Committee for Risk Assessment

RAC

Annex 1

Background document

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

pinoxaden (ISO); 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

EC Number: -

CAS Number: 243973-20-8

CLH-O-0000001412-86-127/F

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

Adopted

16 September 2016

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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Version: **2** **Date:** **September 2015**

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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

RAC general comment
Pinoxaden (ISO) is a pesticide active substance used as a grass-weed control herbicide. It has currently no existing entry in Annex VI to the CLP Regulation and is therefore subject to Article 36(2) of CLP.

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

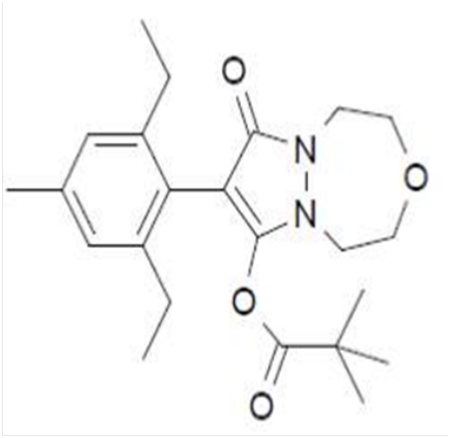
1 IDENTITY OF THE SUBSTANCE

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		

RAC evaluation of physical hazards

Summary of the Dossier Submitter's proposal

In a standard flammability study (EEC method A.10), pinoxaden was found not to be flammable and does not meet the criteria for classification as a flammable solid. Further, 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 A.16) and no spontaneous ignition was observed. Pinoxaden was tested in a standard explosivity study (EEC method A.14) 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 (Oxidising properties (solids); EEC A.17) and was not oxidising.

As such, the Dossier Submitter (DS) concluded that pinoxaden does not meet the criteria for classification for physico-chemical properties according to CLP.

Comments received during public consultation

There were no comments regarding the classification for physico-chemical hazards.

Assessment and comparison with the classification criteria

Pinoxaden does not have a flash point below 78 °C and was shown to decompose before reaching the boiling point. Therefore, pinoxaden does not meet the classification criteria for a flammable liquid. Examination of the chemical structure did not indicate that pinoxaden would have any explosive or oxidising properties and so it does not meet the

criteria for classification as an explosive substance or an oxidising liquid.

RAC is in agreement with the DS that **classification is not required for physical hazards**.

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

RAC evaluation of acute toxicity

Summary of the Dossier Submitter's proposal

The DS presented three studies performed in accordance with OECD Test Guidelines (TG) for acute toxicity, one for each route of exposure.

Oral

Pinoxaden was tested for oral acute toxicity in male and female Wistar rats according to OECD TG 401, following GLP (DAR B.6.2, 2000a). Five animals per sex and group (control or 5000 mg/kg bw) were used in the study. Mortality was observed in 1/5 males and 0/5 females at 5000 mg/kg bw; 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, and hunched posture in two males and two females in the in the 5000 mg/kg group. All surviving animals appeared normal by day 1. Necropsy examinations revealed reddish small intestine, large intestine and caecum in the 5000 mg/kg group male that was found dead; there were no other remarkable necropsy observations.

An acute oral LD_{50} of >5000mg/kg bw was derived. The DS did not propose to classify pinoxaden for acute oral toxicity.

Inhalation

Pinoxaden was tested for acute inhalation toxicity according to OECD TG 403 in Wistar rats (nose only, dust aerosol, 4h/day) following GLP (DAR B.6.2.2, 2001). Five animals per sex and group were used (control group or exposed to 2.2 mg/L, 3.7 mg/L or 5.4 mg/L Pinoxaden). No deaths occurred in the low dose group, while in the 3.7 mg/L group

2/5 males and 1/5 females were found dead two days after exposure. At 5.4 mg/L, 3/5 males and 2/5 females were found dead (3 males and 1 female died the day after exposure and 1 female died three days after exposure).

The principal clinical signs consisted of effects on breathing (laboured respiration and breath sounds or rales) preceded by tachypnea in the mid dose and bradypnea in the high dose, salivation and ruffeled fur, hunched posture at low and high dose, decreased spontaneous activity (mid and high dose), and restlesseness at the mid dose. Red secretion from the nose was seen in one low dose female and swollen abdomen in one female survivor of the mid dose group.

Marked transient losses in mean body weight were evident in male and female animals of the low dose group and in the survivors of mid and high dose.

Necropsy revealed dark red and/or reddish discoloration of the lungs in two of the three premature deaths at the mid dose and four of the five premature deaths at the high dose. Incompletely collapsed lungs were seen in one male survivor of the high dose group. No other pathology findings attributable to treatment were reported.

The following 4h LC_{50} values were derived: males, 4.63 mg/L (90% confidence limits: 3.35 – 20.68 mg/L); females, 6.24 mg/L (confidence limits not determined), males and females, 5.22 mg/L (95% confidence limits 4.07 – 18.00 mg/L).

Dermal

Pinoxaden was tested for dermal acute toxicity according to OECD TG 402 in Wistar rats, following GLP (DAR B.6.2.3, 2000b). Five animals per sex and group (control or treated with 2000 mg/kg bw pinoxaden; 24 h, semi-occlusive) were used. There were no mortalities, no clincial signs or signs of irritation at the application sight. A slight body weight loss was noted in three treated females.

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

Conclusion

According to the DS, pinoxaden meets the criteria for classification for acute toxicity by inhalation as Acute Tox. 4 (H332, Harmful in inhaled) with an Acute Toxicity Estimate (ATE) between 1.0 and 5.0 mg/L (1.0 mg/L - ATE - 5.0 mg/L). No classification was proposed for acute oral or dermal toxicity.

Comments received during public consultation

One Member state Competent Authority (MSCA) supported the DS's proposal to classify pinoxaden for acute inhalation toxicity (Cat. 4, H332), and no classification for acute toxicity via oral and dermal route.

Assessment and comparison with the classification criteria

Oral

Via the oral route, classification is required when the LD_{50} is < 2000 mg/kg bw. Based on the rat LD_{50} for pinoxaden which is > 5000 mg/kg bw no classification for acute toxicity

via the oral route would be warranted.

However, in contrast to the DS RAC is of the opinion that the severe toxicity and mortality seen in the preliminary range finding study for an OECD 414 study in rabbits after only a few doses have to be considered for acute toxicity classification via the oral route. Considerable toxicity and mortality was seen in pregnant rabbits, shortly after first exposure. Doses of 0, 30, 150, 300, 700 or 1000 mg/kg bw/d were administered to 8 time-mated female Russian rabbits per group on gestation days (GD) 7-28 via gavage. Initial weight loss, reduced food consumption (62% at GD 7-12) and considerable reduction of weight gain (↓ 87%) were already seen at 150 mg/kg bw/d. One out of 8 animals was found moribund after 8 doses and another animal showed reduced activity and hunched posture on days 15-19. No clinical signs were seen in the other animals or at lower doses. The test groups dosed at ≥300 mg/kg bw/d were terminated early as all animals were moribund, i.e. hunched posture, reduced activity and body weight loss and animals were found dead after only a few doses: at 300 mg/kg bw/d 1/8 was found dead after 12 doses, at 700 mg/kg bw/d 2/8 were found dead after 5 and 6 doses, respectively, and at 1000 mg/kg bw/d 2/8 were found dead after 1 and 2 doses, respectively (see table 1).

In four developmental toxicity studies in rabbits (using doses up to 100 mg/kg bw/d, 24 time-mated females per group, gavage dosing on GD 7-29) considerable reductions in weight gain and food consumption were seen at 100mg/kg bw/d pinoxaden, but no other clinical signs were described. At this dose also a few animals died, but deaths occurred after several doses (i.e. more than 14) and in the majority of cases they was related to abortion (see table below).

Table: Summary of the effects / deaths observed in the preliminary dose range findings study and four developmental toxicity studies conducted in rabbits.

Dose (mg/kg bw/d)	**# of deaths**	**Approx. time to death**	**Additional information**
Preliminary range findings study			
0	-	-	
30	-	-	
150	1	GD 15 / after 8 doses	found in moribund condition
300	1	GD 19 / after 12 doses	found dead, group terminated early
700	2	GD 12 and 13 / after 5 and 6 doses	found dead, group terminated early
1000	2	GD 8 and 9 / after 1 and 2 doses	found dead, group terminated early
1st OECD 414 study			
No deaths up to 100 mg/kg bw/d			
2nd OECD 414 study			
No deaths up to 30 mg/kg bw/d			
100	2	2 on GD 27 / after 20 doses	related to abortion
	1	GD 26 / after 19 doses	terminated in moribund condition (emaciated due to severe bw loss and recumbent, reduced activity

			the days before termination), considered treatment related
1st investigative study (single buck study); single dose of 100 mg/kg bw/d			
100	1	1 on GD 26 / after 19 doses	related to abortion
2nd investigative study (multi buck study); single dose of 100 mg/kg bw/d			
100*	1	1 on GD 23 / after 16 doses	found dead, considered treatment related
	1		injury, not treatment related
	1	1 on GD 27 / after 20 doses	related to abortion

* One further female aborted on the last day of dosing i.e. GD 29.

Due to the early termination of the preliminary range findings study, it cannot be assessed if further deaths would have occurred and no LD_{50} value can be determined. However, as all animals were moribund at doses ≥ 300 mg/kg bw/d, RAC assumed that further animals would have died, if the study would have been continued.

In contrast to the results from the rabbit developmental and investigative toxicity studies, no deaths were seen in the rat developmental toxicity study (dosing on GD 6-20) up to a dose of 800 mg/kg bw/d or in the 2-generation study (OECD TG 416) in rats up to a dose of 500 mg/kg bw/d. This indicates that the rabbit is more sensitive towards pinoxaden than the rat.

According to the CLP regulation (Annex I, 3.1.3.6.2.1) and the CLP guidance on the Application of CLP Criteria (Nov. 2015, pp 255 - 256) it is possible to also use other types of toxicity studies than those designed for acute toxicity testing. The guidance further states that these studies will not usually provide an LD_{50}/ATE value that can be used directly for classification, but they may provide enough information to allow an estimate of acute toxicity to be made, which would be sufficient to support a decision on classification. No LD_{50} value can be derived on the basis of the available data, because the study groups resulting in severe toxicity were terminated early. It should, however, be noted that contemporary study protocols, such as the fixed dose procedure, use signs of evident toxicity rather than lethality as indications of acute toxicity (see CLP guidance, section 3.1.2.1.2).

The effects seen were clinical signs preceding mortality and mortality (resulting in early termination of the affected animals) at doses relevant for classification as Acute Tox. 4. As STOT SE classification is reserved for effects other than lethality no STOT SE classification is supported by the described effects.

Relevant for classification as Acute toxicicity via the oral route are the deaths observed in the rabbit preliminary dose-range finding study, at doses ≥ 150 mg/kg bw/d after only a few doses. These deaths appear to be caused by acute toxicity which is in contrast to the deaths observed after a longer time period (i.e. after 16 to 20 doses) at a dose of 100 mg/kg bw/d, which were related to abortion in the majority of cases. There were 7 deaths of which 4 were directly related to abortion, 1 was due to an accidental death (injury) and 1 additional abortion occurred on the last day of study.

At 150 mg/kg bw/d only 1 out of 8 animals died after 8 doses and another showed signs of toxicity (hunched posture and reduced acitivty during days 15-19 of exposure). No

other animals in this group showed clinical signs. Severe acute toxicity in all animals, including deaths, was seen at doses of 300, 700 and 1000 mg/kg bw/d. These doses correspond to the dose range supporting Acute Tox. 4 classification (300 < ATE ≤ 2000 mg/kg bw). The proposed classification as Acute Tox. 4 oral is supported by the Acute Tox. 4 classification for the inhalation route.

Therefore, RAC concludes that classification of pinoxaden as **Acute Tox. 4; H302 (Harmful if swallowed)** is warranted.

Dermal

Via the dermal route, classification is required where the LD_{50} is < 2000 mg/kg bw. The LD_{50} was > 2000 mg/kg bw. RAC agrees with the DS that **no classification is warranted for pinoxaden for acute dermal toxicity**.

Inhalation

Via the inhalation route, the 4h LC_{50} (aerosol) in male rats was 4.63 mg/L. RAC agrees with the proposal of the DS to classify pinoxaden for acute toxicity by inhalation as **Acute Tox. 4; H332 (Harmful in inhaled) with an ATE of 4.63.**

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

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

Summary of the Dossier Submitter's proposal

The DS concluded that there were no indications of specific target organ toxicity that would warrant a classification as STOT SE 1 or 2 in the single exposure acute studies. Indeed, in the acute toxicity studies via oral, dermal and inhalation route clinical signs of systemic toxicity were non-specific and, where deaths occurred, necropsy findings were related to lethality. See section on acute toxicity above.

Regarding respiratory tract irritation the DS concluded based on evidence from both an acute inhalation study in rats and from human experience that classification as STOT SE 3 (H335) is warranted.

Signs of possible respiratory irritation (laboured respiration, breath sounds, tachypnea, bradypnea, dark red and/or redish discoloration of the lungs and on collapsed lung) were observed in the acute inhalation study (see section on acute inhalation toxicity).

During the late development phase of pinoxaden in 2004 and subsequent commencement of large scale production of pinoxaden in 2005 up to 2011, incidents of respiratory tract irritation (28 among 306 employees) have been observed among the workforce. 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, 1 worker was diagnosed with occupational asthma but the cause was not established. Since 2012 further coughing incidents and very isolated incidents of asthma-like symptoms (including wheezing) have been reported. Based on the above elements, the DS therefore proposed that pinoxaden should be classified as STOT SE 3, H335.

Comments received during public consultation

One MSCA supported the proposed classification as STOT SE 3 (H335) for respiratory tract irritation mainly based on human data. The same MSCA judged the information from the acute inhalation study as difficult to interpret because it was not possible to distinguish between inhalation toxicity and irritation.

Assessment and comparison with the classification criteria

The hazard class STOT SE covers 3 sub-categories. Categories 1 and 2 are assigned for non-lethal 'significant and/or severe toxic effects', reflecting the dose level required to cause the toxic effect occurring in a specific target organ. Category 3 covers 'transient effects' occurring after single exposure, specifically respiratory tract irritation (RTI) and narcotic effects (NE) (see Sections 3.8.2.4.3 and 3.8.2.4.2 of the CLP Guidance, November 2015).

Regarding STOT SE 3, 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 repeated dose animal tests, including the observation of clinical signs of toxicity (dyspnea, rhinitis,…) with histopathology (e.g. hyperemia, edema, inflammation, thickened mucous layer,…).

Pinoxaden has shown some evidence for respiratory tract irritation in humans i.e. intermittent coughing, wheezing and sneezing.

According to the more detailed information submitted by industry on 1 July 2016, 38 incidents affecting the respiratory tract of exposed workers have been reported from 2004 - 2016 (among a total of 306 employees). It is reported that among the respiratory cases asthma-like symptoms (including wheezing, shortness of breath) have also occurred. The respiratory symptoms resolved completely upon removal of the workers from the workplace, though only in very few cases it has been indicated how long the reactions/symptoms lasted in the affected individuals. The symptoms were observable when incidental acute exposure occurred. Nevertheless, based on the nature of the described symptoms and since some symptoms repeatedly occurred in some individuals, it cannot be unambiguously excluded that the observed irritation effects are also related to respiratory hypersensitivity developed by the workers.

Although, there are no objective measurements in humans, some supportive information of an irritation potential can be extracted from an acute inhalation study in rats where signs of RTI and/or injury (laboured respiration, breath sounds, tachypnea, bradypnea, dark red and/or reddish discoloration of the lungs and one collapsed lung) were observed.

The CLP criteria for STOT SE 3 are listed below (in grey) and compared with the available human information.

3.8.2.2. *Substances of Category 3: Transient target organ effects*

3.8.2.2.1. Criteria for respiratory tract irritation

The criteria for classifying substances as Category 3 for respiratory tract irritation are:

(a) respiratory irritant effects (characterised by localised redness, oedema, pruritis and/or pain) that impair function with symptoms such as cough, pain, choking, and breathing difficulties are included. This evaluation will be based primarily on human data;

Effects fitting those described under point (a) were described in the workforce exposed to pinoxaden (see text above, as well as section on respiratory sensitisation).

(b) subjective human observations could be supported by objective measurements of clear respiratory tract irritation (RTI) (such as electrophysiological responses, biomarkers of inflammation in nasal or bronchoalveolar lavage fluids);

No measurements are available.

(c) the symptoms observed in humans shall also be typical of those that would be produced in the exposed population rather than being an isolated idiosyncratic reaction or response triggered only in individuals with hypersensitive airways. Ambiguous reports simply of 'irritation' shall be excluded as this term is commonly used to describe a wide range of sensations including those such as smell, unpleasant taste, a tickling sensation, and dryness, which are outside the scope of classification for respiratory irritation;

In total, 23 different individuals showed respiratory symptoms after workplace exposure to pinoxaden i.e. 7,5% of the workfore (23/306). In 11 of 23 individuals affected the effects seem to be clearly irritant. For the 13 remaining individuals, it appears that asthma-like symptoms were predominant (see text above and section on respiratory sensitization).

(d) there are currently no validated animal tests that deal specifically with RTI, however, useful information may be obtained from the single and repeated inhalation toxicity tests. For example, animal studies may provide useful information in terms of clinical signs of toxicity (dyspnoea, rhinitis etc) and histopathology (e.g. hyperemia, edema, minimal inflammation, thickened mucous layer) which are reversible and may be reflective of the characteristic clinical symptoms described above. Such animal studies can be used as part of weight of evidence evaluation;

Some supportive information of an irritation potential can be extracted from an acute inhalation study in rats where signs of possible RTI (laboured respiration, breath sounds, tachypnea, bradypnea, dark red and/or reddish discoloration of the lungs and one collapsed lung) were observed. However, it should be noted that the observed effects could also be related to acute inhalation toxicity.

RAC agreed to propose classification as Eye Irritant 2 based on a clearly positive OECD TG 405 study. The study can also be regarded as supportive for a classification as STOT SE 3, H335.

(e) this special classification would occur only when more severe organ effects including in the respiratory system are not observed.

No such more severe effects were seen for pinoxaden.

As drowsiness or dizziness and/or related clinical signs (lethargy, underactivity) were not observed in any of the available studies, the DS proposed not to classify pinoxaden as STOT SE 3; H336.

In conclusion, the DS's proposal to classify pinoxaden as **STOT SE 3; H335 (May cause respiratory irritation)** is supported by RAC.

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

RAC evaluation of skin corrosion/irritation

Summary of the Dossier Submitter's proposal

The skin irritation potential of pinoxaden was tested in a standard guideline study (OECD TG 404) in rabbits, following GLP (DAR B.6.2.4, 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. It is concluded that pinoxaden is not a skin irritant in rabbits.

In a guideline dermal 28-day study in rats (GLP, OECD TG 410; DAR B.6.3.4, 2001) slight erythema formation was observed at the application site in 2/10 males and 3/10 females at 100mg/kg bw/d and also in 2/10 females at 10mg/kg bw/d. However, no such effects were seen at the high dose (1000mg/kg bw/d) and therefore, the effects observed at low and mid dose were considered not treatment-related.

Since commencement of large scale production of pinoxaden in 2005, incidences of skin irritation (redness, itchiness and rashes) have been observed among the workforce at the manufacturing sites. The manufacturer considers these data to be accurate and reliable.

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 was a total of 54 adverse events out of a total of 306 employees. Among these adverse events, 15 cases of skin irritation were reported. Other effects were eye irritation and respiratory tract irritation. In all dermal cases, the symptoms exhibited were minor and resolved completely without the need for medical intervention.

Therefore, given the incidences of skin irritation seen in the workforce at the manufacturing sites, the DS proposed that pinoxaden should be classified as Skin Irrit. 2, H315.

Comments received during public consultation

One MSCA supported the proposed classification for skin irritation based on human data.

Assessment and comparison with the classification 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.

Moreover, in the Guinea pig maximisation test (see section on skin sensitisation) a 50% preparation was shown to be non-irritant.

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, these effects observed in the repeated dose study are not considered to be treatment related.

However, classification can also be based on human data (CLP Annex I, section 3.2.2.1 and 3.2.2.4) and where adequate and reliable information are available this shall take precedence.

From 2004 to 2013 a total of 54 skin, eye and respiratory tract irritations in 306 employees were described among the workforce at the pinoxaden manufacturing sites. Among these adverse events, 15 events involved skin reactions (redness, itchiness and rashes) in a total of 306 employees were reported by the company.

The data do not point towards corrosive properties of pinoxaden, as the effects were minor and fully reversible without medical intervention. RAC notes that pinoxaden is a strong skin sensitiser (see RAC evaluation of skin sensitisation) and thus it can be assumed that the observed skin effects in humans might be caused by an irritation and/or a sensitisation mode of action (MoA).

Considering the negative results in the animal studies and the fact that it is not possible to clearly identify an irritant mode of action, RAC does not support DS's proposal to classify pinoxaden **as a Skin Irritant**.

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

RAC evaluation of serious eye damage/irritation

Summary of the Dossier Submitter's proposal

The eye irritation potential of pinoxaden was tested in a standard guideline study (OECD TG 405) in rabbits, following GLP (DAR B.6.2.5, 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; however, in the third animal, corneal opacity increased in severity on day 7 and persisted as moderate to marked to day 21 and finally cleared at the 28 day reading. No abnormal findings were observed in the iris at any reading. Slight reddening of 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.

From 2004 to 2013 a total of 54 skin, eye and respiratory tract irritations in 306 employees was described among the workforce at the pinoxaden manufacturing sites. Among these adverse reactions, 6 incidences of eye irritation in a total of 306 employees have been observed, i.e. 1.9%. No information on severity of the effect is available.

Comments received during public consultation

One MSCA supported the proposed classification for eye irritation based on positive results in a guideline animal study, supported by human data.

Assessment and comparison with the classification 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.

Therfore, classification of pinoxaden as an eye irritant under CLP as Eye Irrit 2; H319 proposed by the DS on the basis of animal data and reports of eye irritation in the workforce is supported by RAC.

RAC supports the DS's proposal to classify pinoxaden as **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

RAC evaluation of respiratory sensitisation

Summary of the Dossier Submitter's proposal

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 (see section on respiratory tract irritation, STOT SE) were observed among workers. In most respiratory irritation cases the typical symptoms included sneezing or intermittent coughing, which resolved completely upon removal of the worker from the workplace. In one case, following exposure during formulation activities with pinoxaden, a worker was diagnosed with occupational asthma but the cause was not established. Since 2012, further coughing incidents and *very isolated* incidents of asthma-like symtoms (including wheezing) were reported.

It is unclear whether these symptoms represent respiratory tract irritation or respiratory sensitisation. Therefore the DS pointed out that it is already proposed to classify pinoxaden for respiratory irritation and overall, there is no clear evidence that pinoxaden has the potential to induce allergic respiratory sensitisation.

Comments received during public consultation

One MSCA proposed that in view of the strong skin sensitisation potential in the LLNA study and the observed effects in humans (i.e. one case of occupational asthma and isolated incidents of asthma-like symptoms), pinoxaden should be considered as a respiratory sensitiser in category 1B (Resp. Sens. 1B; H334: May cause allergy or asthma symptoms or breathing difficulties if inhaled).

Assessment and comparison with the classification criteria

Pinoxaden is a potent skin sensitiser according to the results of a recent LLNA study. RAC notes that the ECHA guidance document on information requirements (Chapter R7a) states that substances positive in the LLNA should be considered for classification as respiratory sensitiser. Therefore, available human data, *in vitro* data, structural alerts and QSARs data should be assessed.

The use of the knowledge-based expert system DEREK confirmed the alert for skin sensitisation, but no alerts for respiratory irritation or respiratory sensitisation were identified.

A search with the latest version of the respiratory sensitisation profiler of the OECD Toolbox (Vers. 3.4.0.17, July 2016) did not produce any matches for pinoxaden with any of the 41 newly introduced structural alerts for respiratory sensitisation. It should be noted that neitherwas an alert given for skin sensitisation.

The complex structure of pinoxaden, corresponding to its relatively high molecular weight and several functional groups, can be considered as a hindrance for modelling this substance. In addition, the data base of the OECD Toolbox was used for the search of structurally similar substances with the aim to use them for read-across and trend analysis. Similar substances with data on respiratory sensitisation were not found. It

should be noted that the ECHA draft CLP guidance chapter R.7a states that this profiler should be used with caution due to the limited data available for the development of structural alerts, due to the lack of a standardised assay (in vivo or in vitro) suitable for identifying potential respiratory sensitisers.

Currently there are no *in vitro* or *in vivo* data or other objective measurements available for pinoxaden concerning respiratory sensitisation potential.

Upon request, detailed information on humans exposed at the workplace were received from industry. In total, 38 adverse incidents on the respiratory tract were reported between 2004 and 2016 among 306 employees involved into the manufacture, handling and bagging of pinoxaden. Symptoms included wheezing, sneezing, coughing and shortness of breath. Based on the described symptoms summarised in the table below, it is difficult to distinguish between irritation and/or sensitisation potential.

Table: Summary of Syngenta's information on workers exposed to pinoxaden, number of individuals affected and measured air exposure levels at the different sites.

Site	**Number exposed**	**Number of individuals with respiratory effects**	**Measured exposure levels (where available)**
Grangemouth (Active ingredient manufacturing site)	**65**	**3** GM2: 3x; between 2005 and 2009 GM8: 1x; Feb. 2009; GM9: 1x; Oct. 2008 (one case with eye and skin involvement, no respiratory effects, confirmed allergic dermatitis: Feb. 2011)	Before 2011 (before installation of hygiene booth): - average personal monitoring levels: 0.5 mg/m^3 - average static monitoring levels: 0.23 mg/m^3. Use of air-fed suit (filter mask NPF 40) would result in further reduction of exposure to 0.0125 mg/m^3 (no info. whether suits were used) 2012 / 2013: 0.2 mg/m^3 personal and 0.08 mg/m^3 static monitoring (without PPE) 2016: both monitoring data < 0.01mg/m^3 Activities: 155 – 325 minutes
Monthey (Formulation)	**109**	**1** (same individual affected twice: 2008 and Jan. 2009)	2009: personal monitoring, after consideration of PPE: 0.166 – 0.206 mg/m^3 (considering that NPF 40 was used the values correspond to effects without PPE. Activities: 64 – 105 minutes
Omaha (Formulation)	**27**	**8** Individuals with multiples occurrences of symptoms between 2010 and 2013: OM1: 10x, OM2: 2x, OM3:	Formulation started in 2006: - highest personal monitoring level: 0.3 mg/m3

		2x, OM8[1]: 2x Individuals with single occurrence of symptoms between 2009 &and2013: OM4, OM10, OM11, OM12	In 2009 – modification to control for levels < 0.1 mg/m^3: - subsequent monitoring levels < 0.05 mg/m^3 2011 monitoring data for activities of connecting the FIBC to the formulation vessel using glovebox technology, untying the discharge spout and emptying the content: - levels between 0.064 – 0.093 mg/m^3
Greensboro (Formulation)	**20**	**-**	-
Münchwilen (Formulation)	**60**	**6** (all had single incidents, all in Oct. and Nov. 2004)	-
Goa (Formulation)	**15**	**-**	Formulation started in 2012: - < 0.1 mg/m^3, - loss of containment resulted in levels between 0.15 – 0.45 mg/m^3 Use of PPE (NPF 40): < 0.1 mg/m^3 at all times
Contact formulators (3rd party in Canada)	**10**	**5** (2006: 5 individuals were exposed when a big bag containing pinoxaden was heavily placed on the ground, all 5 reported coughing)	-
Sum	**306**	**23**	

Regarding the air concentrations of pinoxaden at the workplace it should be noted that an OEL of 10 mg/m^3 was originally implemented by industry when pinoxaden production was started in 2004. Because of the reported respiratory effects this OEL was reduced to 0.1 – 1 mg/m^3 in 2005. However, based on an increase in skin & respiratory effects in the last quarter of 2008 and the first half of 2009, despite rather low exposure levels (below 1 mg/m^3), a ceiling OEL of 0.1 mg/m^3 (8-hr time-weighted average) was introduced in September 2009. Industry's explanation for this approach was, that an allergic MoA could be involved and that it was decided to treat pinoxaden "as if it was an asthmagen" (Information from Industry, received in March 2016).

[1] One of two individuals with pre-exsting asthma.

A total of 23 individuals were affected, 5 of them presented repeatedly with respiratory tract effects (one individual 10 times, 3 individuals 2 times and one individual 3 times). Based on the reporting it is not possible to know whether individuals with one incidence only where exposed later to the substance to some degree.

Some of the reported single incidents point towards an irritation effect, like e.g. five cases reported from a 3rd party located in Canada. Five workers were exposed to pinoxaden dust liberated when a big bag containing pinoxaden was 'heavily placed' on the floor, and all five reported coughing. Other incidents indicate an allergic MoA. For instance the primary operator for a unit from the Omaha site (OM10) had intermittent cough and sneeze when not wearing a respirator (skin: tight feeling). Another incidence from the Grangemouth site was described as tickle in throat and cold-like symptoms when the employee (GM8) stood next to an open drier that was undergoing maintenance by 3rd party contractors.

For those incidences which involved repeated occurrence of symptoms in the same individuals it should be noted that the repeated occurrence as such points towards a possible allergic MoA or at least an hypersensitivity developed by the worker. Additionally some of these cases involved only minor exposure levels. For example, in 2010 at the Omaha site workers from the production area wearing plant clothes entered the office and two individuals working in the office (OM1[2] and OM2) showed symptoms – sneezing, coughing, wheeze). Also in 2009 the individual OM2 showed symptoms after walking through the unit close to operators working with substance. Very low exposure levels can be expected as concentration in the working area would be less than 0.1 mg/m^3 and transfer from clothing is expected to be much less, unless dust clouds are formed from e.g. tapping clothing.

For another individual from the Omaha site (OM3) two incidences are reported in 2009 and 2011. Both involving shortness of breath and wheezing while performing maintenance activities in the formulation unit. The exact exposure levels are not reported, however, based on the response from industry monitored data indicated exposures less than 0.05 mg/m^3. It is not reported how long the symptoms lasted.

Among the described incidents three asthma like symptoms were reported and detailed information on these three cases was provided by industry.

In 2009 one case of occupational asthma was diagnosed based on clinical history by the site occupational physician and the accident insurance fund. The individual (OM1) reported coughing, sneezing, shortness of breath and wheezing after working with big pinoxaden bags. It was stated that occupational asthma was caused by respiratory irritation. No further incident was reported in this individual and no clinical investigations were conducted as the individual was relocated and remained fit and well. Originally (2008) these effects were considered to be respiratory irritation and were thought likely to be attributable to pivalic acid (a degradation product of pinoxaden). [3]

A second individual (GM2) showed symptoms on four occasions (skin rash, cough,

[2] Individual OM1 had pre-existing non-occupational asthma (one of two individuals with pre-existing asthma).

[3] Also pivalic acid is a skin sensitiser according to the results from the LLNA, however, less potent than pinoxaden itself (Information from Industry).

sneezing), with the first symptoms occurring in 2005. A putative diagnosis of occupational asthma was made in 2008 based on work history. Exposure to pinoxaden occurred after the individual leaned against a contaminated plant structure thereby dislodging dust (sneezing), when inspecting plant equipment (sneezing, cold symptoms), when discharging of a big bag which lost containment (rash, cough, sneezing) and when inspecting plant equipment (red face). The effects were thought to be attributable to pinoxaden but this was not confirmed by appropriate testing. The individual was relocated to a different working area, after the last incident in 2009. No further incident was reported in this individual and he remained fit and well.

A third individual (OM1) working in the office, which had pre-existing non-occupational asthma reported symptoms on 10 occasions. Symptoms were coughing, sneezing, wheezing, short breath, itchiness, swelling around eyes, itchy eyes. On two occasions the individual used his inhaler. Exposure occurred when the individual was visiting a unit that was not handling pinoxaden (symptoms reversed in the evening without use of inhaler), when a colleague from the formulation unit came to the office for 5 minutes (solvent smell on uniform, hooded by a winter coat), when the individual walked past an area where colleagues were breaking down boxes that had been around pinoxaden bags, when the individual was speaking with colleagues from the formulation unit, still wearing the plant uniform, when the individual went to the pinoxaden formulation unit (in contravention to workplace restriction due to pre-existing non-occupational asthma), when the individual stood next to workers from a formulation unit who were wearing their plant uniform that smelled as if it had been contaminated with solvent, when the individual worked with bag baler equipment with no visible contamination, when the individual walked by a formulation unit where two bulk bags of pinoxaden had recently been taken past on fork lift truck, and when colleagues from the production unit visited the office wearing plant clothing.

Although detailed reporting was provided by Industry, only in very few cases it has been indicated how long the reactions/symptoms lasted in those affected individuals and which were the exposure concentrations, information which could be helpful in the assessment.

Occupational asthma can be induced by irritants (non-immunological stimuli) and by sensitisers (immunological stimuli). Thus, the sole information that asthma-like symptoms have been observed, does not allow to conclude that pinoxaden provokes immunological reactions through inhalative exposure. According to the CLP Regulation substances that induce symptoms of asthma are considered respiratory sensitisers, for preventive measures (Footnote 2 to 3.4.2.1.3.1 of Annex I). Immunological mechanisms do not have to be demonstrated.

Summary of effects seen at the workplace (manufacture and formulation site from 2004 to 2013)

Among the 306 workers exposed to pinoxaden 38 incidents of respiratory tract effects in 23 individuals were reported.

Five incidents at the 3rd Party in Canada and 6 incidents at the site in Munchwhilen, displayed symptoms indicating irritant action of pinoxaden on the respiratory tract (coughing following relatively high dust exposures which resolved within minutes after exposure was stopped). No further incidents were reported in these individuals.

For 9 of the affected individuals the information received from Syngenta points towards a respiratory hypersensitivity with asthma-like symptoms, based on the described

symptoms (wheezing, sneezing, tickle in throat, cough, shortness of breath, tightness of chest, which were sometimes accompanied by effects on skin and eyes which could also be related to a sensitisation MoA: itchiness, rashes, swelling around eyes, red eyes, itchy eyes) which occurred after relatively low exposure levels (e.g. walking through production site or being in the office when workers from the production area wearing plant clothes enter the office). The repeated occurrence of symptoms in single individuals as such can be regarded as indicative for a sensitisation MoA.

For 5 incidents at different sites the information was insufficient to draw any firm conclusions on the symptoms and the according exposure levels.

	Individuals (number of incidents)	Number of incidents / number of individuals during 12 years of exposure
Indicative of respiratory hypersensitivity (asthma like symptoms) (based on described symptoms and because low exposure can be expected based on description)	OM1* (10x), OM8* (2x), OM3 (1st incident), OM2 (2x), OM10 (1x), GM8 (1x), GM2** (3x), GM9 (1x), MO1** (1x)	22 / 9
Indicative for irritation (based on described symptoms and because relatively high exposure levels expected based on description)	MU1-6 (all single incidents), 5 individuals at 3rd Party (Canada)	11 / 11
Unclear (regarding effects and / or exposure)	OM11 (1x), OM12 (1x), OM4 (1x), OM3 (2nd incident), MO1 (1st incident)	5 / 5***

* individuals with pre-existing asthma

** relocated to other working area, remains fit and well ➔ for all other individuals it is not known whether they remained at their workplace

*** The individuals underlined (OM3, MO1) had one incident supporting respiratory hypersensitivity and one unclear incident each. The total number of individuals affected is 23.

Data from Asthma UK established that the prevalence of asthma in the UK population (adults and children combined) is approximately 9% (Asthma UK, facts and statistics, date unspecified). However, the prevalence of asthma in the UK population in 2010 ranged from 7,51% to 11,18% (Asthma UK, database consulted on 13 July 2016, data from 2010). This incidence is clearly above the incidence of effects at the workplace, when only considering those respiratory effects likely caused by a sensitising MoA.

Insufficient information is available on whether workers with a history of such health effects left their workplace, nor it is known whether the workers were carrying out the same work (resulting in comparable exposure levels) over the whole time period. A "healthy worker effect" can therefore not be excluded.

No objective measurements (e.g. electrophysiological responses, biomarkers of

inflammation in nasal or bronchoalveolar lavage fluids) are available to confirm that the observed effects were related to immunological changes. The individuals involved declined bronchial challenge tests and suitable reagents were not available.

Further details of the human data were requested from the industry representative at RAC 37. The additional information was provided ahead of the RAC-38 plenary meeting which enabled RAC to evaluate the human data. During RAC plenary discussion, when further details were asked again, the industry representative stated that they had provided as much information as they could without breaching confidentiality.

Exposure to the formulated product

In their statement Industry also informed that they were not aware of adverse effects related to the handling of the formulated product. Syngenta has received 4 reports of adverse health incidents attributable to respiratory exposure, none of which were consistent with "asthma-like" symptoms or clearly attributable to pinoxaden. In a further document from Syngenta (September 2016) it is stated that there are about 200.000 end users with contact to pinoxaden containing products across the EU. It is, however, uncertain how efficiently symptoms from the end users can be monitored by industry.

ExAn incident of eposure to pinoxaden was also reported for 45 cadets crawling in a field freshly treated with pinoxaden formulation of unknown composition[4]. Seven of them developed symptoms described as wheezing, facial swelling, swelling of the throat without skin reactions and bronchospasm and individuals were treated with steroids and adrenalin. It is unknown whether the cadets had been exposed to pinoxaden previously, for instance during a similar exercise as the one described or whether another constituent of the pinoxaden formulation may have induced the symptoms. It can be concluded that sensitisation (induction and subsequent elicitation) cannot be ruled out, but it is unclear if exposure to pinoxaden was the cause of the symptoms.

Comparison of the relevant data with the CLP criteria:

In CLP, Annex I, 3.4.2.1.2.1. it states: "*Evidence that a substance can lead to specific respiratory hypersensitivity will normally be based on human experience. In this context, hypersensitivity is normally seen as asthma, but other hypersensitivity reactions such as rhinitis/conjunctivitis and alveolitis are also considered. The condition will have the clinical character of an allergic reaction. However, immunological mechanisms do not have to be demonstrated"*.

In 9 out of 306 exposed workers, symptoms were observed which fit the description above: Wheezing, sneezing, tickle in throat, coughing, shortness of breath, tightness of chest, which were sometimes accompanied by effects on skin and eyes which could also be related to a sensitisation MoA: itchiness, rashes, swelling around eyes, red eyes, itchy eyes. It is noted that neither in the CLP Regulation nor in the CLP guidance can a description of the symptoms of "asthma" and "rhinitis" be found. Therefore, a published definition (Kimber *et al*., 2006) is used instead:

Asthma: "*Wheezing, chest tightness, coughing, breathlessness, typically after a latent*

4 https://cot.food.gov.uk/sites/default/files/TOX2015-30%20FOLLOW-UP%20PAPER%20on%20skin%20sensitisation%20-%20format.pdf

period of at least several month after onset of exposure (cannot be evaluated from the available information on pinoxaden) and in most instances (early cases) these symptoms are associated with "time spent at work and with improvement away from work" (in most cases described for pinoxaden improvement away from work was noticed)".

Rhinitis definition is: *"Sneezing and blocked and runny nose, with similar time pattern for the occurrence of the symptoms as for asthma."*

RAC notes that these symptoms have the clinical character of an allergic reaction, which supports the conclusion that a sensitising MoA could be the underlying cause of these symptoms.

Furthermore, 3.4.2.1.2.2 states that when considering the human evidence, it is necessary for a decision on classification to take into account, in addition to the evidence from the cases:

(a) the size of the population exposed;

(b) the extent of exposure.

The use of human data is discussed in sections 1.1.1.3, 1.1.1.4 and 1.1.1.5.

Relevant effects (as described in CLP section 3.4.2.1.2.1) were seen in 9 out of 306 exposed employees. Exposure patterns among those employees showing symptoms were rather variable. However, RAC notes that exposure must have been very low (measured values ranged from 0.3 to 0.5 mg/m^3) before the introduction of additional strict control measures and protective equipments that further decreased exposure to concentrations at or below 0.1 mg/m^3. Activities that resulted in the observed effects also suggested very low exposure. They include indirect exposure in the office when workers wearing plant clothing entered the office, walking through production area, standing next to an open drier undergoing maintenance, walking past an area where colleagues were breaking down boxes that had been around pinoxaden bags.

Additionally, a study reported acute symptoms in 7 out of 45 cadets after crawling through a field treated with a pinoxaden formulation. Exposure of these individuals is less clear since the compostion of pinoxaden formulation is not known and previous exposure to pinoxaden is not reported.

3.4.2.1.2.3. The evidence referred to above could be:

(a) clinical history and data from appropriate lung function tests related to exposure to the substance, confirmed by other supportive evidence which may include:

(i) in vivo immunological test (e.g. skin prick test);

(ii) in vitro immunological test (e.g. serological analysis);

(iii) studies that indicate other specific hypersensitivity reactions where immunological mechanisms of action have not been proven, e.g. repeated low-level irritation, pharmacologically mediated effects;

(iv) a chemical structure related to substances known to cause respiratory hypersensitivity;

There is not much information available on clinical history (e.g. no information on smoking, medication or exposure to other substances). Only 2 individuals were identified to have pre-existing asthma (OM1 and OM8), both were among those 9 individuals showing effects fitting the description in section 3.4.2.1.2.1. Two individuals were relocated to another working area without pinoxaden exposure and are reported to remain fit and well, for the rest it is not known whether they were relocated after symptoms had occurred. It is also not known how long the individuals had already worked at their workplace before onset of symptoms.

No lung function tests or tests mentioned under points (i) to (iii) are available.

No related substance known to cause respiratory hypersensitivity could be identified (iv).

(b) data from one or more positive bronchial challenge tests with the substance conducted according to accepted guidelines for the determination of a specific hypersensitivity reaction.

Not available.

3.4.2.1.2.4. Clinical history shall include both medical and occupational history to determine a relationship between exposure to a specific substance and development of respiratory hypersensitivity. Relevant information includes aggravating factors both in the home and workplace, the onset and progress of the disease, family history and medical history of the patient in question. The medical history shall also include a note of other allergic or airway disorders from childhood, and smoking history.

Information is poor, see section 3.4.2.1.2.3.

3.4.2.1.2.5. The results of positive bronchial challenge tests are considered to provide sufficient evidence for classification on their own. It is however recognised that in practice many of the examinations listed above will already have been carried out.

Not available.

3.4.2.1.3. Animal studies

3.4.2.1.3.1. Data from appropriate animal studies (1) which may be indicative of the potential of a substance to cause sensitisation by inhalation in humans (2) may include:

(a) measurements of Immunoglobulin E (IgE) and other specific immunological parameters in mice;

(b) specific pulmonary responses in guinea pigs.

Not available.

(1) At present, recognised and validated animal models for the testing of respiratory hypersensitivity are not available. Under certain circumstances, data from animal studies may provide valuable information in a weight of evidence assessment.

(2) The mechanisms by which substances induce symptoms of asthma are not yet fully known. For preventative measures, these substances are considered respiratory

sensitisers. However, if on the basis of the evidence, it can be demonstrated that these substances induce symptoms of asthma by irritation only in people with bronchial hyper reactivity, they should not be considered as respiratory sensitisers.

Overall it can be concluded that there are some indications that pinoxaden has a respiratory sensitisation potential. There is no objective immunological evidence to confirm that pinoxaden causes allergic respiratory hypersensitivity in the available data on humans. It is noted that according to CLP criteria (3.4.2.1.2.1., Annex I) the immunological mechanisms do not have to be demonstrated in order to classify. However, in the absence of a more detailed description of medical and occupational history of the affected individuals and/or objective measurements, the observed symptoms were considered not sufficient to support a classification.

RAC supports the DS's proposal for **no classification**.

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

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
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

RAC evaluation of skin sensitisation

Summary of the Dossier Submitter's proposal

Pinoxaden was tested in a Guinea pig maximisation test (GPMT, OECD TG 406, 2000c) and in a local lymph node assay (LLNA, OECD TG 429, 2010a).

For the GPMT (DAR B 6.2.6, 2000c), a 5% w/w pinoxaden preparation was well tolerated systemically, was mildly/moderately irritant to the skin in a preliminary study and was selected for intradermal induction. A 50% preparation had been shown to be non-irritant and to be the maximum practical concentration in the preliminary study and was therefore used for the topical induction and challenge. No dermal reactions were induced, neither with test material nor with control (vehicle = CMC and Tween 80). A positive control (2-mercaptobenzothiazole) induced the appropriate response.

In the LLNA (2010a) pinoxaden excerted a stimulation index >3.0 at concentrations of 1, 5, 10 and 25% (vehicle = DMF). An EC3 value of 0.43% was calculated. On the basis of this result, the DS concluded that pinoxaden should be considered to be a strong skin sensitiser and classificed as Skin Sens. 1A; H317: May cause an allergic skin reaction.

A single human case is reported: a manufacturing worker with a putative diagnosis of skin sensitisation based on the exclusion of other causative agents by skin patch testing. Furthermore, it cannot be unambiguously excluded that the observed irritation effects in humans (incidence 4.9%) (see RAC evaluation of Skin Irritation) might represent sensitising properties.

Comments received during public consultation

Two MSCAs supported the proposed classification as Skin sensitiser Cat 1A, based on the positive LLNA.

Additional key elements

No additional key elements

Assessment and comparison with the classification criteria

In the positive LLNA the EC3 value was 0.43%. According to the classification criteria an EC3 value < 2% supports classification as Skin sensitiser Cat 1A (strong sensitiser). An EC3 value of 0.43% triggers the generic concentration limit of 0.1%.

It is unclear why a negative result was obtained in a valid maximisation study in which the substance was tested up to 50%. The DS speculated that the different results could be due to the different vehicles used (CMC and Tween 80 vs. DMF) and/or the different species (Guinea pig vs. mouse).

Based on the results from the valid LLNA RAC supports the DS's proposal to classify Pinoxaden as **Skin Sensitiser 1A; H317 (May cause an 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

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

Summary of the Dossier Submitter's proposal

Pinoxaden was tested for repeated dose toxicity via the oral route in the rat (28-day, gavage; 90-day, gavage; 90-day, dietary; 2-year, gavage), the mouse (90-day, gavage, range finding study) and the dog (28-day range finding study, gavage (caspsule); 90-day, gavage (capsule); 1-year, gavage (capsule)) and a dermal 28-day study in the rat. All studies followed appropriate TG protocols (except for the 28-day range finding study in dogs) and were conducted according to GLP.

Rat

The DS identified the kidneys as main target organ of pinoxaden induced toxicity in the rat.

In a 28-day rat **gavage** study, pinoxaden induced increased kidney weight (f: 17%, rel.), increased water intake (m: 41%, f: 22%), tubular atrophy (m: 5/5, f: 5/5), tubular dilatation (m: 4/5, f: 5/5), tubular casts (m: 2/5), polymorphic infiltration (m: 2/5) and related changes in some urinalysis parameters at the dose of 600 mg/kg bw/d. The histological changes were similar at the high dose of 1000 mg/kg bw/d (tubular atrophy (m: 5/5, f: 5/5), tubular dilatation (m: 5/5, f: 5/5), tubular casts (m: 1/5, f: 4/5), polymorphic infiltration (m: 0/5, f: 3/5), single cell necrosis (m: 3/5)), while the urinalysis parameters were more severly affected and water consumption was further increased (m: 76%, f: 53%). At this high dose general toxicity was increased (i.e. 1 male died on day 11, body weight gain was reduced in males and food consumption was reduced in males and females) and slight leucocytosis was reported in males.

There were toxicologically relevant increases in relative liver weights at 600 and 1000 mg/kg bw/d in males and females and according to the DAR also at 300 mg/kg bw/d in

males (14%). In females a slight decrease in plasma albumin was seen at all doses. The only histopathological change noted in the liver (increased glycogen deposition) was considered not to be adverse. RAC notes that according to the DAR glycogen deposition was also seen in control animals and no significant increase with dose was observed. Overall a NOAEL of 300 mg/kg bw/d could be derived.

90-day **gavage** study in the rat with 28 days recovery period: Some signs of kidney toxicity (increased water intake, changes in urinalysis and clinical-chemistry parameters) were also seen after a gavage dose of 300 mg/kg bw/d after 90 days. Liver weight and associated clinical chemistry parameters were only slightly affected at this dose. The described effects were not seen after 28 days recovery. The NOAEL in this study was set at 100 mg/kg bw/d, a dose at which urinalsysis parameters were affected (f: 540% increase in ketones, reduced pH) but no effects on organ weights or water consumption were reported.

Also in a rat **dietary** 90-day study, with 28-day interim kill, the kidneys were affected. At the high dose of 890/965 mg/kg bw/d (m/f) water consumption was increased, kidney histology was affected (cortical tubular basophilia/dilatation/atrophy (m: 8/10, f: 6/10 after 90 days and m: 3/5, f: 1/5 after 28 days), renal cysts were seen after 90 days (m: 10/10, f: 7/10)) and urine volume was increased by 54% in females after 90 days. At this dose also body weight and food consumption were decreased and haematology (f: indication for slight anaemia) and clinical chemistry (liver related parameters) were affected. The NOAEL was set at the next lower dose (466/527 mg/kg bw/d (m/f)): at this dose only slight body weight reduction (from 3.2% to 6%) and some changes to liver associated clinical chemistry parameters were noted, but considered not adverse in the absence of any histopathological findings.

12 months **gavage** exposure to 250 and 500 mg/kg bw/d (as part of a chronic toxicity study) induced kidney toxicity (increased water intake, tubular dilatation/atrophy, chronic progressive nephropathy and changes in related urinalysis) next to severe generalised toxicity in rats (reduced survival in males with 24/90 deaths in the high dose males and reduced body weight gain). At both doses haematology was affected in males and females (indication for slight anaemia) and at the high dose absolute and relative liver weight was increased in high dose males and females. In high dose males the incidence and severity of mineralization of "clear cells" in the tail area of the epididymides was increased compared to control (9/10 grade 2.0 in the high dose vs. 6/10 grade 1.0 in control). The NOAEL (53 weeks) in the study was 100 mg/kg bw/d.

No systemic toxicity was seen in rats **dermally** exposed to pinoxaden up to the limit dose of 1000 mg/kg bw/d for 28 days.

Mouse

In a mouse 90-day **gavage** range finding study (no clinical chemistry parameters determined) the high dose of 1000 mg/kg bw/d induced a considerable reduction in body weight gain (m: 67%, f: 60%) and water consumption was increased by 20% in males. Piloerection was clearly increased at the high dose (m: 8/10, f: 5/10), but was also seen in females at 700 mg/kg bw/d (3/10) and at 400 mg/kg bw/d (6/10). At doses ≥ 700 mg/kg bw/d renal tubular basophilia was seen in males and females and absolute and relative liver weight was increased more than 110% in males and females. Females dosed with 400 mg/kg bw/d pinoxaden or more had an altered haematological profile indicating slight anaemia (lower haemoglobin concentration, erythrocyte count, haematocrit and at 1000

mg/kg bw/d higher platelet counts).

Dog

In the 28-day **gavage** (capsule) range finding study 1 animal/sex/dose were tested without including a control group (comparison to pre-treatment levels). Salivation and resistance to dosing at 1000 mg/kg bw/d was considerable (very high incidence of vomiting), leading to the conclusion that systemic exposure was limited. It was considered that 1000 mg/kg bw/d would not be tolerated in studies of longer duration. At 1000 and 500 mg/kg bw/d clinical signs of toxicity (dehydration, pale and thin appearance, decreased activity), reduced food consumption, effects on haematology and clinical chemistry parameters and histopathological changes in the lymphnodes (lymphoid hyperplasia) were described (sometimes only seen in one of the two animals). The DS derived a NOAEL of 250 mg/kg bw/d, however, it seems that the effects on haematology, clinical chemistry and histopathology seen at this dose were comparable to the effects seen at 500 and 1000 mg/kg bw/d. Therefore, RAC concludes that no NOAEL can be derived from this study and that without a control group a comparison with the STOT RE guidance values in the CLP Regulation is not meaningful for this study.

In a 90-day **gavage** (capsule) study increased mortaility was seen at the high dose of 500 mg/kg bw/d (m: 1/4 killed wk 13, f: 4/4 killed wk 5 – due to reduced food consumption and body weight loss). Histopathology of the liver (reduced liver glycogen: m: 1/4, f: 2/4 and increased apoptosis: f: 1/4) and thymus (atrophy in f: 1/4) and absolute liver weight (m: 19%) were also affected at the high dose. At ≥ 250 mg/kg bw/d general toxicity (pale/cold ears/mouth/tongue, cold at touch, dehydrated, decreased activity, thin appearance), gastro-intestinal effects (salivation, retching, vomiting, fluid faeces, regurgitation) reduced body weight and food consumption and clinical chemistry (liver related parameters) were affected (in general severity of these effects increased at the top dose).

Increase in serum albumin (52%, 945%, 87% after 4, 8, 13 weeks, respectively) in the low dose females could be an initial adaptation to adverse liver effects induced at higher doses, however, as no other parameters were effected and no histolopathological findings were described this effect in females only is not considered adverse. Also at the next higher dose (100 mg/kg bw/d) clinical chemistry was affected.

Overall, the DS concluded that classification of pinoxaden with STOT RE is not warranted.

Comments received during public consultation

One MSCA commented that based on the available subacute and subchronic studies in rats and dogs no classification for STOT RE is supported. However, this MSCA mentioned the severe effects, i.e. maternal deaths, seen in developmental toxicity studies in rabbits, as potentially supportive for a STOT RE classification.

Assessment and comparison with the classification criteria

According to the CLP Regulation substances that, on the basis of evidence from studies in experimental animals, can be presumed to have the potential to be harmful to human health following repeated exposure should be classified as STOT RE. Substances are classified in category 2 for target organ toxicity (repeated exposure) on the basis of

observations from appropriate studies in experimental animals in which significant toxic effects, of relevance to human health, were produced at generally moderate exposure concentrations.

Guidance dose/concentration values for different study durations are provided below in order to guide classification as STOT RE 2:

Oral, rat

28-day: 30 < C ≤ 300 mg/kg bw/d

90-day: 10 < C ≤ 100 mg/kg bw/d

1-yr: 2.5 < C ≤ 25 mg/kg bw/d

2-yr: 1.25 < C ≤ 12.5 mg/kg bw/d

In the rat the main target of pinoxaden toxicity is the kidney, however, significant toxic effects occur at doses well in excess of the relevant guidance values.

In the mouse, pinoxaden toxicity was targeted to the kidneys and the blood, however, significant effects were only seen at doses above the relevant guidance values for STOT RE classification.

In the dog gastro-intestinal effects and minor changes in clinical chemistry parameters occurred at dose levels of pinoxaden equivalent to the relevant guidance values, however, in the absence of associated body weight reductions and histopathology findings in any organ, these effects were not regarded as significant in the context of STOT RE classification.

On the basis of the available repeated dose toxicity studies in rats, mice and dogs no classification as STOT RE is supported.

It is noted, that in the rabbit developmental toxicity studies (see section on Reproductive toxicity) considerable maternal toxicity was described. However, as these effects included deaths occurring within relatively short time periods after first exposure (within one week) they are considered supportive for a classification for acute toxicity via the oral route (see section on Acute toxicity, oral).

In line with the DS RAC proposes **no classification for STOT RE**.

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	Negative	Positive controls included;	2001

Method	Results	Remarks	Reference
In vitro OECD 482 GLP Primary hepatocytes (from male rats) 9.38 - 300 ug/mL Pinoxaden technical - Batch No. EZ005006 (purity 97.2%).		Cytotoxicity observed at higher concentrations;	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)
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	+ S9: Positive - S9: Positive	Concentration limited by cytotoxicity. Expt I: +/-S9 4h exposure, 18h	2002 DAR B.6.4.1(e)

Method	Results	Remarks	Reference
In vitro Chinese hamster V79 cells 15 – 100 µg/mL Pinoxaden technical - Batch No. AMS 1055/2 (purity 99.5%).		harvest; Expt II: +S9 4h exposure, 28h harvest; -S9 18h exposure & harvest, also 28h exposure, 28h harvest. Positive controls included.	
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)
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

RAC evaluation of germ cell mutagenicity
Summary of the Dossier Submitter's proposal The genotoxicity potential of pinoxaden was tested in five *in vitro* guideline studies (one unscheduled DNA synthesis test, UDS in primary hepatocytes, one Ames test, one cell

mutation test (eukaryotic system) and two chromosome aberration assays) and two *in vivo* (mouse micronucleus, MN, rat UDS) guideline studies, all following GLP. All tests were negative except for the two *in vitro* chromosome abberation tests, which gave clearly positive results with and without metabolic acitivation of Pinoxaden. In the *in vivo* mouse MN test, which is a test adequate for the detection of clastogenic potential, an increase in micronuclei was seen at the low dose (500 mg/kg bw/d), but not at the two higher doses (1000 and 2000 mg/kg bw/d).

In vitro chromosome aberration tests

In the first test (OECD TG 473, DAR B.6.4.1(d), 2001) technical pinoxaden (purity 97.2%) was used. The clastogenic potential was tested in a series of independent *in vitro* cytogenetic experiments using Chinese hamster V79 cells, treated in the presence or absence of rat liver-derived metabolic actication system (S9). The test substance was dissolved in acetone which was used as the negative control. Ethyl methane sulphonate (in the absence of S9-mix) and cyclophosphamide (in the presence of S9-mix) were used as positive controls, and induced statistically significant increases ($p<0.05$) in cells with structural chromosome aberrations. The cells were exposed over the concentration range of 20 – 125 µg/mL, the highest concentration being limited by the cytotoxicity of the test material. The study met all criteria specified for the OECD TG 473 (1997).

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 system with and without S9.

Table 3: Summary of results of chromosome aberration study (1)

Expt	Harvest time			Polyploid cells (%)	Cell No. (% of control)	Mitotic indices (% of control)	Aberrant cells		
							Incl. gaps	Excl. gaps [a]	Exchanges
Exposure period 4 hours without S9 mix									
I	18 hours	Negative control		1.6	n.t.	100	2.5	0.5	0.0
		Solvent control[1]		1.6	100	100	1.55	0.0	0.0
		Positive control[3]		1.6	Nt	66	20.0	20.0***	5.5
		Pinoxaden (µg/ml)	25	3.5	89	64	2.5	2.5	1.0
			50	2.0	63	109	8.5	7.5***	3.0
			75	2.2	66	97	8.0	6.0***	1.5
			100	2.0	46	85	9.0	6.5***	2.0
III	18 hours	Negative control		3.0	Nt	100	0.5	0.5	0.0
		Solvent control[1]		4.7	100	100	0.5	0.0	0.0
		Positive control[3]		3.8	Nt	102	16.0	14.0***	6.5
		Pinoxaden (µg/ml)	50	2.5	90	100	2.0	0.5	0.0
			75	2.5	81	91	3.5	2.5*	0.5
			125	3.0	54	56	4.5	3.5**	1.0
Exposure period 18 hours without S9 mix									
II	18 hours	Negative control		3.3	Nt	100	0.0	0.0	0.0
		Solvent control[1]		4.6	100	100	0.5	0.0	0.0
		Positive control[2]		1.8	Nt	48	19.5	19.5***	6.5
		Pinoxaden (µg/mL)	40	2.1	64	61	1.5	1.0	0.5
			80	1.8	52	87	7.5	5.5***	2.5
			100	1.5	49	55	6.5	2.5*	1.0

III	18 hours	Negative control		2.2	Nt	100	0.0	0.0	0.0
		Solvent control[1]		3.0	100	100	1.0	0.5	0.0
		Positive control[2]		3.4	Nt	49	13.0	11.5	5.5
		Pinoxaden (µg/mL)	50	4.1	115	131	1.0	1.0	0.5
			100	2.7	106	121	6.0	4.0*	2.0
			125	3.1	80	98	9.5	8.0***	1.5
Exposure period 28 hours without S9 mix									
II	28 hours	Negative control		3.8	Nt	100	0.5	0.5	0.0
		Solvent control[1]		3.8	100	100	3.0	1.5	0.0
		Positive control[2]		3.2	Nt	49	20.0	20.0***	0.0
		Pinoxaden (µg/mL)	40	2.2	42	59	4.5	2.0	0.0
Exposure period 18 hours with S9 mix									
I	18 hours	Negative control		4.7	Nt	100	0.5	0.5	0.0
		Solvent control[1]		3.3	100	100	2.0	1.0	0.5
		Positive control[2]		1.9	Nt	88	11.5	10.0***	5.0
		Pinoxaden (µg/mL)	20	3.9	110	97	1.0	1.0	0.5
			40	3.9	71	94	2.0	2.0	0.5
			80	1.8	57	82	4.0	2.0	1.5
II	28 hours	Negative control		6.3	Nt	100	2.0	0.5	0.0
		Solvent control[1]		6.4	100	100	1.5	1.0	0.0
		Positive control[3]		7.8	Nt	80	12.0	11.0***	4.0
		Pinoxaden (µg/mL)	20	7.0	100	101	3.0	3.0	0.0
			40	10.9	32	70	8.5	7.0***	3.0
			80	8.8	37	66	13.0	11.0***	3.5
III	28 hours	Negative control		4.2	Nt	100	1.5	1.0	0.0
		Solvent control[1]		4.1	100	100	0.5	0.5	0.5
		Positive control[2]		2.2	Nt	102	2.0	19.5***	5.0
		Pinoxaden (µg/mL)	20	2.7	86	111	0.5	0.0	0.0
			40	1.8	95	113	3.0	2.0	0.0
			60	2.9	42	33	14.0	11.5***	5.0

[a] including cells carrying exchanges
n.t.= not tested
*= p<0.05, ** = p<0.01, *** = p<0.001
p*= p<0.05 aberration frequency statistically significant higher than corresponding control values
[1]acetone 0.5 %; [2]EMS 600 µg/mL; [3]EMS 1000 µg/mL

In the second assay (OECD TG 473, DAR B 6.4.13, 2002) analytically pure pinoxaden (purity 99,5%) was tested for clastogenic potential in a series of independent *in vitro* cytogenetic experiments, using Chinese hamster V79 cells, treated in the presence or absence of rat liver-derived metabolic actication system (S9). The test substance was dissolved in acetone which was used as the negative control. Ethyl methane sulphonate (in the absence of S9-mix) and cyclophosphamide (in the presence of S9-mix) were used as positive controls, and induced statistically significant increases ($p<0.05$) in cells with structural chromosome aberrations.

The cells were exposed over the concentration range of 25 – 100µg/ml, the highest concentratin being limited by the cytotoxicity of the test material. The study met all criteria specified for OECD TG 473 (1997).

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 system with and without S9.

Table: Summary of results of chromosome aberration study (2)

Expt	Preparation interval	Test Item		Polyploid cells (%)	Cell No. (% of control)	Mitotic indices (% of control)	Aberrant cells		
							Incl. gaps	Excl. gaps [a]	Exchanges
Exposure period 4 hours without S9 mix									
IA	18 hours	Negative control		3.2	-	100	2.0	0.5	0.0
		Solvent control[1]		2.9	100	100	2.5	1.0	0.0
		Positive control[3]		3.2	-	80	20.0	20.0***	7.0
		Pinoxaden (µg/mL)	45	2.3	79	89	0.5	0.5	0.5
			60	4.2	56	113	3.0	2.0	0.5
			75	2.3	46	117	3.5	2.5	0.5
			90	2.3	46	101	13.0	12.0***	7.0
1B	18 hours	Negative control		2.5	-	100	1.5	1.5	0.0
		Solvent control[1]		3.7	100	100	1.0	0.5	0.0
		Positive control[3]		1.7	-	57	94.0	94.0***	19.0
		Pinoxaden (µg/mL)	30	3.1	116	121	1.0	0.0	0.0
			60	3.1	66	124	4.5	2.5	0.0
			90	3.6	61	56	11.5	11.5***	3.0
Exposure period 18 hours without S9 mix									
II	18 hours	Negative control		3.8	-	100	3.5	3.5	0.0
		Solvent control[1]		2.4	100	100	2.5	2.0	0.5
		Positive control[2]		2.4	-	105	47.0	46.5***	14.5
		Pinoxaden (µg/mL)	40	2.2	95	98	0.0	0.0	0.0
			80	1.9	74	96	3.5	3.0	2.0
			100	2.3	77	79	10.5	8.0**	2.0
Exposure period 28 hours without S9 mix									
1	28 hours	Negative control		2.7	-	100	1.0	0.5	0.0
		Solvent control[1]		2.5	100	100	0.0	0.0	0.0
		Positive control[2]		3.6	-	99	43.0	43.0***	22.0
		Pinoxaden (µg/mL)	80	2.7	43	108	3.0	1.0	0.5
Exposure period 4 hours with S9 mix									
IA	18 hours	Negative control		2.7	-	100	0.5	0.5	0.0
		Solvent control[1]		3.4	100	100	2.0	1.5	0.0
		Positive control[2]		2.2	-	76	15.5	13.5***	7.5
		Pinoxaden (µg/mL)	15	3.5	120	93	2.5	1.0	1.0
			30	2.5	111	80	2.5	2.5	1.5
			60	3.1	78	76	10.5	9.0***	4.0
IB	18 hours	Negative control		3.2	-	100	1.5	0.5	0.0
		Solvent control[1]		3.3	100	100	4.0	3.0	1.0
		Positive control[2]		3.6	-	117	32.0	26.0***	12.0
		Pinoxaden (µg/mL)	15	3.9	110	107	2.0	1.0	0.0
			30	3.2	99	98	5.0	3.0	1.0

			45	3.0	63	105	4.5	3.5	0.5
II	28 hours	Negative control		2.0	-	100	2.0	1.0	0.5
		Solvent control[1]		3.5	100	100	0.0	0.0	0.0
		Positive control[3]		3.0	-	100	18.5	18.0***	3.0
		Pinoxaden (µg/mL)	15	3.5	74	92	3.5	1.0	0.0
			30	3.3	54	97	2.5	1.0	0.0
			45	3.9	39	82	13.0	10.5***	1.0

[a] including cells carrying exchanges
n.t.= not tested
*= p<0.05, ** = p<0.01, *** = p<0.001 aberration frequency statistically significant higher than corresponding control values
[1]acetone 0.5 %; [2]EMS 200 µg/mL; [3]EMS 1000 µg/mL

In vivo mouse micronucleus test

In a 2001 study, the ability of pinoxaden (purity 97.2%), to induce micronuclei in bone marrow polychromatic erythrocytes in orally dosed NMRI mice was tested. The test item was formulated in 40% ethanol in PEG 400. This 40% ethanol in PEG 400 was used as vehicle control. 24 h and 48 h after a single oral administration of the test item the bone marrow cells were collected for micronuclei analysis.

The study met all criteria specified for OECD TG 474 (1997).

Five aninmals/sex/group were evaluated for the occurrence of micronuclei. Two thousand polychromatic erythrocytes were examined for the presence of micronuclei for each animal. Slides were also examined for evidence of cytotoxicity, by determining the ratio of polychromatic to normochromatic erythrocytes.

The following dose levels of the test item were investigated:

24-h preparation interval: 500, 1000, and 2000 mg/kg bw

48-h preparation interval: 2000 mg/kg bw

The highest dose (2000 mg/kg bw, highest recommended dose) was estimated by a pre-experiment to be suitable.

The test system positive control, cyclophosphamide, induced statistically significant and biologically meaningful increases in micronucleated polychromatic erythrocytes, compared to vehicle control values, at the 24-hour time points, in both tests, thus demonstrating the sensitivity of the test system to a known clastogen.

A small but statistically significant (p<0.05) increase 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 compared to controls at either the 1000 or 2000 mg/kg bw dose levels, the small increase observed at 500 mg/kg bw was considered not to be biologically significant.

Table: Frequency of micronucleated polychromatic erythrocytes after treatment of NMRI mice with pinoxaden

Substance	Dose (mg/kg bw)	Sampling time (h)	PCEs with micronuclei (%)	Range[a]	PCE/NCE ratio
Vehicle	-	24 h	0.05	0-3	1.35
Pinoxaden	500	24 h	0.1035*	0-4.7[b]	1.13
	1000	24 h	0.055	0-3	1.34
	2000	24 h	0.040	0-1	1.04
cyclophosphamide	40	24 h	1.440***	14-69	1.05
Pinoxaden	2000	48 h	0.060	0-2	1.04

[a] Number of micronucleated PCEs, [b] Value obtained by two separate countings
*= p<0.05, ** = p<0.01, *** = p<0.001

Under the experimental conditions reported, the test item did not induce micronuclei as determined by the micronucleus test with bone marrow cells of the mouse. Therefore, the DS considered pinoxaden as non-mutagenic in this micronucleus assay.

Comments received during public consultation

One MSCA supported that no classification for germ cell mutagenicity is needed for pinoxaden. Another MSCA stated that based on the information presented in the CLH report no evaluation of the results of the mutagenicity tests is possible. The DS included the relevant information from the DAR in an appendix to the RCOM document.

Assessment and comparison with the classification criteria

According to CLP substances can be classified in Category 1A, 1B or 2 for germ cell mutagenicity. For Category 1A 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 the substance is not a Category 1A or 1B mutagen.

For Category 2, CLP states that a substance is regarded as a Category 2 mutagen if it causes concern for humans owing to the possibility that it may induce heritable mutations in 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 *in vivo* studies or chemical structure activity relationship to known germ cell mutagens.

Pinoxaden was tested negative in three *in vitro* tests (one UDS in primary hepatocytes, one Ames test, one cell mutation test (eukaryotic system), and also in an *in vivo* test (UDS *in vivo*).

However, clastogenic activity is indicated by two positive *in vitro* chromosome aberration tests.

A slight increase in micronuclei was observed at the lowest dose (500 mg/kg bw) of an *in vivo* MN test, but not at the higher concentrations (1000 and 2000 mg/kg bw/day). The statistically significant but low incidence at the lowest concentration is considered as not

biological relevant.

However, the MN *in vivo* test has some methodological drawbacks, which questions the validity of the test. The test substance has been applied only once (without any scientific explanation) although two or more treatments would be recommended and might be needed to detect weak clastogens. Furthermore, the negative control data are not within the range of published control data and the number of evaluated cells is lower than suggested in the TG. Thus, there is some remaining uncertainty related to the clastogenic potential of pinoxaden.

However, RAC agrees with the DS that based on the available data **no classification for germ cell mutagenicity** is warranted.

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* <u>Kidney</u> - ↑ 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><td rowspan="2">Tumour type</td><td colspan="5"></td></tr>
<tr><td colspan="5">Females (mg/kg bw/day)</td></tr>
<tr><td></td><td>0</td><td>5</td><td>40</td><td>300</td><td>750</td></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

RAC evaluation of carcinogenicity

Summary of the Dossier Submitter's proposal

The carcinogenic potential of pinoxaden has been investigated by the oral route in three guideline carcinogenicity studies, one in rats (gavage) and two in mice (one gavage, one dietary), all following GLP. There are also two mechanistic investigations conducted in the mouse to assess whether the lung effects seen in the gavage mouse study were attributable to mis-gavage (i.e. direct application to the lungs).

Rat

In a **gavage** guideline chronic toxicity / carcinogenicity study (OECD TG 453; DAR B.6.5.1(a) 2003) Wistar rats received 0, 1, 10, 100, 250 or 500 mg/kg bw/d pinoxaden. Excessive toxicity (24/90 males died by week 53 vs. 3/90 in controls) in high dose males resulted in early termination of this group at week 61. Survival rate was also significantly reduced in males at 250 mg/kg bw/d (38.33% vs. 71.67% in controls). Significantly reduced body weight gains and increased water intake were noted in both sexes at 250 mg/kg bw/d and in females at 500 mg/kg bw/d. However, as the number of survivors at 1, 10 or 100 mg/kg bw/d (3 dose levels) was similar to control group the study was considered acceptable.

Like in the repeated dose toxicity studies in rats the kidneys where the main target of pinoxaden toxicity. Histopathology performed at the end of the study revealed chronic progressive nephropathy in animals treated with 250 mg/kg bw/d and above, renal tubular atrophy in females at 250 mg/kg bw/d and above, and renal tubular dilatation in males and females at 100 mg/kg bw/d and above. At 250 mg/kg bw/d and above also decreased kidney weights, and related changes in urinalysis and clinical chemistry were reported. Other findings included a tendency towards lower haemoglobin concentrations in males and females at 500 mg/kg bw/d.

<u>Tumours</u>

There was an increased incidence of liver adenoma in females at 500 mg/kg bw/d (8% vs. 3.3% in controls, laboratory historical control data (HCD) range: 0-8%) and of endometrial adenocarcinoma from 100 mg/kg bw/d and up (3.3%, 5% and 7% at 100, 250 and 500 mg/kg bw/d, respectively, vs. 1.6% in controls; lab HCD range 0-8.2%). As these increased incidences were within the HCD range the DS considered these findings as incidental.

A slightly increased incidence of leiomyosarcoma of the non-glandular stomach (malignant tumour of the smooth muscle tissue) was noted in males at 250 mg/kg bw/d (2/60 – 3.3% vs. 0% in controls – lab HCD range (5 studies): 0-0%). (For a complete list of HCD, see section on RAC assessment and comparision with criteria.) The DS considered this increase as not related to pinoxaden treatment, because i) the occurrence of one tumour in females at the low dose group of 10 mg/kg bw/d, but not at a higher dose level, which was judged by the DS as 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 gain (13% decrease in males) and water intake.

Table: Tumours seen in the rat carcinogenicity study.

Tumours	**mg/kg bw/d**					
	0	**1**	**10**	**100**	**250**	**500**
Number of **livers** examined; males	60	60	60	59	60	-
Hepatocellular adenoma	3	0	2	2	1	-
Lab HCD for hepatocellular adenoma (males)	3.2% (range 0.0 – 6.0%) from 5 studies [1]					
RITA data base (males)	1% (range 0 – 7.1%) 40 groups / 2056 animals (1995 – 2005)					
Number of **livers** examined; females	60	60	59	60	60	59
Hepatocellular adenoma	2 (3.3%)	0	0	0	2	5 (8%)
Lab HCD for hepatocellular adenoma (females)	3.2% (range 0.0 – 8.0%) from 5 studies [1]					
RITA data base (females)	1% (range 0 – 14%) 40 groups / 2056 animals (1995 – 2005)					
Number of **stomachs** examined; males	59	60	60	59	60	-
Leiomyosarcoma	0	0	0	0	2 (3.3%)	-
Lab HCD for leiomyosarcoma (males)	0.0% (range 0.0 – 0.0%) from 5 studies [1]					
RITA data base (males)	< 0.1% (range 0 – 1.7%) 49 groups / 2635 animals (1991 – 2004)					
Number of **stomachs** examined; females	60	60	59	60	60	59
Leiomyosarcoma	0	0	1 (1.7%)	0	0	0
Lab HCD for leiomyosarcoma (females)	0.0% (range 0.0 – 0.0%) from 5 studies [1]					
RITA data base (females)	0.0% (range 0.0 – 0.0%) 49 groups / 2595 animals (1991 – 2004)					
Number of **uteri** examined; females	60	59	60	59	60	59
Endometrial adenocarcinoma	1 (1.6%)	0	0	2 (3.3%)	3 (5%)	4 (7%)
Lab HCD for endometrial adenocarcinoma (females)	4.5% (range 0.0 – 8.2%) from 5 studies [1]					
RITA data base (females)	3.1% (range 0.0 – 14.3%) 48 groups / 2785 animals					

[1] Fankhauser H (2004) 24-Month Reference Study in Wistar Rats (Control Diet): Reference Control Data. RCC Ltd. Stein Report number 970043. This is a single study initiated in 1997, involving 5 groups of 50 animals / sex / group fed control diet for 24 months.

The DS concluded that there were no carcinogenic effects up to a dose which exceeded the Maximum Tolerated Dose (MTD) (250-500 mg/kg bw/d) in males and females.

Mouse

In the first mouse carcinogenicity study (OECD TG 451, DAR B.6.5.2(a) 2003) with **gavage** administration of 0, 5, 40, 300 or 750 mg/kg bw/d pinoxaden increased mortality was

observed at doses ≥40 mg/kg bw/d (i.e. the 3 highest doses out of 4 doses tested) in a dose dependent manner (males: 57%, 47%, 47%, females: 69%, 66%, 63%, at 40, 300 and 750 mg/kg bw/d, respectively). The DS considered this observed trend of increased mortality 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. The DS reported that this hypothesis was confirmed by two subsequent investigative studies (see below). A further assessment of the lung effects is also included in the section "Assessment and comparison with classification criteria".

At doses ≥300 mg/kg bw/d bodyweight was decreased in females, haematology (increased platelet counts) was affected in males, liver weights were increased in males and females, which was accompanied by increased glycogen deposition in the livers of males.

At 750 mg/kg bw/d bodyweight was also decreased in males, water intake was increased in males and females and kidney weights were increased in females. At this dose also clinical signs of toxicity were described in males and females.

According to the DS the histopathological lung effects (hyalinosis) were slightly increased at 300 and 750 mg/kg bw/d and an increase in incidence of foamy outflow of the bronchi was noted in males and females that died or were sacrificed intercurrently. For the 40 mg/kg bw/d dose the DS stated that the majority of the increased numbers of "accidental deaths" was later confirmed by macro- and histopathology to be associated with effects on the respiratory tract. No treatment related effects at 5 mg/kg bw/d were reported in the CLH dossier.

In male animals, a statistical trend test (Peto *et al*., 1980) showed a significant increase with time for lung adenoma at 750 mg/kg bw/d (14.3% vs. 11.4% in controls; Lab HCD range: 6-14%) and for lung carcinoma at 40, 300 and 750 mg/kg bw/d (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/d (26% and 17% respectively vs. 16% in controls). In female animals, despite an isolated increase in adenoma at 300 mg/kg bw/d, statistical analysis indicated no significant positive trend for the combined incidence of adenoma and carcinoma.

Table: Tumours seen in the first mouse carcinogenicity study with **gavage** administration

Tumour type	**Males (mg/kg bw/d)**				
	0	5	40	300	750
Lungs examined	70	70	69	70	69
Adenomas	8 (11.4%)	4 (5.7%)	4 (5.7%)	11 (15.7%)	10 ↑ (14.3%)
Lab HCD	10.0% (range 6.0 – 14.0%) from 5 dietary studies				
Carcinomas	3 (4.3%)	5 (7.1%)	8 ↑ * (11.4%)	9 ↑ * (12.9%)	5 ↑ (7.1%)
Lab HCD	8.8% (range 2.0-12.0%) from 5 dietary studies				
Combined	11 (16%)	9 (13%)	11 (16%)	18 ↑ * (26%)	12 ↑ (17%)
Lab HCD	18.8% (range 12-26%) from 5 dietary studies				
Tumour type	Females (mg/kg bw/day)				
	0	5	40	300	750
Lungs examined	70	70	70	70	70
Adenomas	5 (7.1%)	5 (7.1%)	1 (1.4%)	10 (14.3%)	4 (5.7%)
Lab HCD	4.8% (range 2.0-8.0%) from 5 dietary studies				
Carcinomas	5 (7.1%)	4 (5.7%)	8 (11.4%)	0 (0%)	6 (8.6%)

Lab HCD	3.6% (range 0.0-6.0%) from 5 dietary studies				
Combined	10 (14%)	8 (11%)	9 (13%)	10 (14%)	10 (14%)
Lab HCD	8.4% (range 6-14%) from 5 dietary studies				

All statistical analyses were conducted with correction for survival:
↑ = statistically significant positive trend ($p \leq 0.05$, Peto test)
* = Statistically significant pairwise comparison to control ($p \leq 0.05$, Peto test)

The DS concluded that the increase of lung adenoma and carcinoma at 300 and 750 mg/kg bw/d was small and just above the Lab HCD range, with no clear dose response relationship and was seen 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. On this basis the DS concluded that these tumours were not related to oral exposure to pinoxaden.

A second guideline carcinogenicity study in mice (OECD TG 451; DAR B.6.5.2(d) 2005) was conducted with **dietary** exposure in order to assess whether the observed lung effects in the first mouse study were caused by gavage dosing/mis-dosing of test material to the lungs.

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/d in males/females). No treatment releated effects on survival or on clinical signs of toxicity were observed.

Significant reduction in bodyweight, body weight gain and food utilisation at the top dose resulted in early termination of the animals of this group at week 40. At 1500 ppm 19% reduction in body weight was seen in both sexes, while at 500 ppm bodyweight reduction by 6% was only seen in females. No other changes of toxicological significance were reported.

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

In the two investigative studies no guideline protocol was followed but GLP criteria applied.

In an *ex vivo* study excised mouse lungs were treated with 0 (control), 250 µL pinoxaden solution (75mg/mL) in vehicle (0.5% CMC and 0.1% Tween 80 in water; same vehicle as used for the mouse gavage carcinogenicity study) or 250 µL vehicle alone for 1 or 10 minutes (2004).

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 seen in the gavage mouse carcinogenicity study (hyalinosis), but less severe. The reason why the effects in the carcinogenicity study were more severe were explained by the repeated exposure over longer time periods.

In the DAR it is reported that the study authors observed difficulties when applying the test material containing pinoxaden to the mouse lungs compared to vehicle alone. It was described that pinoxden containing test material blocked the lungs. The study authours therefore postulated that the higher incidence of lung changes at higher doses in the gavage carcinogenicity study may be related to the physical nature of dosing solution (being thicker at higher concentrations) which could hinder expulsion from the lungs by physiological means.

In the second investigative study (2004) groups of 5 CD-1 mice were dosed by gavage with the same vehicle used in the mouse gavage carcinogenicity study (0.5% CMC and 0.1%

Tween 80 in water). One groupe remained untreated (control), one groupe received a sinlge 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 at 72 hours after dosing and the lungs removed and prepared for histopathology.

While in the "normal" group vehicle did not enter the lungs and no effects were seen in the lungs, in the animals from the oesophagus group vehicle entered the lungs and slight haemorrhage and minimal hyaline changes were seen in the lungs. The described effects were reported to be consistent with the lung lesions (hyalinosis) observed in the mouse gavage carcinogenicity study (2003).

Comments received during public consultation

One MSCA supported the proposed no classification for carcinogenicity.

The applicant (Syngenta) also supported no classification for carcinogenicity and further submitted Syngenta's position on gastric leiomyosarcoma in rats (see supplemental data below).

Assessment and comparison with the classification criteria

Discussion of tumours observed in the rat study

Liver

In female rats hepatocellular adenomas were increased in the top dose above concurrent control (8% vs. 3.3%), corresponding to the upper range of the lab HCD (0-8%). Hepatocellular adenomas also occurred in male rats but the incidence did not show a dose response (highest incidence in controls, lowest in the high dose). Given the benign nature of the tumours, the fact that they occurred within the HCD range and at a dose with considerable toxicity (doses ≥250 mg/kg bw/d exceeded the the MTD) RAC considers these tumours not supportive for a carcinogenicity classification of pinoxaden.

Uterus

The incidence of endometrial adenocarcinomas was dose dependantly increased at doses ≥100 mg/kg bw/d (3.3%, 5% and 7% at 100, 250 and 500 mg/kg bw/d vs. 1.6% in concurrent controls; Lab HCD: 0-8.2%, mean = 4.5%). Doses of ≥250 mg/kg bw/d exceeded the MTD, but at the dose of 100 mg/kg bw/d the tumours occurred without excessive toxicity. However, as the tumours were clearly within the HCD range RAC concludes that these tumours do not warrant classification, considering the uncertainty caused by the possibility that they are not treatment related.

Stomach

Leiomyosarcomas of the non-glandular stomach were slightly (2/60) increased in high dose males (250 mg/kg bw/d): 3.3% vs. 0% in concurrent control and 0.0% in the lab HCD. There was also a single case of leiomyosarcoma of the non-glandular stomach in the 10 mg/kg bw/d groups in females. Leiomyosarcomas are very rare tumours, as can be derived from colony control data, a data base control incidence (RITA) (see table) or published HCDs (Bomhard and Rinke, 1994 (22 groups, 1240 male animals, 0%); Walsh and Poteracki, 1994 (10 groups, 685 male animals, <3 animals with lesion or <0,4%, range: n.d.)).

Historical control data may slightly underestimate the true occurrence of this tumour type due

to the differing terminologies that have been used to describe it (Syngenta position paper, ref to Takahashi, 1985).

In their discussion of the stomach leiomyosarcomas the DS pointed out that the single tumour seen in females at 10 mg/kg bw/d, with no tumours at higher dose levels, indicates a potential spontaneous nature of this tumour type. Even with rare tumours an increase of tumour incidence at significantly higher dosages (in the present case up to a factor of 50) would be expected.

The DS further argued that no pre-neoplastic lesions were seen in the stomachs, however, RAC asserts that it is not always the case that tumour prestages are also detected. At this point reference is also made to the position paper of Syngenta which states that there were no signs of irritation of the stomach in any of the studies which might precede tumour development. However, they also stated that irritation of the stomach mucosa (including damage and repair) is not expected to precede the development of stomach leiomyosarcomas as they arise from the underlying mesenchymal derived structures.

RAC agrees with the DS that it is important to consider the significant generalised toxicity observed at the higher dose animals (≥250 mg/kg bw/d) when assessing the tumours seen at these doses. However, it is also important to note that survival rates in the males of the 250 mg/kg bw/d dose was significantly reduced (38.33% vs. 71.67%), potentially leading to an underestimation of tumours.

For the evaluation of the leiomyosarcoma Syngenta brought forward a position paper in which they indicate that for tumours arising from mesenchymal tissue it would be expected that tumours would also develop from other mesenchymal tissues, not only from the stomach. They brought forward two citations in support for this argument (Tannehill-Gregg *et al*., 2007; Takahashi, 1985) and it is noted, that also US EPA in their assessment of pinoxaden analysed leiomyosarcomas of different tissues together. Like Syngenta also US EPA concluded that no increase with dose was obvious when leiomyosarcomas in different tissues were analysed together.

RAC notes, however, that no mechanistic explanation for the assumption that there should be a link between leiomyosarcomas of different mesenchymal tissues was presented by Syngenta or US EPA.

Mouse studies

Lung adenomas and carcinomas were described in the first mouse carcinogenicity study with gavage administration. When corrected for mortality there was an increase of lung adenomas at the high dose and an increase of lung carcinomas at doses ≥40mg/kg bw/d in males. However, when adenomas and carcinomas were combined, which is justified as lung adenomas are accepted as prestages of lung carcinomas, this increase in comparison to controls was only evident at the two highest doses (300 and 750 mg/kg bw/d). Pairwise comparison with controls gave no clear dose response relation for adenomas and carcinomas, and the incidences in the different dose groups were within the upper range of the HCD data of just minimally above. In female animals, despite an isolated increase in adenoma at 300 mg/kg bw/d, statistical analysis indicated no significant positive trend for the combined incidence of adenoma and carcinoma.

This study was severely compromised by reduced survival in the dosed groups. The explanation presented by the DS that mis-gavage with direct exposure of the lungs to vehicle /vehicle + pinoxaden was the underlying cause for these preliminary deaths seems plausible:

i) a considerable number of deaths were recorded as accidental deaths (occurring within 1 hour after gavage dosing) and there was macro- and histopathological confirmation of respiratory tract involvement,

ii) no reduced survival or evidence for carcinogenic effects in the second mouse carcinogenicity study with dietary application,

iii) two mechanistic studies (one *ex vivo* and one *in vitro* study) showed that direct exposure of the lungs to vehicle and vehicle + pinoxaden caused the same lung effects as observed in the carcinogenicity study (i.e. hyalinosis and increase of intrapulmonary phagocytes),

iv) the increase of this effect with increasing dose as seen in the mouse carcinogenicity study can be explained by the physical properties of the testing material, increasing viscosity with increasing concentration of pinoxaden within the vehicle leading to hampered expulsion from the lung by physiological means (increased exposure time).

It might be possible that the observed lung tumours were secondary to the described lung changes in the lungs or a site of contact effect, although, as stated in the DAR this is considered unlikely.

There was no treatment related effect on the incidence of other tumour types in this study.

Overall the described incidences of lung adenomas and carcinomas in this study do not point towards a carcinogenic potential of pinoxaden in mice.

As there was no evidence for carcinogenicity in the second mouse carcinogenicity study with dietary exposure the DS concluded that pinoxaden was not carcinogenic in the mouse. RAC supports this conclusion.

Comparison with classification cirteria

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.

Pinoxaden has been tested in one guideline carcinogenicity study in rats (gavage) and two guideline studies in mice (one gavage, one dietary).

It can be conluded that pinoxaden was not carcinogenic in the mouse, as the observed lung tumours in the first mouse carcinogenicity study via gavage are likely to be related to mis-gavage. No other treatment related tumours were seen in the gavage study and no treatment related tumours were seen in the dietary mouse carcinogenicity study. In the rat hepatocellular adenomas and endometrial adenocarcinomas were seen in one sex only in the presence of considerable toxicity and / or clearly within laboratory HCD, and are therefore not considered supportive for a carcinogenicity classification. Although leiomyosarcomas of the stomach are considered a very rare tumour type, the slight increase of these tumours in the high dose males (2/60), in the presence of severe toxicity and mortality, is considered as not supportive for a classification. This is supported by the following arguments: i) occurrence of a single leiomyosarcoma of the stomach in the 10 mg/kg bw/d females, but not at higher doses, points towards a possible spontaneous occurrence of the tumour, ii) the leiomyosarcomas of the high dose males were accompanied by severe toxicity and mortality,

iii) the available historical control data could lead to underestimations of the occurrence of leiomyosarcomas because of inconsistent terminologies that have been used to describe it (Takahashi, 1985).

Pinoxaden is not considered mutagenic. A comprehensive test battery including *in vitro* and *in vivo* genotoxicity tests gave mainly negative results, except for two *in vitro* chromosome aberration tests. However, as the *in vivo* micronucleus test gave negative results, no classification for germ cell mutagenicity is proposed (see section on Germ cell mutagenicity).

In summary it can be conluded that the available carcinogenicity studies give not sufficient evidence to support a clasificatin of pinoxaden as carcinogen.

Overall RAC supports the DS's view that classification for carcinogenicity is not warranted.

Supplemental information - In depth analyses by RAC

Lung effects seen in the first mouse carcinogenicity study with gavage administration

In order to have a clear picture on the observed lung effects a table from the DAR is included here, summarising the effects on the lungs in the different dose groups. It supports the DS's conclusion that the lung effects were related to mis-dosing during gavage application at all doses.

Table: Incidence of alveolar / bronchiolar hyalinosis[b] and intra-alveolar macrophages (phagocytic cells) from the first mouse carcinogenicity study (DAR B.6.5.2(a) 2003)

Finding	**Overall incidence (premature decedents + terminal sacrifice)**									
	Males (mg/kg bw/d)					**Females (mg/kg bw/d)**				
	0	5	40	300	750	0	5	40	300	750
Hyalinosis	2	1	4	8	20	5	1	3	6	19
Phagocytic cells [a]	23	36	20	12	6	22	27	27	15	12
Total affected	25/70	37/70	24/70	20/70	26/70	27/70	28/70	30/70	21/70	31/70
Incidence (%)	36	53	34	29	37	39	40	43	30	44

[a] … without simultaneous hyalinosis (to avoid counting animals twice, mice with concurrent phagocytic cells and hyalinosis were not added to the phagocytic cell totals. Low numbers of animals had these findings concurrently in any particular dose group (males: none; females: 1, 1, 3, 3, 2).

Because phagocytic cells were primarily found in terminal animals, it was considered useful by the authors of the DAR to compare the percent incidence in terminal survivors across the dose groups.

Table: Incidence of intra-alveolar macrophages (phagocytic cells) in terminal survivors

Finding	Incidence in terminal survivors									
	Males (mg/kg bw/d)					Females (mg/kg bw/d)				
	0	5	40	300	750	0	5	40	300	750
Total affected	21/58	31/55	16/40	11/33	5/33	20/53	24/59	21/48	15/46	12/44
Incidence (%)	36	56	40	33	15	38	41	44	33	27

HCD for lung intra-alveolar phagocytic cells (from 5 studies; RCC STEIN): Males: 15 of 250 animals (6%, range 4-8%) and females: 19 of 250 animals (7.6%, 4-10%)

The results of this comparison indicate that the incidence of phagocytic cells, as a percentage in terminal survivors, was similar across all the treatment groups. A slight decrease may be

present in males at 750 mg/kg bw/d. The incidence of phagocytic cell response in lungs from control and treated groups was above HCD values from dietary studies in the conducting laboratory and strain of mice. The study authors concluded that these findings reflected direct exposure of the lung to dosing preparation, rather than a systemic effect of pinoxaden.

The findings of hyalinosis in premature decedents was judged to be the consequence of more marked direct exposure compared to phagocytic cells mostly seen in terminal animals in this study (an effect rarely seen in control animals from other studies) was considered to reflect sub-lethal direct exposure to the lungs.

Syngenta's position on gastric leiomyosarcoma in rats

In this position paper Syngenta recommends to compare the very low incidence of leiomyosarcomas induced by pinoxaden in the rat stomach with tumours in tissues of the same embryological origin, as, according to Tannehill-Gregg (2007) and Toyohiko (2007), consistency of response across such tissues should be expected. Based on the comparison of all mesenchymal tumours in any organ in males and females data across all dose groups of the carcinogenicity study they concluded that there is no treatment related effect.

They further state that there is no conjunction with pre-neoplastic lesions or other tumours of the stomach, which could be suggestive of a local irritant effect.

They therefore conclude that the incidence of gastric leiomyosarcomas in male rats at 250 mg/kg is not related to treatment with pinoxaden. It reflects a chance event of slightly higher incidence of one type of mesenchymal tumour.

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

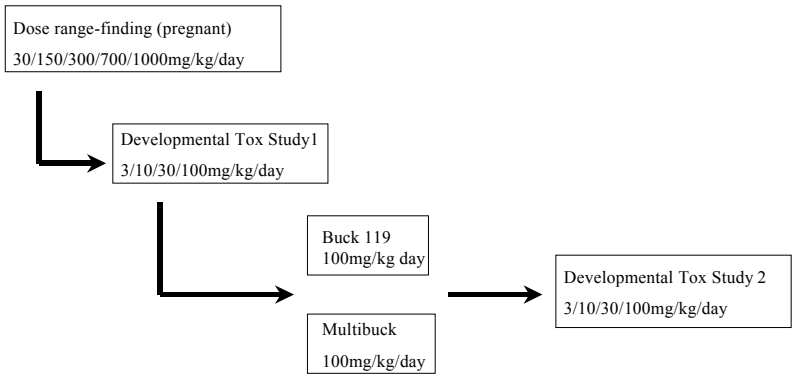
An OECD- and GLP-compliant two-generation rat reproduction toxicity study was conducted using dose levels of 0, 10, 50, 250 or 500 mg/kg bw/day. In this study, no effects on fertility and reproductive performance were seen up to a dose (500 mg/kg bw/day) causing parental toxicity (body weight effects,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

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

The reproductive toxicity of Pinoxaden has been investigated in a guideline two-generation reproduction study in the rat (OECD TG 416), in one guideline developmental toxicity study in the rat (OECD 414), a preliminary dose range finding study in rabbits, and two guideline and two non-standard developmental toxicity studies in rabbits, all following GLP.

Two-generation study

In a guideline **two-generation oral** (gavage) toxicity study (OECD TG 416; DAR B.6.6.1, 5.6.1(a) 2003a) doses of 0, 10, 50, 250 or 500 mg/kg bw/d were administered to **Wistar rats** (30/sex/dosing period).

The effects seen in the F0 and F1 parental generations were comparable to those seen in the

rat repeated dose toxicity studies, with the kidneys being the main target of pinoxaden toxicity. Water consumption was increased at doses ≥ 250 mg/kg bw/day in F1 animals and at 500 mg/kg bw/day in F0 animals. At 500 mg/kg bw/d chronic nephropathy, tubular atrophy was seen in males and females and pelvic dilatation in males of F0 and F1 generation. Relative kidney weight was increased at doses ≥50 mg/kg bw/d in F1 males and ≥250 mg/kg bw/d in F0 males, following a dose response relationship. Also liver weights were increased in females ≥50 mg/kg bw/d and in males ≥250 mg/kg bw/d in F0 and F1 animals. At doses ≥250 mg/kg bw/d F0 and F1 females showed increased glycogen deposition in the liver. In top dose F0 males body weight gain was lower than in controls (8%) although the difference was not statistically significant. No clinical signs were reported at doses up to 500 mg/kg bw/d.

No treatment-related effects on sexual function or fertility (i.e. number of mating animals, number of pregnant females, mean pre-coital time, oestrous cycle and sperm parameters) were reported at any dose level and the NOAEL was set at 500 mg/kg bw/d (highest dose tested).

No treatment-related effects on litter size at birth, or pup viability up to postnatal day (PND) 4 or 21. Offspring toxicity was confined to lower mean pup body weight at 500 mg/kg bw/d from PND 4 in males and females of the F1 and F2 generations. Statistically significant differences compared to controls occurred on PND 4 (F1 females), PNDs 7, 14 and 21 (F1 males and females) and PNDs 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. It is concluded that gondadal function, mating, fertility, gestation, parturition, sperm parameters, regularity of oestrus cycles, histopathology of the reproductive organs, litter size at birth, postnatal pup survival and sexual maturation of F1 pups were not affected by the administration of pinoxaden at any dose level. Lower pup body weight was observed during lactation at the highest dose of 500 mg/kg bw/d.

Developmental toxicity studies

In a guideline prenatal developmental toxicity study (OECD TG 414, DAR B.6.6.1, IIA5.6(a) 2003b) groups of 24 time-mated female **Wistar rats** were dosed via gavage with 0, 3, 30, 300 or 800 mg/kg bw/d pinoxaden on GDs 6 to 20 and terminated on day 21 for evaluation of maternal and developmental toxicity.

Piloerection was seen for 2-7 days in most animals given 800 mg/kg bw/d. At 300 and 800 mg/kg bw/d there was a dose related reduction in body weight gain and food consumption. This was marked at 800 mg/kg bw/d (stat. signif. ↓ body weight gain: 33% on days 6-21, net weight loss after adjustment for gravid uterus weight, stat. signif. ↓ food consumption (max. 28% lower than controls on days 16-21) and minimal at 300 mg/kg bw/d (stat. signif. ↓ body weight gain: 8%; stat. signif. ↓ food consumption (max. 10% lower than controls days 16-21)).

There were no treatment related effects on the number of implanations, pre- or postimplantation loss or the number of viable foetuses. Mean foetal bodyweights were significantly lower (↓ 8%) at 800 mg/kg bw/d.

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 control. Delayed ossification was seen from a dose of 300 mg/kg bw/day in the presence of maternal toxicity (effects on bodyweight gain and food

consumption). Statistical significant reduction in gravid uterus weight (12,4% lower than controls) was seen at the high dose. The developmental effects were considered by the DS to be the secondary, unspecific consequence of the observed maternal toxicity.

In a preliminary **dose-range finding** study in **Russian rabbits** (Himalayan rabbit) (gavage) (DAR B6.6.3, IIA5.6.1 (a), 2003a) doses of 0, 30, 150, 300, 700 and 1000 mg/kg bw/d were administered to 8 time-mated females per group. Treatment started on GD 7 and ended on GD 28, termination was on GD 29. Study groups at doses ≥300 mg/kg bw/d were terminated earlier as all animals were in bad condition (hunched posture, reduced activity and body weight loss and animals were found dead after only a few doses: 1/8 at 300 mg/kg bw/day, 2/8 both at 700 and 1000 mg/kg bw/d). Therefore a dose of 150 mg/kg bw/d was introduced after termination of the dose groups ≥300 mg/kg bw/d.

At 150 mg/kg bw/d one animal was found in moribund condition and was terminated on day 8 of treatment (i.e. GD 15). One 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/d.

At 150 mg/kg bw/d initial weight loss and a 12,7% reduction in weight gain from GD 7-29 for females with viable foetuses and a statistically significant reduction in weight gain of 87% for all pregnant females were observed. Food consumption was statistically significantly reduced by 38,2% from GD 7-12. Four out of 7 females had no live foetuse at this dose. These resportions were reflected by a high incidence of early resorptions and increased post implantation loss and a reduced number of live foetuses for the group.

Foetal body weight was lower than the controls at 30 and 150 mg/kg bw/d, but not statistically significant.

External and visceral examination of the foetuses did not reveal any remarkable findings.

On the basis of these data, dose levels of 0, 3, 10, 30 and 100 mg/kg bw/d were used in a pre-natal developmental study.

In a guideline prenatal **developmental toxicity** study (OECD TG 414, DAR B.6.6.3, IIA5.6.1(b) 2003b) groups of 24 pregnant **Russian rabbits** (Himalayan) were dosed by gavage with 0, 3, 10, 30 or 100 mg/kg bw/d on days 7-28 of gestation.

Maternal body weight gain at 100 mg/kg bw/d was reduced during the treatment period (↓ by 68% days 7-29) but there was no effect on gravid uterus weight. At 30 mg/kg bw/d, the lower body weight gain was not statistically significant. No treatment related deaths or clinical signs were noted.

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

At 100 mg/kg bw/d mean foetal body weight was significantly reduced (11%). RAC considers the lower foetal weight at this dose likely to be caused by the 1.4 x higher mean litter size compared to control. There were no total resorptions at this dose and no increases in pre- or post-implantation loss.

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 the DS concluded that in this study developmental toxicity (reduced foetal body

weight) was seen in the presence of maternal toxicity (effects on body weight and food consumption). These foetal effects were considered by the DS to be secondary and unspecific consequence to the observed maternal toxicity. However, a low incidence of malformations of the diaphragm was seen from a dose of 30 mg/kg bw/d. The DS indicated that no reliable or suitable HCD are available for this finding in this strain of rabbits.

The DS 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 according to the DS. In addition, it was established that all animals showing the diaphragmatic malformation had the same father (i.e. male no 119). It is, however, not known how many other pups were also sired by the same father.

In order to investigate the possible genetic influence on the occurrence of the diaphragmatic lesions, two non-standard prenatal developmental toxicity studies were undertaken.

A **single buck** prenatal developmental toxicity study in Russian rabbits (Himalayan) (DAR B6.6.3, IIA 5.6.1 (c(i), 2003c) was carried out in order to clarify the potential genetic influence on the occurrence of diaphragmatic malformations (hernia, fissure) by using male semen donor no 119 as single buck. The control group and 100 mg/kg bw/d group consisted of 24 female rabbits/group. The dose of 100 mg/kg bw/d was selected as at this dose the diaphragmatic malformations were observed in the previous study.

At 100 mg/kg bw/d body weight gain (↓ 48,5%) and food consumption were significantly reduced during the treatment period.

One female in the dose group aborted on GD 26 (after showing piloerection on GD 24) and was euthanized early. One control female and one dosed female had total resorption of the litter at term. There was no statistically significant increase in pre- or post-implantation loss in the other litters. Litter size and mean foetal body weight was comparable between control and dosed group.

There were no treatment related external foetal findings and no visceral abnormalities, including diaphragmatic hernia or fissure, were reported. No skeletal examination was conducted.

The DS concluded that this study shows that it is unlikely that the diaphragmatic malformations originated as a consequence of the genetic make up of male no 119.

A **multi buck** prenatal developmental toxicity study in Russian rabbits (DAR B.6.6.3, IIA 5.6.1 (C.ii) 2003d) was performed to further clarify the potential role of the sibling status on the occurrence of the diaphragmatic effects. For this prupose male no 119 was excluded as semen donor and matings among siblings were avoided. The control group and 100 mg/kg bw/d group consisted of 24 female rabbits. The dose of 100 mg/kg bw/d was selected as at this dose the diaphragmatic malformations were observed in the first study.

One female was found dead on GD 23. No significant findings (macroscopic findings were considered autolytic) or clinical signs prior to death, but the death was presumed to be treatment related. A second death of a dosed female was attributed to injury and therefore not considered treatment related.

Body weight gain at 100 mg/kg bw/d was reduced during the treatment period (34%), but there was no effect on gravid uterus weight. Food consumption was significantly reduced in dosed animals throughout the early treatment period.

One 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 their litters totally resorbed, with a statistically significant increase in post-implantation loss (25,7% vs. 3,4% in controls). As five control animals and 8 from the dosed group were not pregnant the number of females with viable foetuses at term was 19 in the control group and 11 in the 100mg/kg bw/d group.

There were no treatment related external findings in the foetuses and no visceral findings, including no malformations (hernia or fissure) of the diaphragm. No skeletal examination was conducted.

The DS concluded that the study shows that when the familial 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.

Another guideline prenatal **developmental toxicity** study in **Russian rabbits** (Himalayan) (OECD TG 414, DAR B.6.6.3, IIA5.6.1(d) 2003c) was carried out. A full guideline study using the same strain, Russian rabbits, and the same doses (0, 3, 10, 30 and 100 mg/kg bw/d) as in the first rabbit developmental toxicity study was performed. Potential genetic and familial influences of sibling matings and non-randomised male donors on the results were removed.

One female at 100 mg/kg bw/d was terminated on GD 26 due to its moribund conditions and two females at 100 mg/kg bw/d aborted on GD 27, which was considered treatment related.

Maternal body weight gain at 100 mg/kg bw/d was reduced during the treatment period (63%) but there was no effect on gravid uterus weight. Slightly lower maternal body weight gain seen at 30 mg/kg bw/d following the onset of dosing on day 7 was not statistically significant. Food consumption at 100 mg/kg bw/d was reduced throughout the treatment period. Food consumption at 30 mg/kg bw/d was lower, but not statistically significant different from control during the early treatment period.

There were statistically significant effects on post-implantation loss (38% vs. 0.8% in controls) and number of live foetuses due to early resorptions at the top dose of 100 mg/kg bw/d .

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

The DS concluded that it is highly unlikely that the resorptions observed at the top dose of 100 mg/kg bw/d masked a possible effect of pinoxaden on the diaphragm. The DS argued that hernia and fissure of the diaphragm are not fatal in utero and thus, if they had occurred, they would have been unrelated to the resorptions observed in this study and would have been detected.

According to the DS, developmental toxicity (resorptions and post-implantation losses) was seen at the top dose of 100 mg/kg bw/d in the presence of maternal toxicity (clinical signs of toxicity and effects on body weight and food consumption).

Comments received during public consultation

Four MSCAs commented on the proposal and were in favour of classification as Repr. 2, H361d. They argued that malformations of the diaphragm seen in the first rabbit developmental toxicity study (2003b) could have been masked by increased foetal loss (resorptions and post implantation losses) and / or fewer gravid does in the other studies. Laboratory HCD from 27 separate studies with 5 cases of diaphragmatic hernia (no fissure) were mentioned to further support the relevance of these findings in the first study. It was

further mentioned that the two standard studies were inconclusive to exclude the relevance of the diaphragmatic hernia, as in one study post-implantation loss was considerably increased, which was not seen in the first study. It was also mentioned that the increased incidence of resorptions at 100 mg/kg bw/d should be considered as concern in rabbit in spite of some maternal toxicity.

Another MSCA commented that the diaphragmatic malformations seen in the first rabbit developmental toxicity study were not reproducible under identical experimental conditions and sufficiently investigated to conclude that this malformation, which otherwise would qualify for category 1B classification, was most likely not treatment related. The same MSCA considered the observed resorptions and post-implantation loss to be clearly linked to a strong reduction in food consumption and therefore not supportive for classification as a reproductive toxicant (Cat. 2). However, this MSCA proposed to take the observed maternal toxicity as basis for classification as STOT RE 2 (see section on STOT RE).

The applicant (Syngenta) supported the DS's view that the observed effects (delayed ossification and reduced foetal weights/reduced gravid uterus weight in the rat, and resorptions, post-implantation loss and reduced foetal weights[5] in rabbits) were either caused by maternal toxicity (reduced body weight gain, reduced food consumption in rats and rabbits) or by mating of siblings or other related individuals (diaphragmatic malformations). Syngenta also believes that despite the increased incidence of early post-implantation loss in the second complete developmental toxicity study in the rabbit, sufficient foetuses were available for evaluation from this study and from the two investigative studies. Syngenta summarised it's view in a position paper on developmental toxicity of pinoxaden.

In it's comment EFSA referred to the conclusion of the peer-review meeting for the mammalian toxicology of pinoxaden. The experts noted that even though diaphragmatic malformations were not observed in the second study, other effects were observed at 100 mg/kg bw/d such as post implantation loss and early resorptions that could mask the occurrence of developmental effects. The experts and EFSA proposed a classification as Repr. 2 for developmental effects.

Assessment and comparison with the classification criteria

Fertility

Pinoxaden's potential to cause effects on fertility has been investigated in a OECD TG 2-generation study in rats. In this study, no effects on fertility and reproductive performance were seen up to a dose (500 mg/kg bw/d) causing parental toxicity (body weight effects, increased water consumption, kidney and liver effects).

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 fertility.

Category 1B (presumed human reproductive toxicant) is also not appropriate as there is no clear evidence of an adverse effect on experimental animals that is considered not to be a secondary, non-specific consequence of other toxic effects. No effects on fertility and

[5] It should be noted that according to the original study data, foetal weight was not reduced in rabbits.

reproductive performance were seen in a guideline study up to a dose of 500 mg/kg bw/d causing parental toxicity.

Category 2 (suspected human reproductive toxicant) is also not appropriate because there is no evidence of an adverse effect on fertility. No effects on fertility and reproductive performance were seen in a guideline study up to the dose of 500 mg/kg bw/d causing parental toxicity.

Therefore, RAC supports the DS's proposal **not to classify pinoxaden for fertility**.

Developmental toxicity

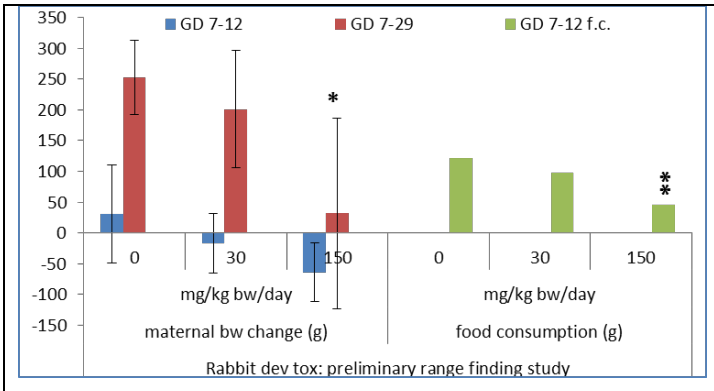
Pinoxaden's potential to cause developmental toxicity has been investigated in five prenatal developmental toxicity studies, one in rats and four in rabbits, including range finding studies.

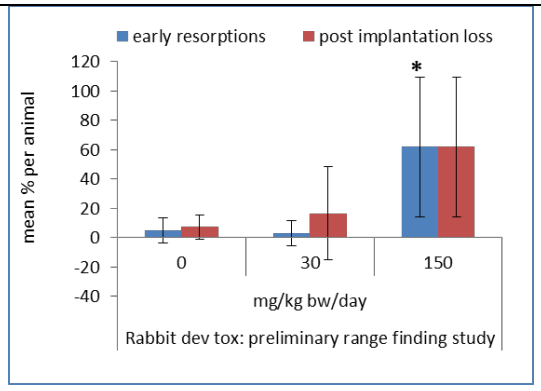
Regarding the rat developmental toxicity study RAC agrees with the interpretation of the DS. In the rat delayed ossification and reduced foetal weight occurred at doses ≥300 mg/kg bw/d. These effects on the rat foetuses were not regarded as severe, and occurred at the same dose level as maternal toxicity (dose dependent effects on body weight gain and food consumption, clinical signs – piloerection at the high dose). No other effects were described.

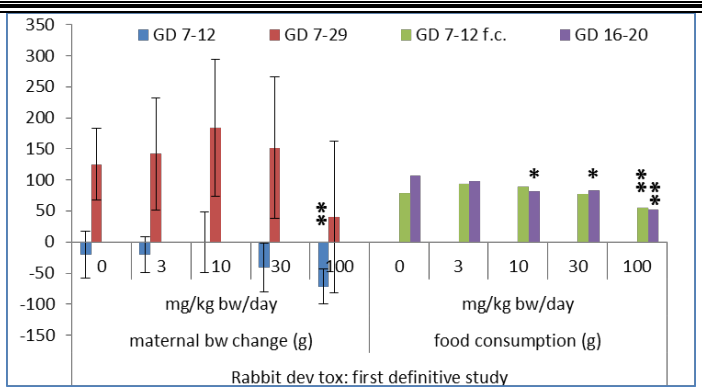
Concerning the rabbit developmental toxicity studies it is necessary to make a detailed analysis of the relation between effects on maternal food consumption and body weight (gain) and the observed implantation losses and abortions as well as the observed malformations.

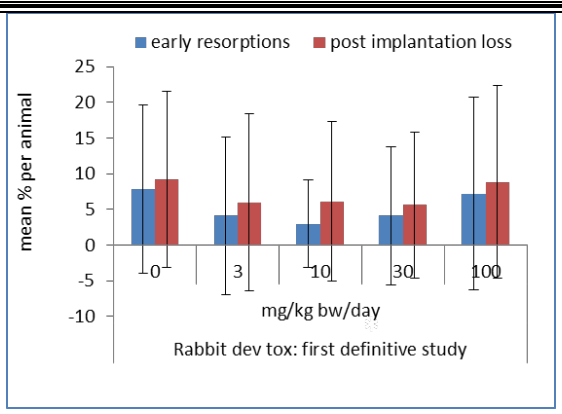
Industry provided an assessment of the pinoxaden developmental toxicity studies (Pinoxaden Developmental Toxicity Assessment, 2014 = Syngenta, 2014), which was included as an annex to the CLH report (Annex I). It contains a detailed overview of the results of the studies in rat and rabbit and it lists the data in a tabular form. Based on this information the following graphs were prepared.

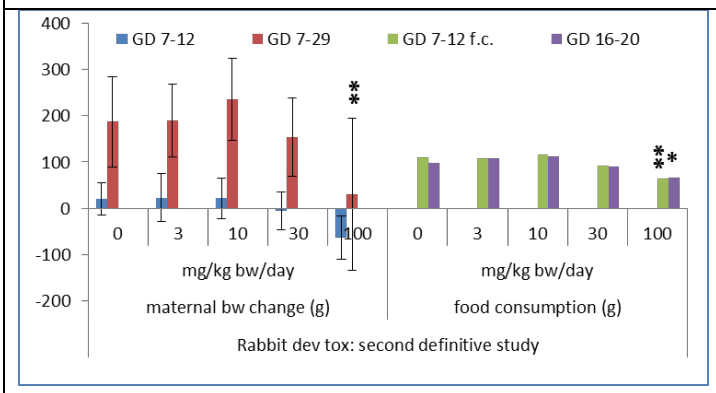
Figure 1: Summary of maternal toxicity data and pregnancy outcome of 5 developmental toxicity studies in rabbits.

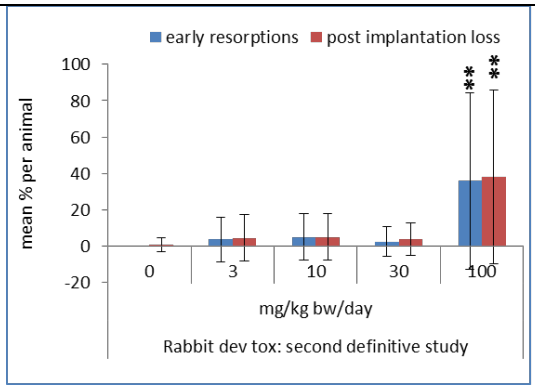


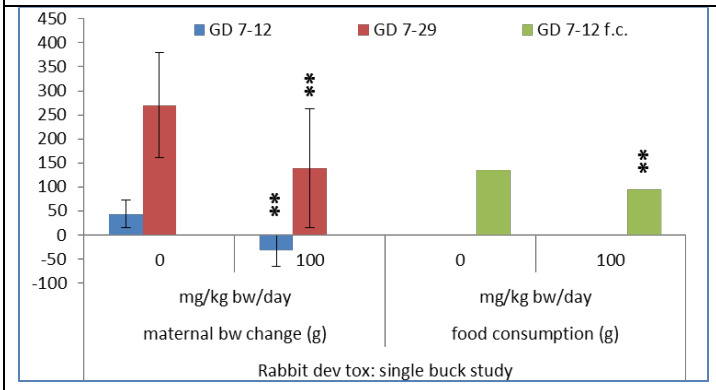


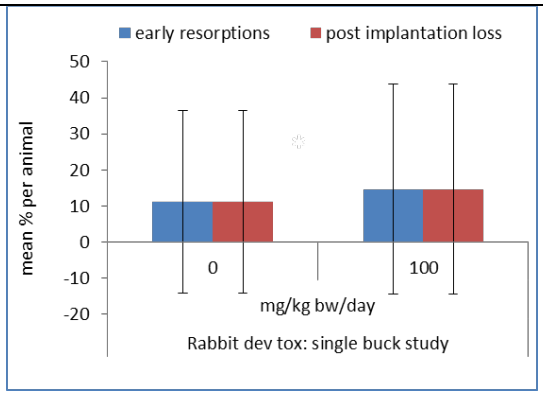


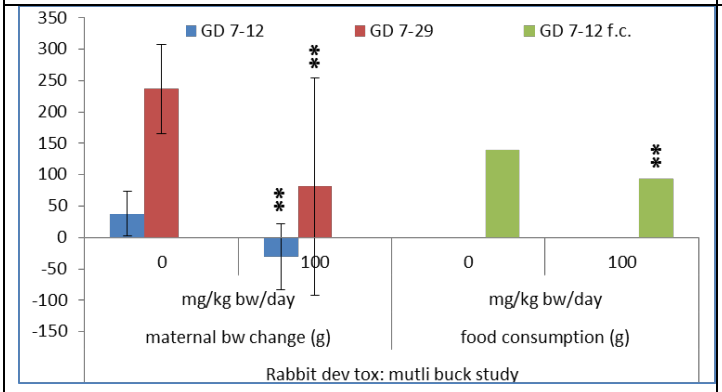


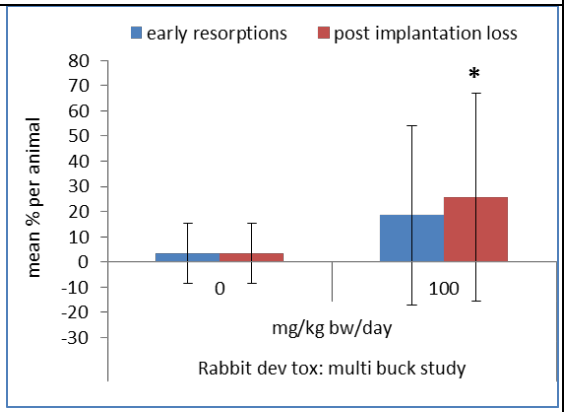












Clinical signs

No clinical signs were reported at doses up until 100 mg/kg bw/d in the rabbit, except in one doe in the single buck study, which showed piloerection before it aborted on GD 26. Three

dams in the multi buck study died, one was sacrificed after abortion on GD 27, another dam aborted on GD 29 and two other dams showed total litter resorptions on GD 29. In the second standard developmental toxicity study three animals were sacrificed in extremis, two of which were showing evidence of abortion. In the preliminary range finding study at 150 mg/kg bw/day one female was terminated in moribund condition and another female showed reduced activity and hunched posture. At doses ≥300 mg/kg bw/d the maximum tolerated dose (MTD) was exceeded (see section on summary of DS's proposal, preliminary range finging study).

Maternal toxicity and post implantation loss

From figure 1 it can be read that in all studies food consumption and maternal weight gain was strongly affected in the high doses (100 and 150 mg/kg bw/d). An intital weight loss was seen in all studies (not always statistically significant) and between GD 7-29 body weight gain of the dams was 12.7%, 32%, 16%, 51,5% and 34% of control in the preliminary range finding study, the 1st and the 2nd full guideline studies, the single buck study and the multi buck study, respectively. Interestingly, post implantation loss was not increased in all animal groups with reduced food consumption and reduced body weight gain. Even significant weight loss did not always result in increased post implantation loss, as for instance in the high dose animals of the multi buck study.

While maternal food consumption and maternal body weight gain were affected in all developmental toxicity study in rabbits, significant increase in post-implantation loss was only seen in the range findings study, the second definitive developmental toxicity study and the multi buck study. The maternal effects can therefore not explain the observed effects on foetal viability. The effects were also different from the results obtained by Cappon *et al*. (2005), who investigated effects of feed restriction on pregnant NZ white rabbits (different from the strain used for the pinoxaden studies). They consistently found reduced foetal body weights and delayed ossification at food rations leading to reduced body weight gain in dams. Increased abortions were only seen in dams that had significant body weight loss. In this regard it is also important to consider the CLP critera under section 3.7.2.4.4: In rabbits the body weight gain may not be a useful indicator of maternal toxicity because of normal fluctuations in body weight during pregnancy.

It can be concluded that the effects on food consumption and maternal body weight were comparable between the different studies. This might indicate that the significant increase in early resorptions / post-implantation loss observed in three studies might not be correlated to the maternal effects.

Abortions and total litter resorption

Two abortions were seen in the 2nd standard developmental toxicity study at the high dose (2 out of 3 animals sacrificed in extremis showed evidence of abortion). Also in the single buck study 1 doe was sacrificed on GD 26 due to abortions and in the multi buck study 1 doe was sacrificed on GD 27 due to abortion and a late abortion was noted on GD 29.

The following table lists % total litter resorptions (number of does with no viable foetus/ number of pregnant does x 100) seen at the high doses of the different studies.

Table: Percentage total litter resorptions in the high dose groups and number of abortions per group

	Range finding study	1st definitive dev tox study	2nd definitive dev tox study	Single buck study	Multi buck study
% total litter resorptions	57,1%	0%	35%	5,6% *	14,3%

Number of abortions	-	-	2	1	2

* … Number of resorptions was the same as in control (one)

Also the observed abortions and litter resorptions cannot be directly linked to the effects on maternal food consumptions and body weight gain across groups.

Gravid uterus weight and foetal weight

Gravid uterus weight and foetal weight were not affected in any of the studies, except a decrease of foetal weight at the high dose of the 1st definite developmental toxicity study. However, this decrease was explained by the 1,4x higher mean litter size at this dose.

For unknown reasons several dams in some studies (see table below) were not gravid. This was, however, not related to treatment as pinoxaden was only administered after implantation (from GD 7 onwards).

Table: Number of gravid does and does with viable foetuses in control and high dose of the single studies

	Range finding study		**1st definitive dev tox study**		**2nd definitive dev tox study**		**Single buck study**		**Multi buck study**	
	mg/kg bw/d									
	0	150	0	100	0	100	0	100	0	100
Number of does / study	8	8	24	24	24	24	24	24	24	24
Number of gravid does	7	7	22	20	22	23	20	19	19	17
Does with viable foetuses	7	3	22	20	22	13	19	17	19	11
Does with viable foetuses / number of gravid does (%)	7/7 (100%)	3/7 (43%)	22/22 (100%)	20/20 (100%)	22/22 (100%)	13/23 (95%)	19/20 (95%)	17/19 (89%)	19/19 (100%)	11/17 (65%)

Malformations

In the 1st definitive developmental toxicity study, visceral examination revealed malformation of the diaphragm in three foetuses from different litters at 100 mg/kg bw/d (diaphragmatic hernia in two and fissure of diaphragm in one). One foetus at 30 mg/kg bw/d had diaphragmatic hernia. There were no treatment related foetal skeletal malformations, anomalies or variations.

This malformation is rare as indicated by the HCD (see tables).

It was argued that this malformation might have been caused by genetic and familial influences (not controlled for sibling matings, all foetuses with the malformation were sired by the same father = animal no. 119). However, in a mechanistic study (single buck study) all animals were sired by animal no. 119 and the same dose of pinoxaden was applied. In this single buck study post-implantation loss was not increased in the dosed animals (see figure 1). As the malformation was not reproducible it can be concluded that the malformation was not

induced by the gentic configuration of male no. 119.

The malformation was not seen in the range finding study, the two mechanistic and the second definitive developmental toxicity studies. However, in these studies post-implantation loss was significantly increased and/or number of does with viable foetuses was reduced, which might have masked possible malformations in the foetuses. It should be noted that in the first definitive developmental toxicity study, where the diaphragm malformations were observed, post-implantation loss was not increased.

In the paper by Syngenta (2014) another malformation is mentioned, i.e. one foetus with spina bifida in the dosed group of the single buck study. Syngenta (2014) further states that no other neural tube findings were recorded in the pinoxaden studies. However, in the second definitive developmental toxicity 2 cases of external hydrocephalus in two litters at 30 mg/kg bw/d were described. It is stated that the findings were within the HCD range and no effects were seen at 100 mg/kg bw/d. However, HCDs from the DAR indicate that these observations were above the HCD (see tables). However, as described for the diaphragmatic malformations, also possible neural tube findings could have been masked by the reduced number of pups available for examination.

Although the 5 developmental toxicity studies were carried out in the same year (2003), using similar test designs and doses and although all animals were retrieved from the same colony, there are considerable differences in the study results. Syngenta (2014) argues that the colony of rabbits was sold and moved over the time period when the experiments for pinoxaden were conducted, which might explain some of the observed differences between studies. Syngenta (2014) also mention that New Zealand rabbits are preferred over Himalayan rabbits because the results in New Zealand rabbits are not so variable across studies as in Himalayan rabbits.

Although Cappon *et al*. (2005) investigated a different strain of rabbit (NZ white rabbit) the effects of food restriction might still be relevant for comparison. They consistently found reduced foetal body weights and delayed ossification at doses leading to reduced body weight gain in dams, and increased abortions only in dams that showed body weight loss. No malformation were induced by feed restriction.

In the rat delayed ossification and reduced foetal weight occurred at doses ≥300 mg/kg bw/d. These effects were not regarded as severe, and occurred at the same dose level as some maternal toxicity (effects on body weight gain and food consumption).

Also in another full guideline study no malformations of the diaphragm were induced at doses of pinoxaden up to 100 mg/kg bw/d. However, in all three of these studies, developmental toxicity (resorptions, post-implantation loss and in total 5 abortions across the studies) was reported at a dose of 100 mg/kg bw/d pinoxaden, in the presence of maternal toxicity (reduction in body weight gain (up to 63%), reduction in food consumption (up to 42%), mortality (up to 8.3%)). Three deaths unrelated to abortion were observed at that dose across the studies (one not treatment related but caused by injury), but no clinical signs were described in the remaining animals.

It should be noted that malformations could have been masked by the reduced number of foetuses available for examination due to increased post-implantation loss and / or low number of dams with viable foetuses.

Based on the above effects, 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 or were covered with some uncertainties.

Delayed ossification and reduced foetal weights in the rat were considered to be a secondary, unspecific consequence of the observed maternal toxicity.

In contrast, the resorptions, post-implantation loss and abortions in the rabbit cannot be judged as unspecific and secondary to maternal toxicity. While maternal food consumption and maternal body weight gain were affected in every single developmental toxicity study in rabbits, significant increase in resorptions, post-implantation loss and abortions were only noted in three of the studies.

In the rabbit malformations of the diaphragm (hernia / fissure) which occurred above laboratory HCD and following a dose response were seen in a single study. In two investigative studies a possible link of these effects to mating among siblings/related animals could not be finally excluded (information not available) but it could be excluded that they were related to the male animal which sired all foetuses showing the malformation in the first study. Although these diaphragmatic malformations seen were not repeated in the other developmental toxicity studies in rabbits, they cannot be neglected as in these other studies resorptions and post-implantation loss were increased (which were not seen in the first study) and / or number of pregnant does was reduced which might have masked the potential occurrence of malformations.

Based on the available data a potential for teratogenicity cannot be excluded and the observed post-implantation loss cannot be regarded as secondary to the maternal effects and are therefore considered to be developmental effects. As there are some uncertainties related to the data base, a classification in Category 1B is not justified, but Category 2 (suspected human reproductive toxicant) is supported.

RAC supports the classification of pinoxaden as **Repr. 2; H361d.**

Supplemental information - In depth analyses by RAC

Table: Summary of historical control data in Himalayan rabbits

	Reference	Laboratory	Number of foetuses / litters	Number of hernias / fissures	% of foetuses / litters
1	Info. From "Syngenta, 2014", Ref. missing	3 different facilities, 1986	8060 / 1273	0	0 / 0
2	Info. from "Syngenta, 2014", Ref. missing	Japanese colony, 1971 - 1991	2883 / 514	0	0 / 0
3	Info. from "Syngenta, 2014", Ref missing	Lab which performed the pinoxaden studies, 1968 - 1999	7707 / 1136	1	0.01 / 0.09
4	Info. from "Syngenta, 2014", Ref. missing; Overlap with 3	Lab which performed the pinoxaden studies ?	3672 / 664	2 (one hiatal hernia of the diaphragm)	0.05 / 0.3
5	Info from "Syngenta position paper", same as 4?	Lab which performed the Pinoxaden studies ?	3672 / 664	1	0.03 / 0.15

To allow direct comparison with the diaphragmatic malformations seen in the pinoxaden studies the following table is included.

Table: Diaphragmatic malformations observed in the pinoxaden studies

From all pinoxaden studies:			
Dose:	Number of foetuses / litters	Number of hernias / fissures	% of foetuses / litters
30 mg/kg bw/d	241 / 48	1 / 0	0.4% / 2.1%
100 mg/kg bw/d	350 / 86	2 / 1	0.9% / 3.5%
From the single pinoxaden study in which the diaphragmatic malformations were observed:			
30 mg/kg bw/d	? / 24	1 / 0	? / 4.2%
100 mg/kg bw/d	? / 24	2 / 1	? / 12.5%

In the Syngenta position paper further information from the laboratory's data is presented (see table below). It states that in 27 studies (1991 – 1995) there were 5 hernias. However, only 1 of these 5 hernias was seen in the control dose, the other 4 doses were seen in dosed groups, and hence might have been induced by the test material of the specific study.

Table: Incidence of diaphragmatic hernias noted in developmental toxicity studies in Himalayan rabbits (Syngenta position paper)

Individual study	**Report data**	**Foetal incidence**	**Experimental group**
911127	7/91	single	Low dose
922822	8/92	single	Control
922847	1/93	single	Mid dose
923154	3/93	single	High dose
942119	8/95	single	Mid dose

Table: Laboratory historical control data for spina bifida (information taken from the DAR)

Malformation	Vehicle controls	Controls including unaffected dosages		
	n	n	% of total malformations	% of foetuses
Litters examined	1027	3098	-	-
Foetuses examined	6992	20551	-	-
Malformations	89	237	-	-
Spina bifida	0	5	2.11	0.02

Table: Laboratory historical control data for external hydrocephalus (information taken from the DAR)

Malformation	Foetal incidence (from 3672)				Litter incidence (from 664/3 litters)			
	n	mean	min	max	n	mean	min	max
External hydrocephalus (visceral)	4	0.1	0.0	1.1	4	0.6	0.0	9.1

Table: Spina bifida and external hydrocephalus observed in the pinoxaden studies

From all pinoxaden studies:				
Dose:	Number of foetuses / litters	Number of external hydrocephalus / spina bifida	External hydrocephalus	Spina bifida
			% of foetuses / litters	
30 mg/kg bw/d	241 / 48	2 / 0	0.8% / 4.2%	-
100 mg/kg bw/d	350 / 86	0 / 1	-	0.3% / 1.2%

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>
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<table>
<tr><th></th><th>Water kw DT50 (days)</th><th>Sediment ks DT50 (days)</th></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'

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier Submitter's proposal

Pinoxaden is not currently listed in Annex VI to CLP. The DS proposed to classify the substance as Aquatic Acute 1 - H400, M=1 and Aquatic Chronic 3 - H412. The proposal was based on the substance being rapidly degradable, non bioaccumulative and very toxic to aquatic organisms. The lowest acute EC_{50} values were 0.80 mg/L (mean measured [mm]) for the algae *Skeletonema costatum* and 0.40 mg/L (mm) for the oyster *Crassostrea virginica*, respectively. The lowest chronic NOEC values were 0.438 mg/L (initial measured [im]) for *Lemna gibba* and 0.52 mg/L (mm) for *Skeletonema costatum*, respectively. Re-calculated and corrected data on several aquatic toxicity studies (endpoints based on mean measured concentrations for pinoxaden only) was submitted as a response to the Public Consultation comments. These data, however, did not result in a change of the DS's initial

classification proposal for pinoxaden as hazardous to the aquatic environment.

Degradation

There is one hydrolysis study available following OECD TG 111 (1981) and US EPA 161-1 (1982) guidelines and performed according to GLP principles using ^{14}C radiolabelled pinoxaden. Hydrolysis was pH and temperature dependent as shown in the table below. The first order half-life was from 0.3 days at pH 9 up to 25.3 days at pH 5 at 20°C. The mass balance was > 94.8% of applied radioactivity (AR) indicating that there was no major production of volatiles.

Table: Hydrolytic half-lives under various laboratory conditions

Temperature	pH 4	pH 5	pH 7	pH 9
15°C	-	-	23.3 days	0.6 days
20°C (calculated)	24.1 days	25.3 days	14.9 days	0.3 days
25°C	17.2 days	17.5 days	9.9 days	0.2 days

Under environmental conditions, pinoxaden is expected to hydrolyse very rapidly only when the surface water pH was relatively high.

Two aquatic photolysis studies both following GLP principles showed that pinoxaden undergoes limited photodegradation and was thus considered photolytically stable under environmentally relevant conditions. An aqueous photolysis study following US EPA 161-2 (1982) guideline gave a DT_{50} of 10.1 days at pH 4.3. The major photolytic metabolite was NOA 407854 (M2), which reached a maximum concentration of 35.2 % AR. The other study following OECD TG 101 and US EPA OPPTS 835.2210 guidelines gave theoretical aquatic photolytic half-lives for pinoxaden ranging from 82.2 days in summer at 30 °N to 954 days in winter at 50°N.

A ready biodegradation study was conducted according to GLP principles and following OECD TG 301B , showing that pinoxaden is not readily biodegrable (12% degradation at day 29).

A water/sediment study following GLP principles and OECD (2000 draft) and BBA (1990) guidelines (equivalent to OECD TG 308) was performed applying phenyl ^{14}C-radiolabelled pinoxaden to a river system (Rhein) with a loam/sandy sediment (water pH 8.3) and a pond system (Rotenfluh) with a silty clay loam sediment (water pH 8.1) over a period of 147 days. Pinoxaden degraded rapidly with DT_{50} 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 only major degradant was M2, identified in both the river and the pond systems, and it was shown to be persistent. The DT_{50} of pinoxaden in sediment was a maximum of 2 days in the pond system. Partitioning to sediment was weak and the maximum of pinoxaden remained in the water phase, where it degraded. The water DT_{50} values for the degradant M2 were 294.4 days for the river system and 128.8 days for the pond system. The sediment DT_{50} values for M2 were approximately 64 days in both systems. Mineralisation was only a minor element of dissipation of pinoxaden. Organic volatiles were below the limit of detection in both systems. Incorporation into non-extracted sediment residues was considered a further route of dissipation, with up to 14.1 % AR being present in sediment organic matter after 147 days in the pond system.

Another aerobic water/sediment study using oxadiazepine-ring radiolabelled ^{14}C-pinoxaden was performed following GLP principles and OECD (2000 draft) and BBA (1990) guidelines

(equivalent to OECD TG 308) in the same river and pond systems as described above over a period of 100 days at 20°C. The study was conducted in the dark and also under both artificial and natural light conditions. The river system used a loam sediment and the water pH was 7.4. The pond system had a silty, clay loam sediment with a water pH of 7.2. Pinoxaden degraded rapidly with a DT_{50} of < 1 day in all compartments, whereas the degradant M2 degraded slowly in the dark (total system DT_{50} values of > 1 year for the river and 270 days for the pond system). The results of the illuminated incubations indicated that, under suitable conditions, photolysis contributed significantly to the degradation of M2 in water/sediment systems (see Table below).

It has been questioned by the DS whether the rapid degradation of pinoxaden seen in the water/sediment studies is representative since at least one of the studies was conducted at a high pH which was significantly seen to increase sterile hydrolysis, which might be a predominant route of degradation. The DS was of the opinion that since the total system DT_{50} values were less than 1 day and similar at pH 7.2-7.4 as at pH 8.1-8.3, pH did not appear to make such a difference to degradation in non-sterile whole water/sediment systems. This may be due to a combination of the influence of biotic degradation, the presence of sediment and photolysis in illuminated systems, although in isolation these processes make less difference.

Table: Half-lives in aerobic water/sediment systems for pinoxaden

	pH 8.1-8.3, dark		**pH 7.1-7.4, dark**		**pH 7.1-7.4 artificial sunlight**	**pH 7.1-7.4 natural sunlight**
Pinoxaden	DT_{50} water (days)	DT_{50} sediment (days)	DT_{50} water (days)	DT_{50} sediment (days)	DT_{50} total system (days)	DT_{50} total system (days)
River	0.268	0.774	0.6	0.1	0.7	0.4
Pond	0.276	2.000	0.4	0.2	0.6	0.2

Table: Half-lives in aerobic water/sediment systems for the major degradant M2

	pH 8.1-8.3, dark		**pH 7.1-7.4, dark**		**pH 7.1-7.4 artificial sunlight**	**pH 7.1-7.4 natural sunlight**
M2	DT_{50} water (days)	DT_{50} sediment (days)	DT_{50} water (days)	DT_{50} sediment (days)	DT_{50} total system (days)	DT_{50} total system (days)
River	No degradation	64.474	>1 year	183.2	112.3	144.7
Pond	No degradation	64.793	154.2	96.7	64.5	151.3

In addition to the major metabolite M2 other minor metabolites including M3 were identified in abiotic and biotic test systems.

The DS considered pinoxaden to be rapidly degradable for classification purposes due to its rapid degradation demonstrated by DT_{50} values below 1 day in the water/sediment systems and due to non classifiable degradation products M2 and M3.

Bioaccumulation

The log Kow of pinoxaden is 3.2 (EEC Method A.10.) and the log Kow values of the degradants M2 and M3 are -1.1 and 1.8, respectively. No bioconcentration studies were

available. Based on the available information the DS concluded that pinoxaden and its degradants have a low potential of bioaccumulation.

Aquatic toxicity

There are acute toxicity data available from three fish studies, three invertebrate studies, four algae studies and two higher aquatic plant studies. Chronic toxicity data is available from 4 algae studies and 2 higher plant studies As well as data on a prolonged fish study (OECD TG 215). The lowest aquatic toxicity values of each trophic level are presented in the table below. Unless otherwise stated, all of the ecotoxicological studies on pinoxaden were performed according to GLP principles and were considered reliable and suitable for hazard classification purposes by the DS. Data are also available on the main degradants M2 (in water, sediment and soil) and M3 (in soil). The DS gave some newly calculated values and one corrected value as a response to the public consultation comments. These values were also included in the tables where appropriate.

No chronic test on fish and aquatic invertebrates was seen necessary because pinoxaden has a whole water/sediment system DT_{50} of < one day and therefore will not exist for long in the aquatic environment. Corresponding chronic studies are available for the more persistent M2 degradant. A prolonged juvenile fish growth test was subsequently submitted for pinoxaden and, whilst not truly chronic, this was seen sufficient for a substance which degrades as rapidly as pinoxaden.

There is no chronic invertebrate endpoint available but the DS did not consider a surrogate chronic classification using an acute invertebrate endpoint being necessary due to the substance being rapidly degradable and not bioaccumulative.

Table: The lowest relevant aquatic toxicity values (key data are highlighted in bold).

Substance (purity)	Species	Test guidelines	Endpoint (conditions)	Toxicity value mg a.s./L
Acute toxicity to fish				
Pinoxaden (97.2%)	*Oncorhynchus mykiss*	OECD TG 203	96hr LC_{50} (flow-through)	10.3 mm[1] pinoxaden only (58-65% of nominal)
Acute toxicity to aquatic invertebrates				
Pinoxaden (97.7%)	*Crassostrea virginica*	US EPA OPPTS 850.1025	96hr LC_{50} **96hr EC_{50} (shell deposition)** 96h NOEC (flow-through)	>0.88 **0.40 mm**[1] *0.046 mm*[6] pinoxaden only (67.7-91.5% of nominal)
Acute toxicity to algae				
Pinoxaden (97.2%)	*Skeletonema costatum*	OECD TG 201	72hr E_rC_{50} (static)	1.72 nominal [2] 0.80 mm[1] pinoxaden only
Acute toxicity to higher aquatic plants[5]				
Pinoxaden (97.2%)	*Phragmytes australis*	Based on draft OECD TG 221	20 d E_rC_{50} (growth-plant height)(static)	8.5[3] nominal im not calc. ***0.63 mm***[1] *pinoxaden only*[5]
Pinoxaden	*Lemna gibba*	Draft OECD TG	7 d E_rC_{50} (frond	13.9 nominal

(97.2%)		221	no.)(static)	9.7 im[3] *1.698 mm*[1] *pinoxaden only*[5]

Table: The lowest relevant chronic toxicity values (key data are highlighted in bold).

Substance (purity)	Species	Test guidelines	Endpoint (conditions)	Toxicity value mg a.s./L
Prolonged toxicity to fish				
Pinoxaden (97.2%)	*Oncorhynchus mykiss*	OECD TG 215	28 d NOEC (growth) 28 d NOEC (mortality) (flow-through)	6.6 mm[1] 3.2 mm[1] pinoxaden only (80-120% of nominal)
Chronic toxicity to aquatic invertebrates not available				
Chronic toxicity to algae				
Pinoxaden (97.2%)	*Skeletonema costatum*	OECD TG 201	72hr NOE_rC (static)	0.94 nominal [2] 0.52 mm[1] pinoxaden only
Chronic toxicity to higher aquatic plants[4]				
Pinoxaden (97.2%)	*Phragmytes australis*	Based on draft OECD TG 221	**20 d NOE_rC (height, biomass and chlorosis)(static)**	3.0[3] nominal im not calc. ***0.17 mm***[1] ***pinoxaden only***[5]
Pinoxaden (97.2%)	*Lemna gibba*	Draft OECD TG 221	7 d NOE_rC (frond no.& dry weight)(static)	0.625 nominal 0.438 im[3] *0.23 mm pinoxaden only*[5]

[1] mm = mean measured concentration
[2] mean measured concentration decreased significantly during the test. Concentrations of M2 were tested → sum of pinoxaden and M2 above 80% of nominal → toxicity due to both → nominal pinoxaden concentrations used.
[3] mean measured concentration decreased significantly during the test. Concentrations of M2 were tested → sum of pinoxaden and M2 above 76% of nominal → toxicity due to both → nominal pinoxaden concentrations used → by the end of the test pinoxaden plus M2 dropped → initial measured concentration (im) of pinoxaden used.
[4] In *Lemna gibba* and *Phragmytes australis* studies the low recovery of pinoxaden plus M2 was explained due to uptake and further metabolism as well as uptake into the soil for *P. australis*.
[5] Information received from the DS in replies to the Public Consultation comments.
[6] An error that has been corrected in the DS's reply to the Public Consultation comments.

Pinoxaden is a herbicide and algae and aquatic plants are the most sensitive trophic group. Acute L/EC_{50}s for fish, invertebrates and mysid shrimp are > 1 mg/L. An acute LC_{50} is available for the oyster *Crassostrea virginica* of > 0.88 mg/L which was the highest level tested and no mortality was seen. A 96 hour EC_{50} for shell deposition of 0.4 mg/L was also reported from the same oyster study. The DS was uncertain in using this value because the notifier under the pesticides regime had argued that it is not relevant for such purposes as it is based on growth rather than the usual mortality or immobilisation.

All of the algal/plant studies were confounded by being static and showing rapid degradation of pinoxaden to M2. The nominal pinoxaden concentrations have been seen acceptable by the DS since, in most cases, the mean measured concentrations of pinoxaden plus M2 were 80% of the nominal and toxicity was assumed to be due to both substances. In the case of the algal studies M2 was, however, of relatively low toxicity compared to pinoxaden and thus this combined approach would not reflect the hazard of pinoxaden.

Studies on higher aquatic plants/microphytes*, L. gibba* and *P. australis* were also static and affected by substantial losses of pinoxaden even so that total mean measured concentrations of pinoxaden plus M2 dropped below 80 % and initial measured

concentrations were re-calculated.

For *P. australis* it was assumed that pinoxaden and M2 posed a similar toxicity and the initial measured sum of the two chemicals was calculated to be > 80% of nominals and the results are expressed as nominals.

The DS was of the opinion that it could have been argued that, ideally, mean measured pinoxaden-only endpoints (without consideration of M2) should be recalculated for the algal/plant species, but these were not available at the time the proposal was submitted. They were, however, provided in the response to the Public Consultation comments. The re-calculated values were added to the table above and the consequent key study results are indicated in bold.

The main degradant of pinoxaden is M2. The acute L/EC_{50}s for fish, *Daphnia* and algae are greater then 100 mg/L. The 7 days E_rC_{50} (frond no.) for *L. gibba* is 14.6 mg/L. The chronic NOEC for fish *Daphnia* and algae range between ≥ 1 mg/L (highest nominal concentration tested) and 100 mg/L. The 7 days NOE_rC (frond no. and dry weight) for *L. gibba* is 4.0 mg/L.

In soil M2 degrades further to M3. The acute L/EC_{50}s for fish, *Daphnia,* algae and *L. gibba* are greater then 100 mg/L. The 72 hours NOE_rC for algae is 15 mg/L and the 7 days E_rC_{50} (frond no.) for *L. gibba* is 50 mg/L.

Comments received during public consultation

Four Member State Competent Authorities (MSCAs) supported the DS's proposal. One MSCA questioned the use of the prolonged fish study to assess chronic toxicity. They also had questions about the concentrations used to calculate the results of the *L. gibba* and *P. australis* tests. Another MSCA pointed out that for algae and aquatic plants studied in static exposure conditions, mean measured concentrations should be used to calculate the results. An MSCA paid attention to the lack of chronic data on *C. virginica* which has a lowest acute toxicity value.

The DS felt that performing a chronic fish test is not needed because pinoxaden is rapidly degrable and the prolonged fish growth test has been conducted on a sensitive life stage. There is also a chronic fish test (according to US EPA OPPTS 850.1400) available for the more persistant main degradant M2. Furthermore the DS submitted re-calculated results based on mean measured concentrations of pinoxaden for *L. gibba* and *P. australis* endpoints. These have been added to the lowest acute and chronic toxicity tables in the opinion, where relevant. An error has also been corrected concerning the *C. virginica* oyster study, in particular the acute (shell deposition) NOEC should be 0.046 mg pinoxaden/L as mean measured concentrations instead of 0.46 mg pinoxaden/L as mentioned in the CLH Report.

Additional key elements

The pH in the two water/sediment studies were around 7 and 8 and no hydrolysis dependency could be seen in the results although this could have been expected based on the results of the hydrolysis study. There are, however, soil simulation studies performed also at lower pH values. According to the DAR (Volume 3, Annex B, B.8, part 2) degradation in soil in also very rapid with DT_{50} values of < 2 days. There are studies performed at pH values ranging from 5.1 to 8.0. Initial degradation of pinoxaden seems to

be abiotic. No or little pH dependency was discovered in soil degradation studies. Two major metabolites are formed. The principal metabolite is M2 which forms M3. M2 may be considered low to moderate persistent and M3 moderate to high persistent. Photolysis may contribute to the degradation of M2 and M3. The degradation of pinoxaden is not specifically dependent on microbial activity in soils. Degradation of M2 is principally microbially mediated.

Assessment and comparison with the classification criteria

RAC agrees with the DS conclusion that pinoxaden is rapidly degrable. Although it is not readily degradable, its dissipation half-life in water, sediment and total system both in dark, artificial sunlight and natural sunlight is less than 2 days. The dissipation half-life in soil is also less than 2 days. The main degradation product is M2 which is not classifiable for aquatic environmental hazards. M2 is not rapidly degradable (DT_{50} > 64 days) but the acute and chronic toxicity values, the lowest being an acute E_rC_{50} of 14.6 mg/L and chronic NOE_rC of 4.0 mg/L for *L. gibba*, do not warrant classification. In soil M2 forms M3 which is more persistant than M2. The acute L/EC_{50}s for fish, *Daphnia,* algae and *L. gibba* are greater then 100 mg/L. The lowest chronic NOE_rC for algae is 15 mg/L. Thus M3 is also not classifiable for environmental hazards. Although pinoxaden does not pass the ready biodegradation test, it is demonstrated to be primarily degraded biotically or abiotically in the aquatic environment with a half-life < 16 days (corresponding to a degradation of > 70% within 28 days), and it is demonstrated that the degradation products do not fulfil the criteria for classification as hazardous to the aquatic environment. Furthermore, pinoxaden has no potential to bioaccumulate based on the log Kow of 3.2.

The DS has provided re-calculated toxicity values based on mean measured pinoxaden concentrations for several algae and higher plants study endpoints. These re-calculated values have been added to the aquatic toxicity tables above. Consequently, by taking these data into account the lowest acute toxicity value is a 96 hours EC_{50} (shell deposition) of 0.40 mg/L for the oyster *C. virginica*. There are two further acute toxicity results in the same range (> 0.1 mg/L but ≤ 1.0 mg/L), namely a 20 days E_rC_{50} of 0.63 mg/L for the higher plant *P. australis* and a 72 hours E_rC_{50} of 0.80 mg/L for the algae *S. costatum* warranting a classification as Aquatic Acute 1 with a corresponding M-facotr of 1. No chronic test on the acutely most sensitive species, *C. virginica*, is available. However, the 20 days NOE_rC of 0.17 mg/L for *P. australis* is the lowest chronic value. The NOEC of 0.52 mg/L for *S. costatum* and a 7 days *L. gibba* NOE_rC of 0.23 mg/L are in the same range (> 0.1 mg/L but ≤ 1.0 mg/L). Considering pinoxaden to be rapidly degradable (to non-classifiable degradants) a classification as Aquatic Chronic 3 is warranted.

The use of a surrogate approach for fish and invertebrates would not have changed the classification of pinoxaden since the chronic algae/higher plants data is the key data for chronic classification. For fish there is data from a prolonged test but for invertebrates the chronic data is missing. RAC can accept that a chronic fish study was not needed in this case. The substance is a herbicide and the available data showed oysters, algae and higher plants to be the most sensitive species. The lack of chronic data on *C. virginica* and other invertebrates might lead to the use of a surrogate approach for invertebrates. This would not, however, change the classification since algae/higher plants data in the same range are available for both acute and chronic endpoints. RAC appreciated the re-calculated values as a better basis for classification purposes. The surrogate system would mean no chronic classification with the invertebrate data because pinoxaden is rapidly degradable

and non bioaccumulative. Consequently, RAC agrees with the DS's proposal to classify pinoxaden as **Aquatic Acute 1; H400, M=1** and **Aquatic Chronic 3; H412**.

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