in these tests such that total mean measured concentrations of pinoxaden plus M2 dropped below 80%. In the case of *Lemna*, even the Day-0 combined recoveries dropped below 80% and so the Notifier re-calculated the endpoints based on initial measured (rather than mean measured) concentrations of both substances. For *Lemna*, degradant M2 does appear to be of similar toxicity to the parent substance (7 day E_rC50 of 14.6 mg/L and NOE_rC of 4.0 mg/L, see Table 58) and so the combined toxicity approach is more plausible for hazard classification. Based on these initial measured pinoxaden concentrations, the 7 day E_rC50 value for *Lemna* was 9.7 mg a.s./L and the initial measured NOEC was 0.438 mg a.s./L.

For *Phragmytes* it was assumed but not tested that pinoxaden and M2 posed a similar toxicity - although the combined levels of these substances dropped well below 80% by the end of the 20 day study. The initial measured sum of the two chemicals was however calculated to be >80% of nominals; hence the author justified the expression of endpoints as nominals and this gave an E_rC50 of 8.5 mg/L and a NOEC of 3.0 mg/L. Since *Lemna* and *Phragmytes* are both biologically monocotyledenous plants the Notifier was asked if any other aquatic plant data were available in case pinoxaden specifically targeted other taxa. They have responded that 'dicotyledons have not been shown to be sensitive to pinoxaden in other plant studies' and the herbicide is intended for control of grass weeds.

It could be argued that ideally mean measured pinoxaden-only endpoints should be re-calculated for each of these algal/plant species - but these are not available. The Dossier Submitter does however consider that the mean measured pinoxaden-only E_rC50 for *Skeletonema costatum* of 0.8 is likely to be the most sensitive and reliable acute endpoint for all tested taxa even if these re-calculations were done. This E_rC50 is >0.1 mg/L but ≤1.0 mg/L requiring that pinoxaden be classified as category **Acute 1 (H400) with an acute M-factor of 1**. In case they are considered relevant, the oyster acute LC50 of >0.88 mg/L and shell deposition EC50 of 0.4 mg/L would fall in to the same classification category.

Pinoxaden chronic toxicity and hazard

During pesticide registration it was proposed (and agreed) that due to the rapid degradation of pinoxaden to M2, chronic toxicity studies on the parent substance were not required. For fish and invertebrates these were conducted on M2 instead and indicated a low chronic toxicity for this degradant (see Table 58). A prolonged juvenile fish growth test was subsequently submitted for pinoxaden and, whilst not truly chronic, this is sufficient for a substance which degrades as rapidly as pinoxaden (whole system DT50 of <1 day). This study gave a 28 day mean measured NOEC for

Oncorhynchus mykiss of 3.2 mg a.s./L which would not lead to a chronic classification. It could be argued that an adequate chronic data set is still not available since there is no chronic invertebrate endpoint, however it is proposed that pinoxaden be considered 'rapidly degradable' since it does degrade rapidly, not entirely to CO_2 but to unclassified degradants. It also does not bioaccumulate and neither do its degradants (log Kow <4). Therefore undertaking a surrogate chronic classification using an acute invertebrate endpoint is not considered necessary.

For algae and plants, as previously discussed for acute toxicity, these static studies are affected by rapid degradation of pinoxaden to M2 - and NOECs are variously derived based on nominal, initial measured or mean measured pinoxaden concentrations. For the most sensitive alga/diatom tested, *Skeletonema costatum*, a 72 hour mean measured NOE_rC of 0.52 mg a.s./L was derived for pinoxaden only. This is appropriate since algae were not especially sensitive to M2. For higher aquatic plants a 7 day NOE_rC of 0.438 mg a.s./L was determined for *Lemna gibba* which appears to have some sensitivity to M2, however Day-0 levels of both were low so this was based on initial measured concentrations of pinoxaden. For *Phragmytes australis* a nominal 20 day NOE_rC of 3.0 mg/L was provided but whilst initial combined concentration of pinoxaden and M2 were >80% levels of both had dropped by the end of the study. Each of these taxa were exposed under static conditions to high initial concentrations of pinoxaden followed by increasing concentrations of M2 and in this respect comparing nominal endpoints would still give an indication of relative sensitivities. The alga *Skeletonema* with a nominal NOE_rC of 0.94 mg/L and mean measured NOE_rC of 0.52 mg/L and *Lemna* with a nominal NOE_rC of 0.625 mg/L and initial measured NOE_rC of 0.438 mg/L appear to be the most sensitive species and pertinent endpoints for chronic classification. They are each in the range >0.1 mg/L to ≤1.0 mg/L and, given that pinoxaden is considered to be rapidly degradable (to unclassified degradants), it is proposed that it be classified as category **Chronic 3 (H412)** - with no chronic M-factor required.

5.6 CONCLUSIONS ON CLASSIFICATION AND LABELLING FOR ENVIRONMENTAL HAZARDS (SECTIONS 5.1 – 5.5)

Category Acute 1; H400 'Very toxic to aquatic life'; Acute M-Factor = 1

Category Chronic 3; H412 'Harmful to aquatic life with long lasting effects'

OTHER INFORMATION

This substance has been reviewed under Council Regulation 1107/2009, with the rapporteur Member State being the United Kingdom. The studies evaluated in this dossier were taken from the pesticide assessment report; where necessary, the full study reports were consulted, but these are generally not publicly available. Where other information from additional references has been sourced, this is indicated.

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7 ANNEXES

1. Pinoxaden Developmental Toxicity Assessment