Committee for Risk Assessment

RAC

Annex 2

Response to comments document (RCOM)

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

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

EC Number: -
CAS Number: 243973-20-8

CLH-O-0000001412-86-127/F

Adopted

16 September 2016

ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON 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

COMMENTS AND RESPONSE TO COMMENTS ON CLH: PROPOSAL AND JUSTIFICATION

Comments provided during public consultation are made available in the table below as submitted through the web form. Any attachments received are referred to in this table and listed underneath, or have been copied directly into the table.

All comments and attachments including confidential information received during the public consultation have been provided in full to the dossier submitter (Member State Competent Authority), the Committees and to the European Commission. Non-confidential attachments that have not been copied into the table directly are published after the public consultation and are also published together with the opinion (after adoption) on ECHA's website. Dossier submitters who are manufacturers, importers or downstream users, will only receive the comments and non-confidential attachments, and not the confidential information received from other parties.

ECHA accepts no responsibility or liability for the content of this table.

Substance name: 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
Dossier submitter: United Kingdom

GENERAL COMMENTS

Date	Country	Organisation	Type of Organisation	Comment number
16.11.2015	Norway		MemberState	1
Comment received				
We agree with the classification proposals for : Acute Tox 4; H332 - Harmful if inhaled Skin Irrit 2; H315 - Causes skin irritation Eye Irrit 2; H319 - Causes serious eye irritation STOT SE 3; H335 - May cause respiratory irritation Skin Sens 1A; H317 - May cause an allergic skin reaction				
Dossier Submitter's Response				
Thank you for your support.				
RAC's response				
Noted				

Date	Country	Organisation	Type of Organisation	Comment number
13.11.2015	Germany		MemberState	2
Comment received				
The German CA supports the proposed classifications of pinoxaden. In addition, however, classification for STOT RE based on mortality in developmental toxicity studies is suggested and a further one for respiratory sensitisation should be at least considered.				

ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON 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

Editorial Comments: Within the scope of the assessment of active substances in plant protection products pinoxaden is referred to as „8-(2,6-Diethyl-4-methylphenyl)-1,2,4,5-tetrahydro-7-oxo-7H-pyrazolo[1,2-d][1,4,5]oxadiazepin-9-yl 2,2-dimethylpropanoate"
Dossier Submitter's Response
Thank you for your comments. We disagree that classification with STOT-RE 2 based on mortality in pregnant rabbits and with respiratory sensitisation is warranted. See further comments later. We can agree with the editorial comment.
RAC's response
RAC believes that the severe effects seen in the rabbit developmental toxicity studies (mortality, animals in bad conditions, considerable weight loss and reduced food consumption) have to be considered. However, in contrast to the comments from the German CA, RAC consideres these effects as supportive for a classification as Acute Tox 4, H302, as the deaths observed at the relevant doses occurred within a very short period after first exposure. Considerable toxicity and mortality was seen in a preliminary range finding study for a developmental toxicity study 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 GD 7-28, via gavage. Initial weight loss, reduced food consumption (62% on GD 7-12) and considerable reduction of weight gain (↓ 87%) were already seen at 150mg/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. Study groups at doses ≥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. Due to the early termination of the 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 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 100 mg/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. According to the CLP Regulation (Annex I, 3.1.3.6.2.1) and the CLP guidance (p 255 - 256) it is possible to also use other types of toxicity studies than those designed for acute toxicity testing and it should 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). 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 on 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).

Therefore, RAC supports a classification of pinoxaden as Acute Tox. 4 (H302: Harmful if swallowed) The classification as Acute Tox. 4 oral is supported by the Acute Tox. 4 classification for the inhalation route.

CARCINOGENICITY

Date	Country	Organisation	Type of Organisation	Comment number
13.11.2015	Germany		MemberState	3
Comment received				
It is agreed not to classify pinoxaden for carcinogenicity because the increases in different tumour types in the rat were partly covered by historical control data and confined to a dose exceeding the MTD. In the mouse, there was no evidence of carcinogenicity when the test substance was fed to the animals. Validity and reliability of the first (gavage) study appears questionable because of the lung lesions that have been presumably produced by the application technique.				
Dossier Submitter's Response				
Thank you for your support.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
13.11.2015	United Kingdom	Syngenta	Industry	4
Comment received				
Syngenta agrees with the conclusion on page 60 of the CLH report that the slightly increased incidence of leiomyosarcomas in the non-glandular stomach of the male rat is not a specific treatment-related effect of pinoxaden. In addition to the reasons for this conclusion stated in the CLH report, Syngenta should like to add that when assessing the significance of very low incidence findings it can be valuable to look at findings in tissues of the same embryological origin to understand potential target tissue sensitivity and consistency of response across these tissues. Hence Syngenta evaluated the total number of tumours of mesenchymal origin and found no evidence of an increased incidence. In addition, the US EPA evaluated the total number of tumours of the smooth muscle and again found no increased incidence of this tumour type. This supports the view that the gastric leiomyosarcomas are not related to treatment. The data to support the Syngenta position are include in the attached position statement. *ECHA comment* - The following attachment was provided with the comment above: 1. PINOXADEN-Syngenta position on gastric leiomyosarcoma in rats				
Dossier Submitter's Response				
Many thanks for this additional information which further supports non-classification for carcinogenicity.				
RAC's response				
Noted. RAC took the document provided during the PC into account in their assessment.				

MUTAGENICITY

Date	Country	Organisation	Type of Organisation	Comment number
16.11.2015	Sweden		MemberState	5
Comment received				
No experimental values are indicated for any of the studies referred to (neither in the summary table of relevant studies, nor in the text). It is therefore not possible for the reader of the CLH report to evaluate the results on mutagenicity other than by taking general statements about an observed effect or no observed effect into account. Regarding the studies measuring the induction of chromosome aberrations it seems to be particularly important to have access to the experimental values for the evaluation, since the substance was considered to be clearly positive in two in vitro cytogenetic studies in mammalian cells, but induced a statistically significant increase in micronuclei in vivo only at the lowest dose used (500 mg/kg). Without having the raw data for all three doses used in the in vivo study (500, 1000 and 2000 mg/kg) it is not possible to make an overall conclusion, including an analysis to determine whether there was a statistically significant positive trend or not.				
Dossier Submitter's Response				
Relevant details of these studies are in the DAR. An Extract from the DAR is presented in Appendix 1 to this RCOM.				
RAC's response				
Noted. The relevant information is included in the RAC opinion.				

Date	Country	Organisation	Type of Organisation	Comment number
13.11.2015	Germany		MemberState	6
Comment received				
We agree that no classification is needed. There was evidence of clastogenic effects in vitro but pinoxaden proved negative in vivo.				
Dossier Submitter's Response				
Thank you for your support.				
RAC's response				
Noted.				

TOXICITY TO REPRODUCTION

Date	Country	Organisation	Type of Organisation	Comment number
16.11.2015	Sweden		MemberState	7
Comment received				
In one OECD 414 GLP developmental toxicity study on Russian rabbits ("Developmental Tox Study 1", 2003b) a dose-related increase of diaphragmatic malformations in the offspring was observed, in 1 foetus at 30 mg/kg bw/day and in 3 foetuses (from 3 different litters) at 100 mg/kg bw/day. At the dose 100 mg/kg bw/day maternal toxicity was manifested as a 68% decrease in gestational body weight gain and a 36% decrease in food intake. At 30 mg/kg bw/day no significant maternal toxicity was evident. No resorptions or increases in pre- or post-implantation loss occurred. The diaphragmatic malformations in Developmental Tox Study 1 all arose from the same male (119) and were suggested to be due to genetic and familiar influences. However, a				

new investigative modified OECD 414 GLP study ("Buck 119 study", 2003c) using only that male as semen donor failed to induce any such malformations. In another investigative modified OECD 414 GLP study in which male 119 was excluded ("Multibuck study", 2003d) and one new OECD 414 GLP study ("Developmental Tox Study 2", 2003c) no diaphragmatic malformations were observed. However, the follow-up studies showed increased litter losses and/or fewer gravid does which reduces their power and where the litter losses may have masked the occurrence of diaphragmatic malformations.

The dossier submitter concludes that that the malformations in the first study were not treatment-related and thus no classification is warranted. We do not support this conclusion. We consider the dose-related increase in diaphragmatic malformations that was identified in Developmental Tox Study 1 (2003b) to be of concern. This concern has also previously been expressed during the EFSA peer review of Pinoxaden (EFSA 2013). We do not think that there is sufficient support to disregard these findings. There is some evidence for developmental toxicity and thus classification in Cat 2 seems justified.

Dossier Submitter's Response

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

Although it is true that the resorptions observed at the top dose of 100 mg/kg bw/day in "Multibuck study", 2003d might have masked a possible effect of pinoxaden on the diaphragm, this is highly unlikely because the diaphragmatic malformations (hernia and fissure) seen with pinoxaden in the first study are not fatal in utero, and thus, if they had occurred, they would have been unrelated to the resoprtions observed in this study and would have been detected.

Overall, it is our opinon that classification for developmental toxicity in Cat 2 is not warranted.

RAC's response

RAC agrees with the dossier submitter that the first full rabbit developmental toxicity study has drawbacks as it failed to control for matings between siblings or other related individuals. The fact that all foetuses with the diaphragmatic malformation were sired by the same father (male no 119) needs to be considered, however, it also has to be noted that the effects were not repeated in the single buck study (only male no 119). Further it is not known how many other foetuses in the first study that were also sired by male no 119.

RAC does not agree with the DS's conclusion that the observed resorptions / post implantation loss or fewer numbers of does having foetuses are unlikely to mask diaphragmatic malformations. Although, it might be true that such malformations are not fatal in utero, it is not possible to detect such malformations if the foetuses are simply not there.

It is also noted that the EFSA experts considered the results of the developmental toxicity studies in rabbits to warrant a classification as Repr. 2, for development.

RAC concluded that based on the available data a weak potential for teratogenicity cannot be excluded and the observed post-implantation losses 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) and H361d is supported.

Date	Country	Organisation	Type of Organisation	Comment number
10.11.2015	Finland		MemberState	8

Comment received

In a teratogenicity study with rabbit (2003b; DAR B.6.6.3; IIA 5.6.1 (b)), diaphragmatic hernia (left side) was found in two foetuses (one male, one female) and fissure of diaphragm (right side) in one male foetus at 100 mg/kg bw/day. All of these occurrences were noticed in different dams. Diaphragmatic hernia (left side) was also noticed in one female foetus at 30 mg/kg bw/day. The NOAEL for maternal toxicity was 30 mg/kg bw/day based on the reduction of the body weights of the dams on the days 7-12. However, the dams with malformed foetuses did not show any substantial decrease in body weights.

In a repeat of the above mentioned teratogenicity study (2003c; DAR B.6.6.3; IIA 5.6.1 (d)), no evidence for foetal malformations was observed. However, in this study, there were seven females with total resorptions, two abortions and post implantation losses at the maternally toxic dose level of 100 mg/kg bw/day. Foetal deaths may mask the malformations and, thus, the results of this repeat study are not comparable with the results of the first study.

In the historical data from the conducting laboratory between 1989 and 2000, there were only 5 single occurrences of diaphragmatic hernia in 27 separate studies. Diaphragmatic fissure had not previously been noted. Thus, the incidence of diaphragmatic hernia in the study of 2003b cannot be ignored by the results of the repeat study 2003c or by the incidence of diaphragmatic hernia or fissures in historical controls. Moreover, we think that increased incidence of resorptions at 100 mg/kg bw/day in a repeat study should be considered as concern in rabbit in spite of maternal toxicity. Therefore we are of the opinion that RAC should carefully consider classification of Pinoxaden for developmental effects.

Dossier Submitter's Response

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

Although it is true that the resorptions observed at the top dose of 100 mg/kg bw/day in "Multibuck study", 2003d might have masked a possible effect of pinoxaden on the diaphragm, this is highly unlikely because the diaphragmatic malformations (hernia and fissure) seen with pinoxaden in the first study are not fatal in utero, and thus, if they had occurred, they would have been unrelated to the resoprtions observed in this study and would have been detected.

The increased incidence of resorptions seen at 100 mg/kg bw/day in the 2003c study was observed in the presence of significant maternal toxicity (one death, 2 abortions, 63% reduction in body weight gain, reduction in food consumption). Therefore, the increased resorptions are the unspecific, secondary consequence of the observed maternal toxicity.

Overall, it is our opinion that classification for developmental toxicity in Cat 2 is not warranted.

RAC's response

See also RAC's response to comment number 7.

RAC agrees with the view that the observed malformations would be relevant for classification, however, the drawbacks with regard to familial relation (all foetuses with malformation were sired by the same father and it is not known whether there was pairing between siblings or otherwise related animals) need to be considered.

However, it also has to be noted that the effects were not repeated in the single buck study (only male no 119). Further it is not known how many other foetuses in the first study were also sired by male no 119. Therefore it is unlikely that male no. 119 was responsible for the occurrence of the malformations

RAC does not agree with the DS's conclusion that the observed resorptions / post-implantation loss or fewer numbers of does having foetuses are unlikely to mask diaphragmatic malformations. Although it might be true that such malformations are not fatal in utero, it is not possible to detect such malformations if the foetuses are simply not there.

RAC wants to emphasise that the observed diaphragmatic malformations are rare among Himalyan rabbits, also in the laboratory in which the pinoxaden studies were conducted. Regarding the 27 studies with 5 cases of diaphragmatic hernias mentioned by the FIN CA it should be noted that only one of these 5 cases of diaphragmatic hernias was seen in control animals. It is rather unusual to include findings from groups other than the control group to the historical control data. A table from the Syngenta comment submitted during PC is included here in order to describe these data:

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

It should be noted that other sources of HCD show that diaphragmatic malformations are even less frequent than indicated by the numbers in the table above (in the range of 0% to 0.3%).

From the data on the single rabbit studies it can be read that in all studies food consumption and maternal weight gain was strongly affected in the high doses (100 & 150 mg/kg bw/d). An intital weight loss on GD 7-12 was seen in all studies (not always statistically significant) and on GD 7-29 body weight gain of the dams was 12,7%, 32%, 16%, 51,5% and 34% of controls 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.

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.

Overall RAC concluded that based on the available data a weak potential for teratogenicity cannot be excluded and the observed post-implantation losses 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) and H361d is supported.

See also RAC's response to comment number 7.

Date	Country	Organisation	Type of Organisation	Comment number
16.11.2015	Norway		MemberState	9

Comment received

In the main developmental toxicity study by Altman (2003b) there was found diaphragmic hernia at 100 and 30 mg/kg bw/day and reduced foetal weight (11%) at 100 mg/kg bw/day. There were no maternal effects at 30 mg/kg bw/day. Mean maternal body weight gain was reduced at 100 mg/kg bw/day (68%), but there was no effect on the gravid uterus weight.
In the developmental toxicity study in the rabbit by Altman (2003d) there were seen increased post implantation loss and early resorptions at doses that gave only slight maternal effects. High post implantation loss may also mask possible teratogenic effects. The developing foetus thus seems to be more vulnerable to pinoxaden than the adult rabbit and a classification with Repro cat 2: H361d may be warranted.

Dossier Submitter's Response

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

Although it is true that the resorptions observed at the top dose of 100 mg/kg bw/day in

"Multibuck study", 2003d might have masked a possible effect of pinoxaden on the diaphragm, this is highly unlikely because the diaphragmatic malformations (hernia and fissure) seen with pinoxaden in the first study are not fatal in utero, and thus, if they had occurred, they would have been unrelated to the resoprtions observed in this study and would have been detected. Also, it is incorrect that in this study resorptions occurred only in the presence of slight maternal effects. In this study, at 100 mg/kg bw/d there was 1 death, 1 abortion, 35% decrease in body weight gain and reduction in food consumption. Therefore, the increased resorptions are the unspecific, secondary consequence of the observed maternal toxicity.

Overall, it is our opinion that classification for developmental toxicity in Cat 2 is not warranted.

RAC's response

Please see also RAC's response to comments number 7 & 8.

Overall RAC concluded that based on the available data a weak potential for teratogenicity cannot be excluded and the observed post-implantation losses 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) and H361d is supported.

Date	Country	Organisation	Type of Organisation	Comment number
13.11.2015	France		MemberState	10

Comment received

Point 4.10.2 Developmental toxicity p68-79

No new data was provided by applicant since pinoxaden peer review (Efsa 2013). FR considers that non-standard developmental toxicity studies in the rabbit (prenatal developmental toxicity in the rabbit: single buck IIA 5.6.1 (c(i)) and multiple buck IIA 5.6.1 (c(ii))) are inconclusive to exclude the diaphragmatic hernia relevance.
Moreover, the 2 full developmental toxicity studies (IIA 5.6.1 (b) and IIA 5.6.1 (d)) cannot be judged similar because it can be noted an increase of post-implantation loss in one of the study, not observed in the other study at same dose levels.
Therefore, it cannot be excluded that diaphragmatic hernia could be related to pinoxaden administration.
To conclude, FR supports a classification for reproductive toxicity cat. 2 H361d as proposed by Efsa experts.

Dossier Submitter's Response

Many thanks for your comments. We disagree that the single buck study and the multibuck study are inconclusive. These investigative studies show that the diaphragmatic malformations seen in the first study might have arisen from matings between siblings or other related individuals. Failure to control for these factors in the first study brings into question the reliability of such findings. Overall, taking a WoE approach, it is considered that pinoxaden has no teratogenic potential or specific developmental effects in the rabbit.

The lack of complete similarity between the two full developmental studies could be the consequence of experimental variation.

Overall, it is our opinion that classification for developmental toxicity in Cat 2 is not warranted.

RAC's response

Please see RAC's response to comments 7 & 8.

Further, in an independent expert view submitted by Syngenta it is stated that the colony of rabbits was sold and moved during the time the experiments for pinoxaden were conducted, which might explain some of the observed differences between studies. The same expert also mentioned 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.

Overall RAC concluded that based on the available data a weak potential for teratogenicity cannot be excluded and the observed post-implantation losses 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) and H361d is supported.

Date	Country	Organisation	Type of Organisation	Comment number
13.11.2015	Germany		MemberState	11

Comment received

The outcome of the reproduction as well as developmental studies in rats did not suggest a need for classification. Accordingly, only developmental toxicity in the rabbit might be of concern. The relevant effects were diaphragmatic hernia (malformations) in the first study and an increase in resorptions and post-implantation losses in general. With regard to hernia, considerable efforts have been taken to investigate this finding in more depth. In three studies, this malformation was not reproducible under identical experimental conditions. Thus, we agree that the hernia that would otherwise qualify for category 1 B was most likely not treatment-related. Resorptions and post-implantation losses were so closely related to a strong reduction in food consumption of the does that classification (cat. 2) is not warranted. However, maternal toxicity itself is of concern and, therefore, we have proposed STOT RE 2.

Dossier Submitter's Response

Many thanks for your comments and support.
Your proposal for STOT-RE 2 is discussed later under comment number 21.

RAC's response

See also RAC's response to comments number 7 & 8.

The reason why the diaphragmatic malformations were not repeated in 3 subsequent studies might be that in the subsequent studies post-implantation loss / resoprtions were increased, with the possibility that malformations could have been masked.

Overall RAC concluded that based on the available data a weak potential for teratogenicity cannot be excluded and the observed post-implantation losses 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) and H361d is supported.

Regarding a possible classification as STOT RE 2 see RAC's response to comment number 2 & 21.

Date	Country	Organisation	Type of Organisation	Comment number
13.11.2015	United Kingdom	Syngenta	Industry	12

Comment received

Syngenta supports the conclusion on page 78 of the CLH report that delayed ossification and reduced foetal weights in the rat and resorptions, post-implantation loss and reduced foetal weights in the rabbit are secondary, unspecific consequences of the maternal toxicity and that diaphragmatic effects seen in one rabbit study are unrelated to treatment with pinoxaden, being likely to have arisen from matings between siblings or other related individuals. In addition, Syngenta believes that, despite an increased incidence of early post-implantation loss in the second full developmental toxicity study in the rabbit, sufficient foetuses were available for evaluation from this study and from the two investigative studies where the relationship between animals was controlled to ensure that any treatment related effect on the diaphragm would have been evident. Further information supporting Syngenta's position is included in the attached file.

ECHA comment - The following attachment was provided with the comment above:
2. PINOXADEN- Syngenta position on developmental toxicity

Dossier Submitter's Response

Thank you for this additional information.

RAC's response

The position paper from Syngenta is considered by RAC.

Date	Country	Organisation	Type of Organisation	Comment number
13.10.2015	Italy	European Food Safety Authority (EFSA)	EU regulatory body	13

Comment received

During the peer-review meeting for the mammalian toxicology of pinoxaden submitted as a plant protection product, the experts noted that, even though diaphragmatic malformations were not observed during the second study by Khalil (2003), other effects were observed at 100 mg/kg bw per day (such as post implantation loss and early resorptions) that could mask the occurrence of developmental effects.
The experts concluded that there was not sufficient evidence to disregard the effects observed in the study by Altmann (2003) and the majority of them agreed to propose Repro Cat 2 for the developmental effects.

Dossier Submitter's Response

Many thanks for your comments.
A low incidence of malformations of the diaphragm was seen from a dose of 30 mg/kg bw/day (1 foetus in 1 litter at 30 mg/kg bw/day and 3 foetuses in 3 litters at 100 mg/kg

bw) in the first study. However, this was not repeated in three subsequent studies (using groups of 24 pregnant females and the relevant dose of 100 mg/kg bw/day) in which genetic and familial influences of sibling matings and non-randomised male donors were removed. Overall, the available evidence suggests that the diaphragmatic malformations seen in the first study might have arisen from matings between siblings or other related individuals. Failure to control for these factors in the first study brings into question the reliability of such findings. Overall, taking a WoE approach, it is considered that pinoxaden has no teratogenic potential or specific developmental effects in the rabbit.

Although it is true that the resorptions observed at the top dose of 100 mg/kg bw/day in "Multibuck study", 2003d might have masked a possible effect of pinoxaden on the diaphragm, this is highly unlikely because the diaphragmatic malformations (hernia and fissure) seen with pinoxaden in the first study are not fatal in utero, and thus, if they had occurred, they would have been unrelated to the resoprtions observed in this study and would have been detected.

Overall, it is our opinion that classification for developmental toxicity in Cat 2 is not warranted.

RAC's response

RAC agrees with EFSA's conclusion, that the observed post implantation loss and early resorptions could have masked possible malformations. Although it is not assumed that diaphragmatic malformations are fatal in utero, these malformations might still be missed if the foetuses are simply not there.

Please see also RAC's response to comments 7 & 8.

Overall RAC concluded that based on the available data a weak potential for teratogenicity cannot be excluded and the observed post-implantation losses 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) and H361d is supported.

RESPIRATORY SENSITISATION

Date	Country	Organisation	Type of Organisation	Comment number
13.11.2015	Germany		MemberState	14

Comment received

No suitable data is available neither to prove nor completely exclude a potential for respiratory sensitisation. It might be difficult to distinguish between respiratory irritation (for which a proposal for classification has been made) and sensitisation. At least, one worker was diagnosed with occupational asthma. The cause was inconclusive but there was a temporal relationship with his activities to prepare a formulation of pinoxaden. In addition, isolated incidents of asthma-like symptoms have been reported (see section on respiratory tract irritation, 4.4.3.2). In addition, pinoxaden was identified a strong skin sensitiser in the LLNA. Taken this evidence together, the RAC should consider assignment of category 1B for respiratory sensitisation to pinoxaden.

Dossier Submitter's Response
Many thanks for your comments. the weight of evidence did not support a respiratory sensitisation classification for pinoxaden. The reasons for this conclusion are summarized below: • Three cases of asthma-like symptoms were identified in the manufacturing workforce of approximately 330 (an incident rate of <1%). The number of cases, in proportion to the size of the exposed population, is well below the level suggested as being significant by the UK HSE in their publication on occupational asthma[1]. • For these cases, there is no evidence that they are caused by an allergic mechanism as no bronchial challenge or immunological tests were performed. • Pinoxaden is a respiratory irritant and the asthma-like symptoms could therefore have been due to its irritant properties; a classification is proposed for this endpoint. • Following the identification of pinoxaden as a skin sensitiser, further hygiene controls were put in place in the manufacturing/formulating plants. There have been no new cases of asthma-like symptoms since these control measures were implemented 5 years ago. [1] HSE (2001): Asthmagen? Critical assessments of the evidence for agents implicated in occupational asthma. HSE first published 1997 reprinted with amendments 1998, 2001. http://www.hse.gov.uklasthmaJasthmagen.pdf
RAC's response
RAC notes that there is a concern regarding the possible respiratory sensitisation potential of pinoxaden. It is a strong sensitiser according to a recent LLNA study. The ECHA guidance document states that substances positive in the LLNA should be considered for classification as respiratory sensitiser. In order to substantiate this conclusion one should rely on structural alerts, human data, in vitro data or QSARs. In the present case human data are available which give some indication for a respiratory sensitising potential. In response to a questionnaire prepared by RAC secretariat and rapporteurs more information regarding the human data was submitted by Syngenta: The most relevant information comes from 306 workers exposed to pinoxaden, over a period of 12 years (the duration of exposure for the single individuals is not known for most of them). Among these 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, where individuals displayed symptoms indicating an 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

[1] HSE (2001): Asthmagen? Critical assessments of the evidence for agents implicated in occupational asthma. HSE first published 1997 reprinted with amendments 1998, 2001. http://www.hse.gov.uklasthmaJasthmagen.pdf

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 mode of action.

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

For the 9 individuals it can be concluded that the symptoms had the clinical character of an allergic reaction and in many of the affected individuals symptoms were observed at several occasions, which supports the conclusion that a sensitising MoA could be the underlying cause of the observed symptoms.

However, only limited information on occupational and medical history is available and no objective measurements (e.g. electrophysiological responses, biomarkers of inflammation in nasal or bronchoalveolar lavage fluids) are available. Overall RAC 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, and it is noted that according to CLP criteria (3.4.2.1.2.1., Annex I) the immunological mechanism for classification do not have to be demonstrated. However, in the absence of a detailed description of medical and occupational history of the affected individuals and/or objective measurements, the observed symptoms were considered not sufficient to support classification.

RAC supports the DS's proposal for no classification. However, RAC notes, that the company is required to self-classify the substance for respiratory sensitisation if further evidence on respiratory sensitisation would come from the production sites.

OTHER HAZARDS AND ENDPOINTS – Acute Toxicity

Date	Country	Organisation	Type of Organisation	Comment number
13.11.2015	Germany		MemberState	15
Comment received				
The proposed classification for acute inhalation toxicity (Cat. 4, H332) is supported because it is both necessary and appropriate. For the oral and dermal routes, classification is not warranted				
Dossier Submitter's Response				
Thank you for your support.				
RAC's response				
Noted.				

OTHER HAZARDS AND ENDPOINTS – Skin Hazard

Date	Country	Organisation	Type of Organisation	Comment number
13.11.2015	Germany		MemberState	16
Comment received				
The proposed classification of pinoxaden for skin irritation (Skin Irrit. 2, H315) because of clear human evidence is supported despite the negative outcome of the skin irritation test in rabbits. Hovewer, these observations in humans do no point to corrosive properties. Accordingly, cat. 2 is in fact most appropriate.				

Dossier Submitter's Response
Thank you for your support.
RAC's response
Skin effects were seen in pinoxaden exposed workers, however, based on the available information it was not possible to clearly identifiy an irritant mode of action and it was concluded that the observed effects could not be clearly explained by either a sensitising or an irritant mode of action. In contrast to classification of pinoxaden as skin sensitiser, which is based on a clearly positive LLNA, the results of the animal studies relevant for skin irritation were all negative. No signs of irritation were observed in the rabbit skin irritation test. Moreover, in the Guinea pig maximisation test 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. On that basis RAC decided not to classify pinoxaden as Skin Irritant.

OTHER HAZARDS AND ENDPOINTS – Eye Hazard

Date	Country	Organisation	Type of Organisation	Comment number
13.11.2015	Germany		MemberState	17
Comment received				
The proposed classification of pinoxaden for eye irritation is supported. It is based on a positive study in rabbits and further substantiated by observation in humans, i.e., in manufacturing personnel. The severity of effects in the animal test was not that strong and confined to corneal opacity and conjunctival oedema. Thus, category 2 (H319) is appropriate.				
Dossier Submitter's Response				
Thank you for your support.				
RAC's response				
Noted.				

OTHER HAZARDS AND ENDPOINTS – Skin Sensitisation Hazard

Date	Country	Organisation	Type of Organisation	Comment number
16.11.2015	Sweden		MemberState	18
Comment received				
The Swedish CA supports classification of Pinoxaden (CAS No 243973-20-8) in Skin Sens. 1A as specified in the proposal. SE agrees with the rationale for classification into the proposed hazard class and differentiation.				
Dossier Submitter's Response				
Thank you for your support.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
13.11.2015	Germany		MemberState	19
Comment received				
On the basis of the LLNA, pinoxaden should be considered a strong sensitizer even though				

the maximisation test was negative. The proposed classification (Skin Sensitiser 1A, H317) is supported.
Dossier Submitter's Response
Thank you for your support.
RAC's response
Noted.

OTHER HAZARDS AND ENDPOINTS – Specific Target Organ Toxicity Single Exposure

Date	Country	Organisation	Type of Organisation	Comment number
13.11.2015	Germany		MemberState	20
Comment received				
It is agreed that the categories STOT SE 1 or 2 are not applicable. The proposed classification and labelling for respiratory tract irritation (STOT SE 3, H335) is supported, based mainly on human evidence. With regard to animal data, it seems difficult to distinguish between inhalation toxicity and irritation.				
Dossier Submitter's Response				
Thank you for your support.				
RAC's response				
Noted.				

OTHER HAZARDS AND ENDPOINTS – Specific Target Organ Toxicity Repeated Exposure

Date	Country	Organisation	Type of Organisation	Comment number
13.11.2015	Germany		MemberState	21
Comment received				
If only subacute and subchonic studies in rats and dogs are considered, there is indeed no sufficient reason for assigning a classification for STOT RE. However, severe effects were seen in the developmental studies in rabbits. Maternal deaths were noted from 100 mg/kg bw/day onwards. In two studies (i.e., in one of the explorative studies and in the second full study), 3 out of 24 does were found dead or had to be sacrificed in extremis. In the range-finding study, the number of dead or humanely killed does accounted for 1/8 at 150 and 300 mg/kg bw/day and 2/8 both at 700 and 1000 mg/kg bw/day. Clinical signs became apparent first at 150 mg/kg bw/day and were much more pronounced at the higher dose levels. Even though a developmental study is not the same as a subacute feeding or gavage study, it should be taken into account that the "guidance value" for classification in a 28-day study is ≤ 300 mg/kg bw/day. With pinoxaden, maternal toxicity became apparent at lower dose levels following only small number of applications. Based on this data, RAC should consider possible classification and labelling (STOT RE2).				
Dossier Submitter's Response				
Many thanks for your comments. These are interesting observations. No effects triggering classification with STOT-RE 2 were seen in non-pregnant animals (rats, mice and dogs) in subacute, subchronic and chronic studies. These effects potentially triggering classification with STOT-RE 2 have only been seen in pregnant rabbits (but not in pregnant rats). It is possible that the rabbit is particularly sensitive to the effects of chemicals during pregnancy.				

Neither the classificaiton criteria not the CLP guidance document refer to adverse effects below the guidance values in pregnant animals only triggering STOT-RE. Overall, we are of the opinion that the available evidence does not meet the criteria for classification with STOT-RE2, but we would welcome a discussion of this point by RAC.
RAC's response
Noted.

OTHER HAZARDS AND ENDPOINTS – Hazardous to the Aquatic Environment

Date	Country	Organisation	Type of Organisation	Comment number
10.11.2015	Finland		MemberState	22
Comment received				
Basically we support the proposed classification for environmental hazards Aquatic Acute 1; H400 – with M-factor of 1 and Aquatic Chronic 3; H412. However, we still have some questions and comments concerning the proposal. The dossier submitter has suggested in the CLH proposal that available prolonged acute toxicity study on fish (OECD 215) should be considered as chronic study in this case. We are not convinced that the available test is suitable for assessing chronic toxicity for pinoxaden. In the description of the Lemna-test (Study 5 p. 99) it is said that due to the low recoveries of pinoxaden and its metabolite M2, endpoints were recalculated using the initial measured concentrations of pinoxaden. However, in the summary (p. 102) it is said that the results were recalculated based on initial measured (rather than mean measured) concentrations of both substances. For clarity reason could you please confirm whether the results are based on pinoxaden only or combined levels of pinoxaden and M2? In addition, we wonder why the initial measured concentrations were used instead of geometric mean measured concentrations as recommended in the CLP guidance. We think that using initial measured concentrations might underestimate the toxicity of pinoxaden to Lemna, since the measured concentrations decreased significantly during the study, from 70-98% of nominals at Day-0 to 0.75-18.4% of nominals at Day-7. Considering the study with Phragmytes australis (Study 6, p. 100), we also wonder why the results are not based on the measured concentrations. We think that using the nominal concentrations in the situation where the measured concentrations exist and have decreased significantly < 80 % of nominals during the study, challenge the reliability of the study for classification purpose.				
Dossier Submitter's Response				
Thank you for your comments. • With regards to the chronic toxicity to fish. Pinoxaden has a whole system DT50 of <1 day and may be considered 'rapidly degradable'. Because of this, the focus of chronic assessment was on the more persistent main M2 degradant, on which a 32-day fish early-life stage test has been conducted. Nevertheless, a flow-through 28-day OECD 215 fish growth test has been conducted on a sensitive life stage using pinoxaden, the most sensitive endpoint was mortality. Neither the pinoxaden nor M2 test indicate chronic toxicity at 1 mg/L. Given both of these results, along with the rapid degradation of pinoxaden, we do feel there is a chronic hazard to fish or that further chronic testing of pinoxaden would be warranted.				

- Re: The *Lemna* and *Phragmytes* tests and how their endpoints were determined. Because of how rapidly pinoxaden degraded to M2 in these static tests, it was felt appropriate in the DAR to base the endpoints on nominals since initial measured levels of pinoxaden plus M2 were generally >80% of nominals (being predominantly pinoxaden at this point in the test). Toxicity over the duration of the test would also have reflected the combination of both pinoxaden and M2. However, even assuming this, combined levels of pinoxaden plus M2 had dropped below 80% of nominals by the end of the test and we agree that, for classification purposes, toxicity should ideally be based on mean measured concentrations of the substance in question. Because of this, the *Lemna* and *Phragmytes* endpoints have since been recalculated by the applicant using mean measured concentrations of pinoxaden; these are..:
  - *Lemna gibba*: 7-d ErC50 = 1.698 mg/L (mm); 7-d NOErC = 0.23 mg/L (mm).
  - *Phragmytes australis*: 20-d ErC50 = 0.63 mg/L (mm); 20-d NOErC = 0.17 mg/L (mm).

  However, use of these endpoints based on mean measured concentrations of pinoxaden does not alter the classification proposal.

RAC's response

RAC agrees with the DS's reply and welcomes the recalculated values.

Date	Country	Organisation	Type of Organisation	Comment number
13.11.2015	France		MemberState	23

Comment received

We agree with the classification and M factor proposed for Environmental hazards.

Dossier Submitter's Response

Thank you. No further comment.

RAC's response

Noted.

Date	Country	Organisation	Type of Organisation	Comment number
13.11.2015	Germany		MemberState	24

Comment received

Page 98, point 5.4.3 algae and aquatic plants, Study 2 (Grade,R 2003a). The study result should not be used for classification purposes because the growth in the control after 96 hours does not fulfil validity criteria of the guideline 201 (biomass increasing by a factor of 16 within the 72 hour test period)

In general for all studies with algae and aquatic plants with static exposure conditions we would prefer recalculation of ErC50/NOEC to mean measured pinoxaden concentration, due to the rapid degradation of pinoxaden to a relatively non-toxic degradant (M2).

Dossier Submitter's Response

Thank you for your comments.
We accept that the *Anabaena flos-aquae* study is below the growth rate criteria in the guidelines. In further information provided by the applicant, they note that these growth criteria were adopted after this study was performed and that they believe the reduced growth was likely to be due to the high initial cell density of 20,000 cells used in this study.

We accept that basing the endpoints on nominal concentrations may not reflect the toxicity of the parent for hazard classification purposes. Therefore, the endpoints for algae and

aquatic plants with static exposure conditions have been recalculated using mean measured concentrations of pinoxaden; these are..:

- *Pseudokirchneriella subcapitata*: 72-h ErC50 = 7.46 mg/L (mm); 72-h NOErC = 1.43 mg/L (mm).
- *Anabaena flos-aquae*: 96-h ErC50 = 11.81 mg/L (mm); 96-h NOErC = 0.75 mg/L (mm).
- *Navicula pelliculosa*: 72-h ErC50 = 11.17 mg/L (mm); 96-h NOErC = 5.87 mg/L (mm).
- *Lemna gibba*: 7-d ErC50 = 1.698 mg/L (mm); 7-d NOErC = 0.23 mg/L (mm).
- *Phragmytes australis*: 20-d ErC50 = 0.63 mg/L (mm); 20-d NOErC = 0.17 mg/L (mm).

However, use of these endpoints based on mean measured concentrations of pinoxaden does not alter the classification proposal.

RAC's response

RAC welcomes the recalculated values.

Date	Country	Organisation	Type of Organisation	Comment number
09.10.2015	Denmark		MemberState	25

Comment received

Denmark agrees with the CLH proposal.
There is some concern because the lowest short-term EC50 is for the bivalve Crassostrea, and this EC50 is lower than the lowest recorded EC10 or NOEC (which is for algae). As there is no long-term value for the acutely most sensitive group the chronic classification ought to be based on the short-term data. However, as the substance is not regarded as having bioaccumulation potential, and is regarded as rapidly degrading it would not be classified for chronic effects based on short-term data, while it will be classified Chronic 3 based on the long-term data.
The substance is not "readily biodegradable", but transforms rapidly to M2 and M3, which will not be classified. M2 is persistent, but the toxicity to aquatic species is low, and QSAR estimate gives a log Kow = 2.2 (KOWWIN, ver. 1.68), so is not likely to be bioaccumulating.

Dossier Submitter's Response

Thank you for your comments.
Note - we have spotted an error in the CLH Report text for the *Crassostrea virginica* oyster study; the acute 96-h NOEC (shell deposition) should be 0.046 mg pinoxaden/L (mm).
In further information provided by the applicant, they note that they do not consider the acute oyster EC50 for shell deposition of 0.4 mg/L to be relevant for acute classification as it based on growth rather than the usual mortality or immobilization endpoints. However, we agree that it (and the acute NOEC of 0.046 mg/L) could give grounds for concern relating to chronic effects - *if* pinoxaden were persistent in the aquatic environment. This was a flow-through study and pinoxaden would in reality degrade rapidly in natural water systems (DT50 <1 day). A chronic NOEC is not available for oyster. We think it may be useful for the RAC to discuss whether/how to use endpoints from oyster studies for classification as a generic issue - as this has come up previously.

Currently however, pinoxaden is considered 'rapidly degradable' for CLP purposes and there appears to be general agreement with the proposal for an Acute 1 and Chronic 3 classification.

RAC's response

Thank you for the comments.

ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON 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

NON-CONFIDENTIAL ATTACHMENTS RECEIVED

1. **PINOXADEN-Syngenta position on gastric leiomyosarcoma in rats** - comment submitted by Syngenta on 13/11/2015 *[please refer to comment No 4]*
2. **PINOXADEN- Syngenta position on developmental toxicity** - comment submitted by Syngenta on 13/11/2015 *[please refer to comment No 12]*

Appendix 1 to RCOM on pinoxaden

In vitro chromosome aberration test in the Chinese hamster V79 cells (1)

In 2001 pinoxaden (NOA 407855) (Batch No. EZ005006; analysed purity 97.2%.) was evaluated for its clastogenic potential in a series of independent *in vitro* cytogenetic assays, using Chinese hamster V79 cells, treated in the presence and absence of a rat liver-derived metabolic activation system (S9-mix). 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. The cells were exposed to pinoxaden (NOA 407855) over the concentration range 20 – 125 µg/ml. The highest concentration being limited by the cytotoxicity of the test material. A summary of the treatment regimes used is shown in the table below;

Study Design	without S9			with S9	
	Exp I&III	Trial II&III	Trial II	Trial I	Trial II&III
Exposure period	4 h	18 h	28 h	4 h	4 h
Recovery	14 h	-	-	14 h	24 h
Harvest time	18 h	18 h	28 h	18 h	28 h

The study met all criteria specified in the guidelines detailed in OECD 473 (1997).
In each experimental group two parallel cultures were set up. For each culture100 metaphase plates were scored for structural chromosome aberrations. With respect to the solubility of pinoxaden (NOA 407855), test item concentrations between 11.7 and 1500 µg/ml (with and without S9 mix) were chosen for the evaluation of cytotoxicity in a pre-test. Dose selection of the cytogenetic experiments was performed considering the toxicity data.

Toxic effects indicated by clearly reduced cell numbers and/or mitotic indices below 60 % of control were observed in all experimental parts except in experiment III in the absence of S9 mix after 18 hrs continuous treatment.

In the cytogenetic study with pinoxaden (NOA 407855), statistically significant and biologically relevant increases in the number of cells carrying structural chromosomal aberrations were observed after treatment with the test item.

In the presence of S9 mix at interval 28 hrs a dose related increase was observed in experiment II after treatment with 60 and 80 µg/ml (7.0 and 11.0 % aberrant cells excluding gaps). In experiment III an increased frequency of aberrant cells excluding gaps (11%) was seen after treatment with 60µg/ml. All these effects were associated with strong cytotoxicity.

In the absence of S9 mix, a dose related increase was observed only in experiment III after 18 hrs continuous treatment with 100 and 125 µg/ml. These cultures showed 4.0 and 8.0 % aberrant cells excluding gaps. In contrast, under the same experimental conditions an increased incidence of aberrations was only observed at 80 µg/ml (5.5%) but not at 100 µg/ml (2.5%) in experiment II. At interval 18 hrs after 4 hrs treatment with 75 and 125 µg/ml a slight increase was observed (2.5 % and 3.5 % aberrant cells excluding gaps) although this was within the historical control data range (0.0 - 4.0%). No increase in the frequencies of polyploid metaphases was found after treatment with the test item as compared to the frequencies of the controls.
Appropriate mutagens were used as positive controls. They induced statistically significant increases ($p < 0.05$) in cells with structural chromosome aberrations

Table B.6.1. Summary of results of chromosome aberration study (1)

Expt	Harvest time	Test Item		Polyploid cells (%)	Cell No. (% of control)	Mitotic indices (% of control)	Aberrant cells		
							Inc. gaps	Excl. gaps [a]	exchanges
Exposure period 4 hours without S9 mix									
I	18 hours	Negative control		1.6	nt	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 (NOA 407855) (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 (NOA 407855) (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 (NOA 407855) (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 (NOA 407855) (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 (NOA 407855) (µg/ml)	40	2.2	42	59	4.5	2.0	0.0

* including cells carrying exchanges

n.t. not tested

*= $p<0.05$, ** = $p<0.01$, *** = $p<0.001$, n.a. not applicable

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

Table B.6.2. Summary of results of chromosome aberration study (1)

Expt	Harvest time	Test Item		Polyploid cells (%)	Cell No. (% of control)	Mitotic indices (% of control)	Aberrant cells		
							Inc. gaps	Excl. gaps [a]	exchanges
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 (NOA 407855) (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 (NOA 407855) (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 (NOA 407855) (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
*= $p<0.05$, ** = $p<0.01$, *** = $p<0.001$ aberration frequency statistically significant higher than corresponding control values
[1]acetone 0.5 %; [2]EMS 600 µg/ml; [3]EMS 1000 µg/ml

Conclusion Under the experimental conditions reported, the test item induced structural chromosome aberrations as determined by the chromosome aberration test in V79 cells (Chinese hamster cell line) *in vitro* in the presence of metabolic activation. Likewise, in absence of metabolic activation there is some evidence for the induction of chromosomal aberrations. Therefore, pinoxaden (NOA 407855) was considered to be clastogenic in this chromosome aberration test in the absence and presence of S9 mix.

Czich, A (2001)

e. In vitro chromosome aberration test in the Chinese hamster V79 cells (2)

In 2002 a high purity lot of pinoxaden (NOA 407855) (Batch No. AMS 1055/2; analysed purity 99.5%.) was evaluated for its clastogenic potential in a series of independent *in vitro* cytogenetic assays, using Chinese hamster V79 cells, treated in the presence and absence of a rat liver-derived metabolic activation system (S9-mix). 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.

The cells were exposed to pinoxaden (NOA 407855) over the concentration range 20 – 125 µg/ml with the highest concentration being limited by the cytotoxicity of the test material. A summary of the treatment regimes used is shown in the table below;

	without S9			with S9	
	Trial IA&IB	**Trial II**	**Trial II**	**Trial IA&IB**	**Trial II**
Exposure period	4 h	18 h	28 h	4 h	4 h
Recovery	14 h	-	-	14 h	24 h
Harvest time	18 h	18 h	28 h	18 h	28 h

The study met all criteria specified in the guidelines detailed in OECD 473 (1997).

In each experimental group two parallel cultures were set up. For each culture100 metaphase plates were scored for structural chromosome aberrations except for the positive control in experiment IB without metabolic activation, where only 50 metaphase plates were scored. The highest applied concentration 1500 µg/ml (approx. 4 mM) was chosen based on OECD Guideline No. 473 and the solubility of pinoxaden (NOA 407855). In the pre-experiment, test item concentrations between 11.7 and 1500 µg/ml (with and without S9 mix) were applied for the evaluation of cytotoxicity. Dose selection of the cytogenetic experiments was performed considering the toxicity data.).

Toxic effects indicated by reduced cell numbers and/or mitotic indices of below 50 % of control were observed in experiment IA in the absence of S9 mix and in experiment II at preparation interval 28 hrs in the absence and in the presence of S9 mix.

In all experimental parts, in the absence and the presence of S9 mix, statistically significant and biologically relevant increases in the number of cells carrying structural chromosomal aberrations were observed after treatment with the test item, except in experiment IB in the presence of S9 mix and in experiment II after 28 hrs continuous treatment in the absence of S9 mix. No increase in the frequencies of polyploid metaphases was found after treatment with the test item as compared to the frequencies of the controls.

Table B.6.3. 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		
							Inc. 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 (NOA 407855) (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 (NOA 407855) (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 (NOA 407855) (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 (NOA 407855) (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 (NOA 407855) (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 (NOA 407855) (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 (NOA 407855) (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

Conclusion: In conclusion, it can be stated that under the experimental conditions reported, the test item induced structural chromosome aberrations as determined by the chromosome aberration test in V79 cells

(Chinese hamster cell line) *in vitro*. Therefore, pinoxaden (NOA 407855) is considered to be clastogenic in this chromosome aberration
test in the absence and presence of S9 mix.

Schulz, M (2002)

B.6.4.2 *In vivo* studies (IIA 5.4.2)

a. Micronucleus study in the mouse

In a 2001 study, the ability of pinoxaden (NOA 407855) (Batch No. EZ005006; analysed purity 97.2%), to induce micronuclei in bone marrow polychromatic erythrocytes in orally dosed NMRI mice. The test item was formulated in 40% in PEG 400. 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 in the guidelines detailed in OECD 474 (1997).

Ten animals 5/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 he 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, highest recommended dose) was estimated by a pre- experiment to be suitable.

Results

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) dose level at the 24 hour sampling time. As the value obtained was within the historical control range for the laboratory, and there was no increase over controls at either the 1000 or 2000 mg/kg dose levels, the small increase observed at 500 mg/kg was considered not to be biologically significant.

Table B.6.4. Frequency of micronucleated polychromatic erythrocytes at treatment of mice with pinoxaden (NOA 407855)

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 (NOA 407855)	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 (NOA 407855)	2000	48 h	0.060	0-2	1.04

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

Conclusion: 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, pinoxaden (NOA 407855) was considered to be non-mutagenic in this micronucleus assay.

Honarvar N (2001)