Committee for Risk Assessment

RAC

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

Adopted

16 September 2016

16 September 2016

CLH-O-0000001412-86-127/F

OPINION OF THE COMMITTEE FOR RISK ASSESSMENT ON A DOSSIER PROPOSING HARMONISED CLASSIFICATION AND LABELLING AT EU LEVEL

In accordance with Article 37 (4) of Regulation (EC) No 1272/2008, the Classification, Labelling and Packaging (CLP) Regulation, the Committee for Risk Assessment (RAC) has adopted an opinion on the proposal for harmonised classification and labelling (CLH) of:

Chemical name: **pinoxaden (ISO); 8-(2,6-diethyl-4-methylphenyl)-7-oxo-1,2,4,5-tetrahydro-7 H-pyrazolo[1,2-d][1,4,5]oxadiazepin-9-yl 2,2-dimethylpropanoate**

EC Number: **Not assigned**

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

The proposal was submitted by the **United Kingdom** and received by RAC on **14 September 2015.**

In this opinion, all classification and labelling elements are given in accordance with the CLP Regulation.

PROCESS FOR ADOPTION OF THE OPINION

The United Kingdom has submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/harmonised-classification-and-labelling-consultation/*** on **30 September 2015**. Concerned parties and Member State Competent Authorities (MS) were invited to submit comments and contributions by **16 November 2015**.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Christine Hölzl**

Co-Rapporteur, appointed by RAC: **Riitta Leinonen**

The opinion takes into account the comments provided by MSs and concerned parties in accordance with Article 37(4) of the CLP Regulation and the comments received are compiled in Annex 2.

The RAC opinion on the proposed harmonised classification and labelling was adopted on **16 September 2016** by **consensus**.

Classification and labelling in accordance with the CLP Regulation (Regulation (EC) 1272/2008)

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard state-ment Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	No current Annex VI entry										
Dossier submitters proposal	TBD	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	-	243973-20-8	Acute Tox. 4 Skin Irrit. 2 Eye Irrit. 2 Skin Sens. 1A STOT SE 3 Aquatic Acute 1 Aquatic Chronic 3	H332 H315 H319 H317 H335 H400 H412	GHS07 GHS09 Wng	H332 H315 H319 H317 H335 H400 H412		M=1	
RAC opinion	TBD	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	-	243973-20-8	Repr. 2 Acute Tox. 4 Eye Irrit. 2 Skin Sens. 1A STOT SE 3 Aquatic Acute 1 Aquatic Chronic 3	H361d H332 H319 H317 H335 H400 H412	GHS08 GHS07 GHS09 Wng	H361d H332 H319 H317 H335 H400 H412		M=1	
Resulting Annex VI entry if agreed by COM	TBD	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	-	243973-20-8	Repr. 2 Acute Tox. 4 Eye Irrit. 2 Skin Sens. 1A STOT SE 3 Aquatic Acute 1 Aquatic Chronic 3	H361d H332 H319 H317 H335 H400 H412	GHS08 GHS07 GHS09 Wng	H361d H332 H319 H317 H335 H400 H412		M=1	

GROUNDS FOR ADOPTION OF THE OPINION

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.

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**.

HUMAN HEALTH HAZARD EVALUATION

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 toxicity 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. According to Table 3.1.2 of CLP, the converted acute toxicity point estimate is equivalent to 500 mg/kg bw. This value is designed to be used in the calculation of the ATE for classification of a mixture containing pinoxaden and does not represent test results.

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 mg/L.**

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.

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**.

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 $\geqslant$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 $\geqslant$1 and/or iritis $\geqslant$ 1 and/or conjunctival redness $\geqslant$2 and/or conjunctival oedema $\geqslant$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)**.

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	2009: personal monitoring, after

		twice: 2008 and Jan. 2009)	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: 2x, OM8[1]: 2x Individuals with single occurrence of symptoms between 2009 &and2013: OM4, OM10, OM11, OM12	Formulation started in 2006: - highest personal monitoring level: 0.3 mg/m3 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).

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

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

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[1] 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). [2]

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

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

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

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[1]. Seven of them developed

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

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 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**.

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.

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

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: $30 < C \leq 300$ mg/kg bw/d

90-day: $10 < C \leq 100$ mg/kg bw/d

1-yr: $2.5 < C \leq 25$ mg/kg bw/d

2-yr: $1.25 < C \leq 12.5$ 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**.

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.

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 in background document).

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**.

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[1] 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.

---

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

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 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

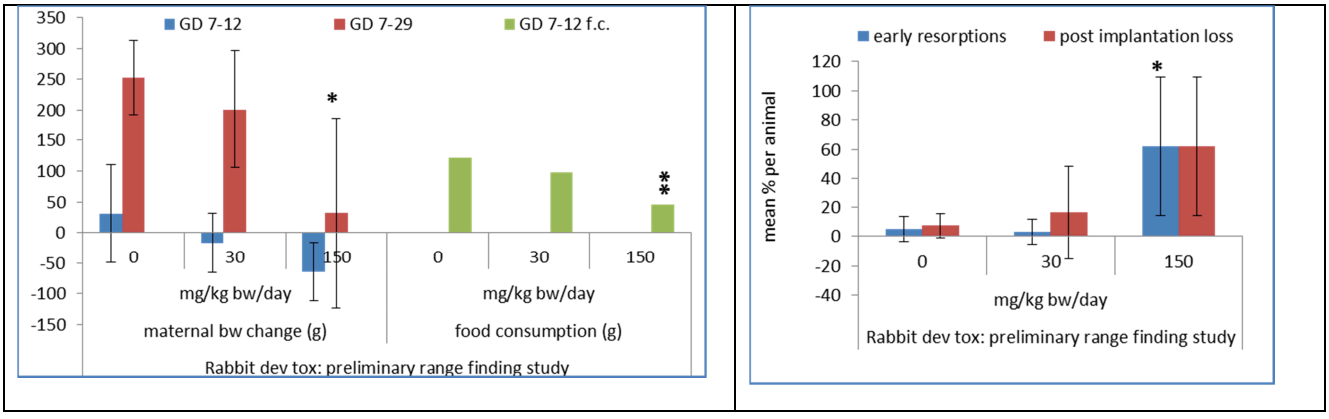
Pinoxaden's potential to cause developmental toxicity has been investigated in five prenatal developmental toxicity studies, one in rats and four in rabbits, including range finding studies.

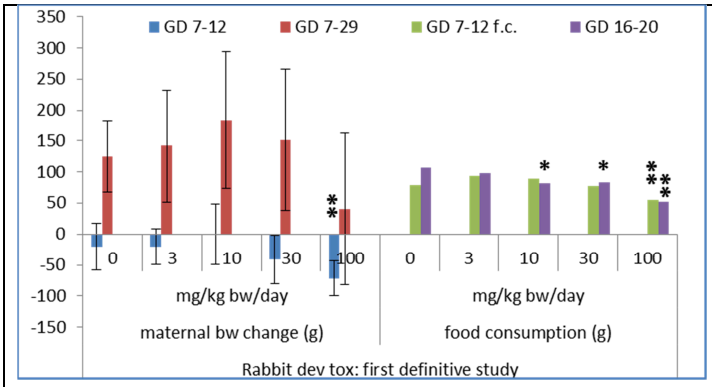
Regarding the rat developmental toxicity study RAC agrees with the interpretation of the DS. In the rat delayed ossification and reduced foetal weight occurred at doses ≥300 mg/kg bw/d. These effects on the rat foetuses were not regarded as severe, and occurred at the same dose level as maternal toxicity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

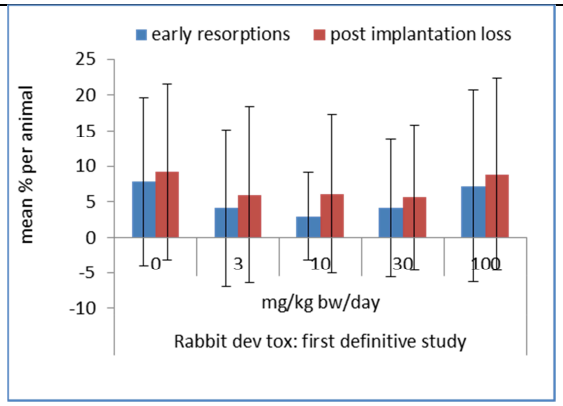
Concerning the rabbit developmental toxicity studies it is necessary to make a detailed analysis of the relation between effects on maternal food consumption and body weight (gain) and the observed implantation losses and abortions as well as the observed malformations.

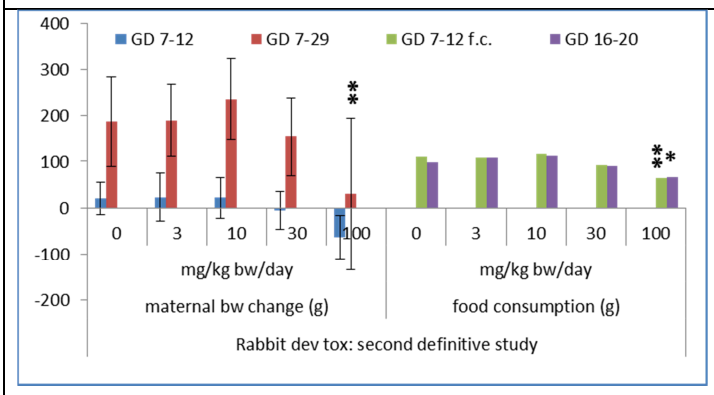
Industry provided an assessment of the pinoxaden developmental toxicity studies (Pinoxaden Developmental Toxicity Assessment, 2014 = Syngenta, 2014), which was included as an annex to the CLH report (Annex I). It contains a detailed overview of the results of the studies in rat and rabbit and it lists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

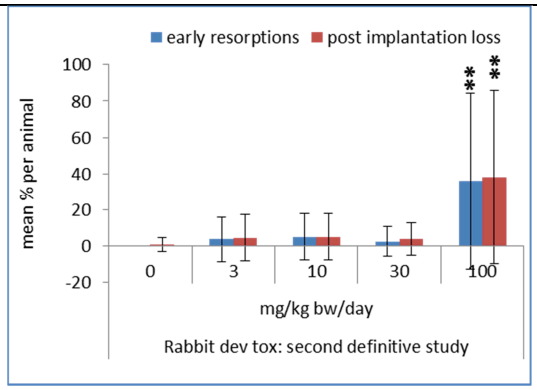
Figure 1: Summary of maternal toxicity data and pregnancy outcome of 5 developmental toxicity studies in rabbits.

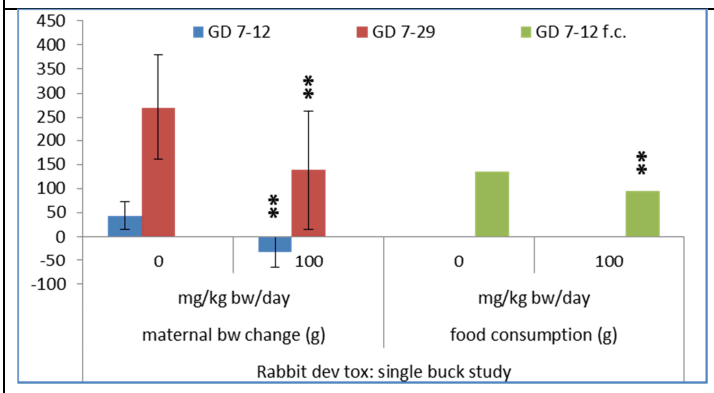


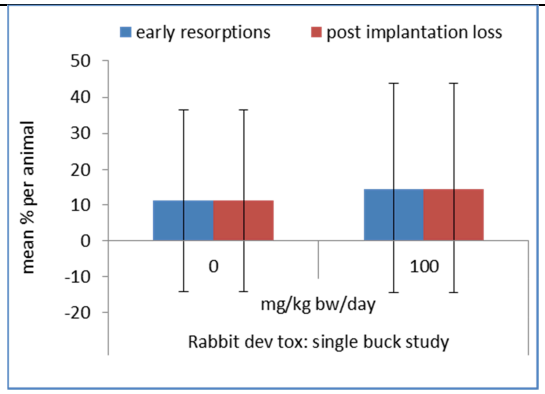


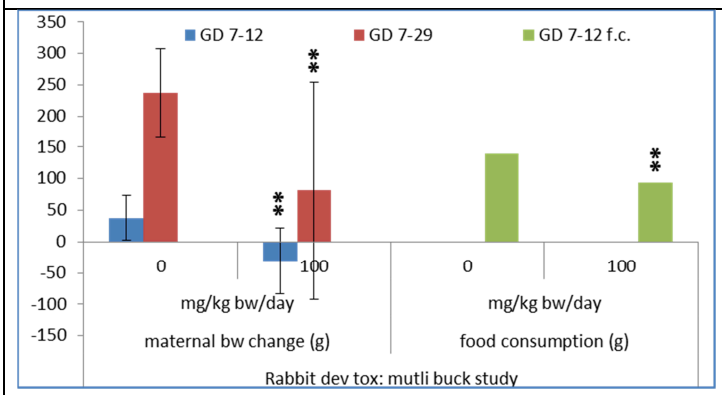


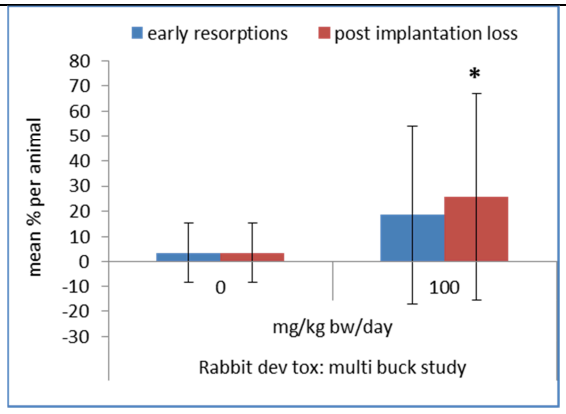












Clinical signs

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.**

ENVIRONMENTAL HAZARD EVALUATION

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 (97.2%)	*Lemna gibba*	Draft OECD TG 221	7 d E_rC_{50} (frond no.)(static)	13.9 nominal 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.

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**.

Additional references

Asthma UK, Facts and Statistics. Date unspecified. www.asthma.data.org.uk. https://www.asthma.org.uk/about/media/facts-and-statistics/

Asthma UK, database consulted on 13 July 2016. Data from 2010. www.asthma.data.org.uk. https://data.asthma.org.uk/SASVisualAnalyticsViewer/VisualAnalyticsViewer_guest.jsp?reportName=Asthma+UK+data+portal&reportPath=/Shared+Data/Guest/&reportViewOnly&_ga=1.161266941.1596414932.1468410778

ECHA, 2008. ECHA Guidance on information requirements and chemical safety assessment. Chapter R.6: QSARs and grouping of chemicals. May 2008.

ANNEXES:

Annex 1 The Background Document (BD) gives the detailed scientific grounds for the opinion. The BD is based on the CLH report prepared by the Dossier Submitter; the evaluation performed by RAC is contained in 'RAC boxes'.

Annex 2 Comments received on the CLH report, response to comments provided by the Dossier Submitter and RAC (excluding confidential information).