Committee for Risk Assessment

RAC

Annex 2

Response to comments document (RCOM)

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

Sulfoxaflor (ISO); [methyl(oxo){1-[6-(trifluoromethyl)-3-pyridyl]-ethyl}-λ6-sulfanylidene]cyanamide

EC number: 250-778-2

CAS number: 31717-87-0

CLH-O-0000004794-65-01/A2

Adopted
5 December 2013

ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON SULFOXAFLOR (ISO); [METHYL(OXO){1-[6-(TRIFLUOROMETHYL)-3-PYRIDYL]ETHYL}-Λ6-SULFANYLIDENE]CYANAMIDE

COMMENTS AND RESPONSE TO COMMENTS ON CLH: PROPOSAL AND JUSTIFICATION

Comments provided during public consultation are made available in this table as submitted by the webform. Please note that some attachments received may have been copied in the table below. The attachments received have been provided in full to the dossier submitter and RAC.

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

Substance name: sulfoxaflor (ISO); [methyl(oxo){1-[6-(trifluoromethyl)-3-pyridyl]ethyl}-λ6-sulfanylidene]cyanamide
CAS number: 946578-00-3
EC number:
Dossier submitter: Ireland

GENERAL COMMENTS

Date	Country	Organisation	Type of Organisation	Comment number
18.03.2013	Germany		Member State	1
Comment received				
The German CA supports the proposed classification and labelling as N; R50/53 (DSD) and H400, H410 (CLP regulation) as well as the M-factors and concentration limits. Furthermore the German CA supports the proposed classification for acute oral toxicity. Concerning the labelling proposal (CLP) there are only Precautionary Statements for the environmental hazards. Therefore we would like to propose some further for the human health hazards for example: P102, P270 and P301 + P312. However, there are few issues on other endpoints we would like to comment on.				
Dossier Submitter's Response				
Thank you for your comment. Yes we agree to adding more precautionary statements.				
RAC's response				
The support is noted.				

Date	Country	Organisation	Type of Organisation	Comment number
25.03.2013	Finland		Member State	2
Comment received				
Editorial comment for page 661: Figure 4.11.3.1 is partially on top of the text and therefore some parts of the page (Figure 4.11.3.1, the figure legend, and the text below the figure legend) are not fully readable. This could be clarified.				
Dossier Submitter's Response				
Thank you, this will be corrected. It seems to have affected the pdf document and a new one will be generated from the original MS Word document.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
22.03.2013	France		Member State	3
Comment received				
FR agrees with the classification proposal for human health and environment.				
Dossier Submitter's Response				

Thank you.
RAC's response
The support is noted.

CARCINOGENICITY

Date	Country	Organisation	Type of Organisation	Comment number
18.03.2013	Germany		Member State	4
Comment received				
It would be helpful for clarification if you could specify whether the observed tumours in rats and mice are not relevant for classification. It is noted that Phenobarbital was not included as a reference compound in the mechanistic studies. Nevertheless it is claimed that sulfoxaflor would induce the same effects as Phenobarbital. A comparison of the effects induced by sulfoxaflor and Phenobarbital in one study under the same laboratory conditions including an analysis according to the IPCS framework for cancer risk assessment would have been more convincing.				
Dossier Submitter's Response				
Thank you for your comments. Many mechanistic studies concentrated on looking at what intracellular receptors were involved in the initial response to treatment. Results confirmed the involvement of the CAR receptor and the downstream effects common to CAR and PXR receptor activation. Comparisons were made with the known responses that phenobarbital elicits since it also involves activation of the CAR receptor albeit by an indirect mechanism. The Dossier Submitter accepted there were sufficient studies available for evaluation that supported a CAR mediated effect and that this was responsible for the observed liver tumours in rats and mice and not relevant for classification with respect to human health.				
RAC's response				
Noted. Although there is always room for (further) improvement of the MoA studies, RAC agreed with the dossier submitter that, all in all, the studies provided in the CLH dossier sufficiently support a CAR-mediated MoA for the development of liver tumours in mice and rats.				

Date	Country	Organisation	Type of Organisation	Comment number
15.03.2013	Denmark		Member State	5
Comment received				
DK agrees with Ireland that no classification is warranted and that the mode of action studies support the non-relevance to humans.				
Dossier Submitter's Response				
Thank you for your comments.				
RAC's response				
The support is noted.				

MUTAGENICITY

Date	Country	Organisation	Type of Organisation	Comment number
15.03.2013	Denmark		Member State	6
Comment received				
DK agrees that no classification is required.				
Dossier Submitter's Response				
Thank you for your comments.				
RAC's response				

The support is noted.

TOXICITY TO REPRODUCTION

Date	Country	Organisation	Type of Organisation	Comment number
18.03.2013	Germany		Member State	7
Comment received				
It would be helpful for clarification if you could specify whether the high post-natal mortality observed in rats is not relevant for classification for developmental effects. It was shown in cross-fostering experiments that this effect was induced by pre-natal exposure and not by exposure via milk.				
Dossier Submitter's Response				
Thank you for your comments. The high post-natal mortality observed in rats is not considered relevant for classification because the study results have shown that mortality coincides with a species-specific activation of foetal type muscle nicotinic receptors as a consequence of pre-natal exposure. At birth this results in respiratory distress because of inhibition of the diaphragm and ancillary skeletal muscles used for breathing. Postnatally, there is a transition to the adult mature type nicotinic receptor by PND 4 that is not susceptible to dosing post partum and consequently there is no lactation effect.				
RAC's response				
RAC is in agreement with the dossier submitter that the neonatal mortality in rats is a result of an agonist effect of sulfoxaflor on foetal type muscle nicotinic receptors (nAChR). This mode of action is however considered not relevant to humans since it was shown that there is no agonist effect on human foetal (and adult) muscle nAChR. In the absence of agonism, (sustained) muscle contracture and the resulting apical endpoints (limb contracture abnormalities, bent clavicles and neonatal death) are not expected to occur in humans. Classification for these effects is therefore not warranted.				

Date	Country	Organisation	Type of Organisation	Comment number
22.03.2013	United Kingdom	Dow AgroSciences	BehalfOfAnOrganisation	8
Comment received				
Dow AgroSciences (DAS) agrees with the dossier submitter (Ireland) that classification for developmental effects is not appropriate for sulfoxaflor. The following comments provide support for this position: • The most comprehensive programme of studies ever conducted for a new active substance demonstrates that developmental effects in rats – foetal abnormalities and reduced neonatal survival - have one mode of action (MoA) that is not relevant to humans. • The MoA programme and Human Relevance Framework (HRF) analysis go far beyond the CLP requirement of "raising doubt over the relevance of the effects to humans" to support a Category 2 classification. • The sulfoxaflor MoA and HRF analysis clearly exceed this requirement and show beyond any reasonable doubt that the effects in rats are NOT relevant to humans. • Neither of these effects occurs in rabbits, even at a maximum tolerated dose (MTD). • Therefore, based on a weight of evidence evaluation, no classification is a balanced and appropriate conclusion. • In summary, all available data provide overwhelming evidence that the two primary developmental effects of sulfoxaflor in rats are not relevant to humans. The CLH report lists 4 'inconsistencies' (page 205) that are addressed in the attached document.				

ECHA's comment: The text below was provided as a separate attachment

The Developmental Toxicity of Sulfoxaflor in Rats and its Non-Relevance to Humans

Dow AgroSciences (DAS) agrees with the dossier submitter (Ireland) that classification for developmental effects is not appropriate for sulfoxaflor. The following comments provide support for this position:

- The most comprehensive programme of studies ever conducted for a new active substance demonstrates that developmental effects in rats – foetal abnormalities and reduced neonatal survival - have one mode of action (MoA) that is not relevant to humans.
- The MoA programme and Human Relevance Framework (HRF) analysis go far beyond the CLP requirement of "raising doubt over the relevance of the effects to humans" to support a Category 2 classification.
- The sulfoxaflor MoA and HRF analysis clearly exceed this requirement and show beyond any reasonable doubt that the effects in rats are NOT relevant to humans.
- Neither of these effects occurs in rabbits, even at a maximum tolerated dose (MTD).
- Therefore, based on a weight of evidence evaluation, no classification is a balanced and appropriate conclusion.
- In summary, all available data provide overwhelming evidence that the two primary developmental effects of sulfoxaflor in rats are not relevant to humans.

The CLH report lists four 'inconsistencies' (page 205) that are addressed below, with more details in Appendix 1. DAS believes that this additional information adds further weight to the conclusion that the non-relevance to humans has been proven beyond any reasonable doubt.

1. CLH: "Sulfoxaflor was shown to have partial agonist activity in recombinant rat foetal muscle nAChR expressed in Xenopus ooctyes using a two-electrode voltage clamp procedure, while agonism was not detected in recombinant human foetal muscle nAChR, recombinant rat adult muscle nAChR, or recombinant human adult muscle nAChR. Preliminary results from a new study using recombinant (rat and human) receptors in HEK (Human Embryonic Kidney) cells confirm specific agonism of the rat foetal receptor only. However, rabbit muscle nAChRs have not been examined due to technical difficulties in the molecular cloning of the rabbit muscle nAChR subunits, thus the lack of effect in the rabbit developmental toxicity study has not been investigated in functional receptor studies".
DAS response: Rabbit muscle nAChRs have not been examined because the nucleotide sequence of the rabbit nAChR subunit genes are not known and are not commercially available. However, it is not necessary to investigate the agonism of sulfoxaflor to rabbit muscle nAChRs to conclude that the rat developmental effects are not relevant to humans. Although the rabbit was the "non-responding" species in vivo and it might be interesting to examine the response of rabbit muscle nAChRs to sulfoxaflor, it is not essential because of the robust MoA that has been shown in the "responding" species (i.e., rat) and the high certainty that the critical Key Events (KEs) leading to the developmental effects have been correctly determined in rat (i.e., KE #2: Agonism at the rat fetal-type muscle nAChR). Therefore, testing the critical KEs in rat and human muscle nAChRs is sufficient to conclude that the rat developmental effects are not relevant to humans.
2. CLH: "The possibility of interaction with other cholinergic receptors (neuronal/nicotinic and muscarinic) has been considered. However, direct evaluations of sulfoxaflor agonism of neuronal receptors has not been conducted because clinical signs of such interactions have not been seen in adult rats or pups and because sulfoxaflor causes rigid contractures without evidence of receptor desensitisation (an effect more strongly associated with neuronal receptors). Clinical signs at birth of neuronal receptor mediated

effects (post-natal respiratory distress) would be impossible to differentiate in the experimental data presented. However, it is noted that foetal lung histopathological analysis study showed that foetal lungs from the 1000 ppm sulfoxaflor treatment group (rat developmental toxicity study) were not different from control foetuses".
DAS response:
Neuronal nAChRs: In contrast to the muscle-type nAChR, there is no postnatal switch in the subunit composition of neuronal nAChRs in rats. Therefore, if neuronal nAChRs caused neonatal death via an effect on respiration, effects on respiration in adults would have occurred and they did not, even at exposure levels more than 25-times the foetal NOEL.
Muscarinic AChRs: As the muscarinic AChRs present at birth are the same as those found in adults, cardinal muscarinic AChR-mediated systemic clinical signs (e.g., diarrhoea, salivation, urination, and tachycardia or bradycardia) would have been observed in other toxicity studies conducted with sulfoxaflor. However, in studies using dose levels similar to those in the developmental studies, no muscarinic AChR-mediated systemic clinical signs were observed, including studies designed to evaluate offspring clinical signs (especially the critical window studies) and the developmental neurotoxicity study, which is uniquely qualified to identify muscarinic acetylcholine receptor-mediated clinical or functional effects.

3. CLH: "The observation of reduced survival in the rat following gestational exposure from 400 ppm is consistent across a number of studies. Some inconsistencies exist in the data with regard to the foetal morphological findings. Such findings were not reported in the one-generation probe study at 1000 ppm (DAR B.6.6.1), although all pups were examined grossly for abnormalities. No sulfoxaflor mediated foetal abnormalities were noted at 1000 ppm in the probe developmental toxicity study in the rat (in which study foetuses were described as 'normal' (DAR B.6.6.10.1)). While it is stated that a detailed foetal examination was not carried out, any external abnormalities would/should have been noted. No pup morphological abnormalities were reported in the rat cross fostering study (DAR B.6.6.12.1) even though all (caesarean-sectioned) pups were examined grossly. Convoluted ureters and bent clavicles were not seen in the critical window studies at the same doses that caused these effects in the developmental toxicity study (DAR B.6.6.12.4-5). This may be related to reversibility of these effects as discussed in the study summary".
DAS response: We agree that there are inconsistencies for detection of foetal abnormalities but 1) the 1-generation probe study did not have a foetal phase and was not designed to detect foetal abnormalities; 2) Dow probe developmental studies were not designed to detect the type of abnormalities seen in the guideline study although our SOP has since been changed to ensure detection in the future; 3) the cross-fostering study was designed to address neonatal survival, not foetal abnormalities. All 3 studies specifically designed to detect foetal abnormalities (main developmental toxicity, critical window 1, and critical window 2 studies) did so, and consistently. The simple reality is that the abnormalities would have been present in studies 1-3, but were not detected for the reasons summarised here and described in more detail in Appendix 1.
Finally, with regard to the comment "Convoluted ureters and bent clavicles were not seen in the critical window studies at the same doses that caused these effects in the developmental toxicity study (DAR B.6.6.12.4-5). This may be related to reversibility of these effects as discussed in the study summary", in this study offspring were evaluated for neonatal survival and externally for limb abnormalities from birth until postnatal day (PND) 4, in contrast to the developmental toxicity study which examined fetuses on GD21. The lack of these observations in pup examinations on PND 4 indicates a reversal of these findings during the early postnatal period, rather than an inconsistency in the database.

4. CLH: "It is noted that the structure of sulfoxaflor leads to specific binding to the rat foetal nAChR with associated post-natal mortality and structural alterations, an effect not previously demonstrated for other structurally related neonicotinoid pesticidal substances. This difference is considered by the notifier to be related to its novel chemical structure, and the unique way in which sulfoxaflor binds with the insect nAChR (different to previous

neonicotinoids). Additionally, sulfoxaflor is metabolised very little unlike other related chemicals."

DAS response: In summary, sulfoxaflor causes the developmental effects in rats, whilst neonicotinoids do not, because:

a. Sulfoxaflor has the unique ability to cause sustained agonism resulting in muscle contracture; this is a critical key event required to produce the effects seen in rats

b. Unlike most neonicotinoids, which are extensively metabolised, sulfoxaflor is not metabolised at all and so is continually present at the nAChR during treatment, especially via the dietary versus the gavage route, which was used for all neonicotinoids

c. Each nAChR agonist has different toxicokinetic and toxicodynamic properties such that the consequences of binding and agonism can and do differ as demonstrated by this case

d. Sulfoxaflor is not a neonicotinoid

List of pending reports that will be finalised by the end of May 2013:

1. XDE-208: CHARACTERIZATION OF THE AGONIST EFFECTS OF XDE-208 ON MAMMALIAN MUSCLE NICOTINIC ACETYLCHOLINE RECEPTORS BY FLUORESCENCE-BASED INTRACELLULAR CALCIUM ASSAY. Neil S. Millar, University College London.

- Aim of the study: To characterise the agonist effects of XDE-208 (sulfoxaflor) on mammalian muscle nAChRs.
- The mechanism under investigation: Developmental toxicity in rats.
- The method: Agonism as detected by fluorescence-based intracellular calcium assay.
- The test organism: Recombinant mammalian muscle nAChR expressed in Human Embryonic Kidney (HEK) cells.
- The preliminary results and conclusions: The results confirm that the developmental effects in rats are not relevant to humans.
- The impact of the study results on the classification of the substance: Data support current proposal: no classification for reproductive effects.

2. XDE-208: MODE OF ACTION EVALUATION AND HUMAN RELEVANCE FRAMEWORK ANALYSIS FOR XDE-208-INDUCED FETAL ABNORMALITIES AND NEONATAL DEATH IN RATS. R. G. Ellis-Hutchings, R. J. Rasoulpour, C. Terry, B. B. Gollapudi, and R. Billington, The Dow Chemical Company.

- Aim of the study: To update the Human Relevance Framework (HRF) analysis for the XDE-208 (sulfoxaflor)-induced developmental toxicity observed in rats.
- The mechanism under investigation: Developmental toxicity in rats.
- The method: Human Relevance Framework (HRF) analysis.
- The test organism: Not applicable.
- The preliminary results and conclusions: The results confirm that the developmental effects in rats are not relevant to humans and sulfoxaflor should not be classified for reproductive toxicity.
- The impact of the study results on the classification of the substance: Data supports current proposal: no classification for reproductive effects.

Appendix 1. DAS Response to 'inconsistencies' listed in the CLH report for sulfoxaflor

The CLH report lists 4 'inconsistencies' (page 205). Dow AgroSciences provides a full response to each of these points below:

1. CLH: "Sulfoxaflor was shown to have partial agonist activity in recombinant rat foetal muscle nAChR expressed in Xenopus ooctyes using a two-electrode voltage clamp procedure, while agonism was not detected in recombinant human foetal muscle nAChR, recombinant rat adult muscle nAChR, or recombinant human adult muscle nAChR. Preliminary results from a new study using recombinant (rat and human) receptors in HEK (Human Embryonic Kidney) cells confirm specific agonism of the rat foetal receptor only. However, rabbit muscle nAChRs have not been examined due to technical difficulties in the molecular cloning of the rabbit muscle nAChR subunits, thus the lack of effect in the rabbit developmental toxicity study has not been investigated in functional receptor studies".

DAS response: Rabbit muscle nAChRs have not been examined because the nucleotide sequence of the nAChR subunit genes from rabbit are not known. There are no reports of the molecular cloning of rabbit nAChR subunits, whereas all 5 subunits have been cloned for rats and humans and are commercially available. Molecular cloning of the cDNAs encoding the 5 rabbit subunits would be possible but would require a considerable amount of additional work by a specialised researcher.

However, it is not necessary to investigate the agonism of sulfoxaflor to rabbit muscle nAChRs to conclude that the rat developmental effects are not relevant to humans. Although the rabbit was the "non-responding" species in vivo and it might be interesting to examine the response of rabbit muscle nAChRs to sulfoxaflor, it is not essential because of the robust MoA that has been shown in the "responding" species (i.e., rat) and the high certainty that the critical Key Events (KEs) leading to the developmental effects have been correctly determined in rat (i.e., KE #2: Agonism at the rat fetal-type muscle nAChR). Therefore, testing the critical KEs in rat and human muscle nAChRs is sufficient to conclude that the rat developmental effects are not relevant to humans.

2. CLH: "The possibility of interaction with other cholinergic receptors (neuronal/nicotinic and muscarinic) has been considered by the notifier. However, direct evaluations of sulfoxaflor agonism of neuronal receptors has not been conducted because clinical signs of such interactions have not been seen in adult rats or pups and because sulfoxaflor causes rigid contractures without evidence of receptor desensitisation (an effect more strongly associated with neuronal receptors). Clinical signs at birth of neuronal receptor mediated effects (post-natal respiratory distress) would be impossible to differentiate in the experimental data presented. However, it is noted that foetal lung histopathological analysis study showed that foetal lungs from the 1000 ppm sulfoxaflor treatment group (rat developmental toxicity study) were not different from control foetuses".

DAS response:

Neuronal nAChRs: In contrast to the muscle-type nAChR, there is no postnatal switch in the subunit composition of neuronal nAChRs. Therefore, if neuronal nAChRs caused neonatal death via an effect on respiration, effects on respiration in adults would have occurred and they did not, even at exposure levels more than 25-times the foetal NOEL.

For sulfoxaflor, at various life-stages in the rat (during lactation, weaning, adolescence, and adults), there has never been any effect on respiration, even at dietary levels exceeding an MTD (e.g., up to ~11,000 ppm, which is almost 30X the neonatal LOEL). Furthermore, there have been no effects at all in terms of neuronal nAChR-mediated clinical signs, including a 90-day dietary neurotoxicity study in rats which, with the most sensitive available validated investigatory methods (e.g., FOB, pre-exposure and prior to necropsy, comprising cageside, hand-held, and open field observations, rectal temperature, fore- and hindlimb grip motor activity) showed no evidence at all of neurotoxicity, even at the HDL of 1500 ppm.

Finally, the developmental neurotoxicity study, which is uniquely qualified to identify neuronal nAChR-mediated clinical or functional effects, showed no such effects with sensitive investigatory methods (e.g., litters were examined daily for survival and any adverse changes in appearance or behaviour, each pup received a detailed physical examination on PND 1, 4 (prior to culling), 7, 11, 14, 17, and 21 and at weekly intervals

thereafter until necropsy, auditory startle response, locomotor activity, learning and memory, brain weight evaluations, neuropathological and brain morphometric evaluations) at doses up to 400 ppm.
The archetypal neuronal nAChR agonist – nicotine, a full agonist of the most widespread neuronal nAChR, α4β2 – does not cause the same effects as sulfoxaflor in neonatal rats. Sulfoxaflor does not cause effects on the fetal lung, which is a known outcome of neuronal nAChR activation (e.g., Dornan et al., 1984; Harding, 1995; Kobayashi et al., 2001).
In conclusion, all of the available data provide no evidence for sulfoxaflor causing neonatal death via neuronal nAChR agonism but overwhelming evidence for a single MoA for limb abnormalities and reduced neonatal survival via agonism at the fetal muscle-type nAChR.
Muscarinic AChRs: As the muscarinic AChRs present at birth are the same as those found in adults, cardinal muscarinic AChR-mediated systemic clinical signs (e.g., diarrhoea, salivation, urination, and tachycardia or bradycardia) would have been observed in other toxicity studies conducted with sulfoxaflor.
However, in studies using dose levels similar to those in the developmental studies, no muscarinic AChR-mediated systemic clinical signs were observed, including studies designed to evaluate offspring clinical signs (especially the critical window studies) and the developmental neurotoxicity study, which is uniquely qualified to identify muscarinic acetylcholine receptor-mediated clinical or functional effects. Importantly, despite the presence of pup deaths in this study, there were no treatment-related effects indicative of muscarinic acetylcholine receptor activation.
3. CLH: "The observation of reduced survival in the rat following gestational exposure from 400 ppm is consistent across a number of studies. Some inconsistencies exist in the data with regard to the foetal morphological findings. Such findings were not reported in the one-generation probe study at 1000 ppm (DAR B.6.6.1), although all pups were examined grossly for abnormalities. No sulfoxaflor mediated foetal abnormalities were noted at 1000 ppm in the probe developmental toxicity study in the rat (in which study foetuses were described as 'normal' (DAR B.6.6.10.1)). While it is stated that a detailed foetal examination was not carried out, any external abnormalities would/should have been noted. No pup morphological abnormalities were reported in the rat cross fostering study (DAR B.6.6.12.1) even though all (caesarean-sectioned) pups were examined grossly. Convoluted ureters and bent clavicles were not seen in the critical window studies at the same doses that caused these effects in the developmental toxicity study (DAR B.6.6.12.4-5). This may be related to reversibility of these effects as discussed in the study summary".
DAS response: We agree that there may be apparent inconsistencies for foetal abnormalities but in reality they simply reflect the different a priori (protocoled) objectives and different Dow SOP's of the differing studies in question.
The first DART study to be conducted for sulfoxaflor was the probe rat developmental toxicity study. The aim of this study is to help choose test concentrations for the main developmental toxicity study. In this study, concern for possible effects was low as there were no structural alerts or information from other neonicotinoids that predicted sulfoxaflor would cause any DART effects. Fetal examinations were not carried out, except for viability on GD21. In addition, although all foetuses were examined grossly, this was conducted after euthanisation and, as described in the main developmental toxicity study, this approach does not allow for subtle effects such as forelimb flexure to be easily observed. Moreover, the foetal gross examination within a developmental toxicity probe study is not performed in as much detail as the standard foetal external examination performed on guideline developmental toxicity studies.
The second DART study to be conducted for sulfoxaflor was the rat reproduction/developmental toxicity probe study. Fetal abnormalities were not detected because the study does not have a fetal phase. It would have been possible to detect abnormalities in neonates if this had been a specific objective, but this was NOT the case in this probe study. This was the first study where we became aware of sulfoxaflor-induced offspring death. As this was obviously a severe finding, the more subtle limb abnormalities

were either missed or at that time likely considered secondary to pup death. Moreover, the clinical observations were performed by animal technicians and not by the specialized staff trained in performing foetal evaluations for developmental toxicity studies.
The third DART study to be conducted was the rat cross-fostering study. The main aim of the cross-fostering study was to allow for as many litters to be cross-fostered as possible to enable a robust conclusion on whether the previously observed effect of neonatal death was caused by gestational or lactational exposure. To this end, offspring were often quickly cross-fostered to avoid compromising the main aim of the study.
The table below summarises the chronology of DART studies:

Table summarising chronology of DART studies conducted for sulfoxaflor relative to offspring observations

#	Study	Date(s) of Offspring Observations	Fetal / Pup Examinations
1	XDE-208: DIETARY DEVELOPMENTAL TOXICITY PROBE STUDY IN CRL:CD(SD) RATS	GD 21 - Mar 18, 2008	Number of viable fetuses on GD 21
2	DIETARY REPRODUCTION/DEVELOPMENTAL TOXICITY SCREENING TEST IN CRL:CD(SD) RATS	PND 0 - May 27 – Jun 10, 2008	Clinical examinations on PND 0, 1, 4, 7, 14, and 21
3	DIETARY REPRODUCTIVE TOXICITY CROSS-FOSTERING STUDY IN CRL:CD(SD) RATS	First PND 0 - Aug 31, 1008 PND 21 - Sep 15-Oct 02, 2008	Clinical examinations on PND 0, 1, 4, 7, 14, and 21 External examinations on PND 21
4	XDE-208: DIETARY DEVELOPMENTAL TOXICITY STUDY IN Crl:CD(SD) RATS	The last group of animals were necropsied on Oct 21, 2008	External and skeletal examinations on GD 21
5	TWO GENERATION DIETARY REPRODUCTIVE TOXICITY STUDY IN CRL:CD(SD) RATS	F1 PND 0 – Jun 21 – Jul 3, 2009 F1 PND 21 - Jul 13-25, 2009 F2 PND 0 – Oct 25 – Nov 7, 2009 F2 PND 21 - Nov 16-29, 2009	Clinical examinations on PND 0, 1, 4, 7, 14, and 21
6	XDE-208: INVESTIGATION OF THE CRITICAL WINDOW OF EXPOSURE FOR FETAL ABNORMALITIES AND NEONATAL SURVIVAL EFFECTS IN Crl:CD(SD) RATS	First PND 0 – Mar 29, 2009 Last PND 4 – Apr 3, 2009	External examinations PND 0, 1, and 4
7	XDE-208: INVESTIGATION OF THE CRITICAL WINDOW OF EXPOSURE FOR FETAL ABNORMALITIES AND NEONATAL SURVIVAL EFFECTS IN Crl:CD(SD) RATS (PHASE 2)	First PND 0 – May 11, 2009 Last PND 4 – May 16, 2009	External examinations from PND 0-4
8	XDE-208: A DIETARY DEVELOPMENTAL NEUROTOXICITY STUDY OF XDE-208 IN RATS	First PND 0 – Jul 14, 2009 Last PND 60 – Sep 29, 2009	Any pup dying from PND 0-4 = external exam and sex Clinical observations PND 1, 4, 7, 11, 14, 17, and 21 DCO PND 4, 11, 21, 35, 45, and 60

LD = Lactation Day
PND = Post Natal Day
BW = Body Weight
DCO = Detailed Clinical Observations

Finally, with regard to the comment "Convoluted ureters and bent clavicles were not seen in the critical window studies at the same doses that caused these effects in the developmental toxicity study (DAR B.6.6.12.4-5). This may be related to reversibility of these effects as discussed in the study summary", in this study *offspring* were evaluated for

neonatal survival and externally for limb abnormalities from birth until postnatal day (PND) 4, in contrast to the developmental toxicity study which examined *fetuses* on GD21. Despite similar plasma concentrations of XDE-208 and incidence of forelimb flexure and hindlimb rotation between the critical window study 1 and the developmental toxicity study, bent clavicle and the ureter findings were not observed in any of the 49 pups on PND 4. Given the significant incidence of these findings in the developmental toxicity study (30 and 71% for bent clavicle and convoluted ureter, respectively) they *would* have been present in a significant number of GD 21 fetuses in the critical window study 1 if fetal examinations would have been conducted.
Their lack of observation in pup examinations on PND 4 indicates a reversal of these findings during the early postnatal period, rather than an 'inconsistency' in the database. Postnatal remodelling of bone is a well-known phenomenon; for example, consistent with the timing of postnatal skeletal change reversibility demonstrated by Collins et al. (1987) with caffeine-induced skeletal effects, for example.

1. CLH: "It is noted that the structure of sulfoxaflor leads to specific binding to the rat foetal nAChR with associated post-natal mortality and structural alterations, an effect not previously demonstrated for other structurally related neonicotinoid pesticidal substances. This difference is considered to be related to its novel chemical structure, and the unique way in which sulfoxaflor binds with the insect nAChR (different to previous neonicotinoids). Additionally, sulfoxaflor is metabolised very little unlike other related chemicals".

DAS response: In summary, it is possible for sulfoxaflor to cause the DART effects in rats whilst neonicotinoids do not because:

a. Sulfoxaflor has the unique ability to cause sustained agonism resulting in muscle contracture; this is a critical key event required to produce the effects seen in rats
b. Unlike most neonicotinoids, which are extensively metabolised, sulfoxaflor is not metabolised at all and so is continually present at the nAChR during treatment, especially via the dietary versus the gavage route, which was used for all neonicotinoids
c. Each nAChR agonist has different toxicokinetic and toxicodynamic properties such that the consequences of binding and agonism can and do differ as demonstrated by this case
d. Sulfoxaflor is not a neonicotinoid

More information on each of these points is given below:

a. Sulfoxaflor has the unique ability to cause sustained agonism resulting in muscle contracture which requires multiple TK and TD factors:
    - Negligible metabolism (discussed further below)
    - Efficient placental transfer to the fetus
    - Efficient tissue distribution
    - Appropriate binding of sulfoxaflor to the rat fetal muscle-type nAChR
    - Selective agonism to the rat fetal muscle-type nAChR
    - No rat fetal muscle-type nAChR desensitisation
b. Sulfoxaflor is not metabolised, therefore the parent compound is continually present at the muscle nAChR and able to cause continued agonism resulting in muscle contracture. In contrast, the neonicotinoids are metabolised in mammals to a varying degree:

	Summary of Metabolism (rat data)	**Reference**
Sulfoxaflor	*Negligible*	*RMS Draft Assessment Report (2012)*
Imidacloprid	**Up to 90%** of the administered dose was metabolised	EFSA Scientific Report (2008) 148, 1-120, Conclusion on the peer review of imidacloprid
Acetamiprid	Approximately **> 90%** metabolised	EU Review Report SANCO/1392/2001 – Final (16th June 2004)
Thiacloprid	**Extensive**: oxidation, hydroxylation, opening of the thiazolidine ring and conjugation.	EU Review Report SANCO/4347/2000 – Final (13th May 2004)
Thiamethoxam	**Completely metabolised at low dose** levels (0.5 mg/kg bw), poorly metabolised **(20 – 30%) at high dose** levels (100 mg/kg bw) in the rat.	EU Review Report SANCO/10390/2002 – Final (14th July 2006)
Clothianidin	Moderate metabolisation (urine, 72h, % of dose): **56-74% parent compound**	EU Review Report SANCO/10533/05 – Final (18 January 2005)

c. Each nAChR agonist has:
   i. Different binding affinity and potency to the muscle nAChR
   ii. Different comparative affinity and potency for neuronal vs. muscle nAChRs
   iii. Different potential to cause general toxicity before specific nAChR-mediated effects would become apparent

   Considering these points together, it seems highly plausible that sulfoxaflor acts differently to another class of nAChR agonists, the neonicotinoids, just as it acts very differently to nicotine itself, for example, the prototypical and very well-known nAChR agonist.

d. Sulfoxaflor is NOT a neonicotinoid. IRAC (Insecticide Resistance Action Committee) classified sulfoxaflor as a nAChR agonist, in a separate sub-group to the neonicotinoids (see table below, taken from http://www.irac-online.org).

IRAC MoA Classification v 7.2, February 2012[1]

Main Group and Primary Site of Action	Chemical Sub-group or exemplifying Active Ingredient	Active Ingredients
4 Nicotinic acetylcholine receptor (nAChR) agonists Nerve action {Strong evidence that action at one or more of this class of protein is responsible for insecticidal effects}	**4A** Neonicotinoids	Acetamiprid, Clothianidin, Dinotefuran, Imidacloprid, Nitenpyram, Thiacloprid, Thiamethoxam,
	4B Nicotine	Nicotine
	4C Sulfoxaflor	Sulfoxaflor

[1] Inclusion of a compound in the classification above does not necessarily signify regulatory approval

A number of publications in the peer-reviewed literature support this separate MoA classification:

i. Book chapter by Peter Jeschke and Ralf Nauen (Bayer) – Table 32.1.1 shows different "agonist classes" for nAChR insecticides - sulfoxaflor is classified as a sulfoximine and not a neonicotinoid.
ii. Book chapter by Peter Jeschke (Bayer) Figure 32.2.1 – shows the basic motif of a neonicotinoid – key is an sp3 nitrogen – sulfoxaflor does not have an sp3 nitrogen

(however this point is not brought up in the chapter) – Although sulfoxaflor was clearly known when this chapter was written it was not included – the caveat is that sulfoxaflor was not yet commercialized.

iii. Paper by Sparks *et al*. showing a lack of metabolism by CYP6G1 is associated with not having an sp3-nitrogen making the sulfoxaflor / sulfoximines distinct from the neonicotinoids in how it interacts with an example of a metabolic enzyme associated neonicotinoid resistance

iv. Paper by Zhu *et al*. 2011 – last paragraph in the discussion describes the fundamental differences between sulfoxaflor and neonicotinoids

v. Paper by Perry *et al*. showing a lack of cross-resistance in Drosophila that have target site resistance to the neonicotinoids – i.e. sulfoxaflor does not interact with the nAChR subunits examined in the same manner as a group of neonicotinoids.

References

IRAC: Mode of Action Classification. Poster edition 3, February 2012. Based on the Mode of Action Classification – Version 7.2, February 2012.

Jeschke, P. (2012). Chemical Structural Features of Commercialized Neonicotinoids. Chapter 32.2 in Modern Crop Protection Compounds, Second, Revised and Enlarged Edition, Volume 3: Insecticides. Eds. Kramer, W., Schirmer, U., Jeschke, P. And Witschel, M.

Jeschke, P. And Nauen, R. (2012). Nicotinic Acetylcholine Receptor Agonists: Target and Selectivity Aspects. Chapter 32.1 in Modern Crop Protection Compounds, Second, Revised and Enlarged Edition, Volume 3: Insecticides. Eds. Kramer, W., Schirmer, U., Jeschke, P. And Witschel, M.

Perry, T., Chan, J. Q., Batterham, P., Watson, G. B., Geng, C. and Sparks, T. C. (2012). Pesticide Biochemistry and Physiology 102, 56-60.

Sparks, T. C., DeBoer, G. J., Wang, N. X., Hasler, J. M., Loso, M. R. and Watson, G. B. (2012). Differential metabolism of sulfoximine and neonicotinoid insecticides by Drosphila melanogaster monooxygenase CYP6G1. Pesticide Biochemistry and Physiology 103, 159-165.

Zhu, Y., Loso, M. R., Watson, G. B., Sparks, T. C., Rogers, R. B., Huang, J. X., Gerwick, C., Babcock, J. M., Kelley, D., Hegde, V. B., Nugent, B. M., Renga, J. M., Denholm, I., Gorman, K., DeBoer, G. J., Hasler, J., Meade, T. and Thomas, J. D. (2011). Journal of Agriculture and Food Chemistry 59, 2950-2957.

Dossier Submitter's Response
All noted and Thank you for your comments.
RAC's response
The detailed response by Industry is appreciated and has been taken into consideration by RAC in its assessment of the data.

Date	Country	Organisation	Type of Organisation	Comment number
15.03.2013	Denmark		Member State	9
Comment received				
Dk agress that there is a strong argument for non-classification as the mode of action studies support the non relevance to humans.				
Dossier Submitter's Response				
Thank you for your comments.				
RAC's response				
The support is noted.				

RESPIRATORY SENSITISATION

Date	Country	Organisation	Type of Organisation	Comment number
15.03.2013	Denmark		Member State	10
Comment received				
No data available.				
Dossier Submitter's Response				
There are no studies for this endpoint. There is no justification to investigate this endpoint. There is no evidence that sulfoxaflor is capable of producing a sensitising response via the nasal route. There is no evidence of such effects from the acute respiratory toxicity study and sulfoxaflor shows no immunogenic activity based on results of the mouse LLNA assay for dermal sensitisation.				
RAC's response				
Noted.				

OTHER HAZARDS AND ENDPOINTS – Specific Target Organ Toxicity Repeated Exposure

Date	Country	Organisation	Type of Organisation	Comment number
18.03.2013	Germany		Member State	11
Comment received				
Necrosis was observed in livers of mice treated with sulfoxaflor for 28-d (at 230 mg/kg bw/d), 90-d (at 98 mg/kg bw/d) and 18-mo (80 mg/kg bw/d). Even though, the effect levels are at the upper range of the guidance values, this effect might qualify for a classification with STOT-RE cat. 2.				
Dossier Submitter's Response				
One incidence of 'very slight' and 2 of 'slight' individual cell necrosis was seen in males only at 1500 ppm (230 mg/kg bw) in the 28-day mouse study. 8/10 males at 750 pp (98 mg/kg bw) had 'very slight' individual cell necrosis in the 90-day study. There was a statistically significant increase in 'very slight' necrosis in males only in the 18 month mouse study at 750 ppm (80 mg/kg bw: **above** the cut-off of 12.5 mg/kg bw)). The effects seen in the 28-day at slightly below the cut-off (≤ 300 mg/kg) and in the 90-day studies (≈ the cut-off of 100 mg/kg) were not considered to be significant morphological changes (in the liver) which are toxicologically relevant.				
RAC's response				
RAC agreed with the dossier submitter that the degree of necrosis observed does not meet the criteria for "significant" or "severe" toxicity under CLP, nor for "serious damage" under DSD. So, no classification under CLP or DSD is warranted.				

Date	Country	Organisation	Type of Organisation	Comment number
15.03.2013	Denmark		Member State	12
Comment received				
Dk agrees that no classification is warranted - although the liver is the target organ with increased weight, certain clinical chemical changes and mild to moderate histopathological changes, the changes are not considered severe, some are reversible and according to dose/exposure time extrapolation (extrapolation to a study of 90 days duration) the NOAEL falls below the cut-off level for classification with STOT RE.				
Dossier Submitter's Response				
Thank you for your comments. Agreed.				
RAC's response				

The support is noted.

OTHER HAZARDS AND ENDPOINTS – Hazardous to the Aquatic Environment

Date	Country	Organisation	Type of Organisation	Comment number
18.03.2013	Germany		Member State	13
Comment received				
Page 213 Environmental hazard assessment: In general the complete chapter gives very extensive data of tests. In our opinion it would be better to reduce information on essential relevant studies such as key studies.				
Dossier Submitter's Response				
The composition of the overall CLH report was discussed with the ECHA Secreteriat prior to and during preparation of the CLH report and the Rapporteur also provided input which was taken into account when preparing the report. In our opinion and considering the time already spent preparing the CLH report it should stand and be assessed as it is currently presented.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
25.03.2013	Finland		Member State	14
Comment received				
We agree with the conclusions that sulfoxaflor is neither readily biodegradable nor rapidly degradable in the environment and that it is considered to have a low bioaccumulation potential.				
Dossier Submitter's Response				
Thank you for your comments.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
25.03.2013	Sweden		Member State	15
Comment received				
SE supports the environmental classification of Sulfoxaflor (Cas Nr:946578-00-3) as specified in the proposal. SE agrees with the rationale for classification into the proposed hazard differentiations. There were two written errors, instead of Chironomus dilutus: LC50 = 0.0.622 mg a.s./L it should be LC 50 =0.622 mg a.s/L and it should only be non rapidly degradable instead of ready biodegradable. The current proposal for consideration by RAC and harmonized classification is: Aquatic Acute 1 ,H400, M factor 1 and Aquatic Chronic 1, H410, M factor 1. H400 follows from the lowest acute toxicity value of the active substance for the most sensitive tested aquatic organism with LC50 < 1 mg a.s./L (Chironomus dilutus: LC50 = 0.622 mg a.s./L). A M-factor of 1 is applicable based on 0.1 < LC50≤1 mg a.s./l. H410 follows from the lowest chronic toxicity value of the active substance for the most sensitive tested aquatic organism with NOEC ≤ 1 mg a.s./L (Chironomus riparius: NOEC = 0.0384 mg/L,) and the fact that the active substance is not readily biodegradable and not rapidly biodegradable. A M-factor of 1 is applicable based on 0.01 < NOEC ≤ 0.1 mg/l. R50 follows from the lowest acute toxicity value of the active substance for the most sensitive tested aquatic organism with LC50 < 1 mg a.s./L (Chironomus dilutus: LC50= 0.622 mg a.s./L, Gerke, 2008d;).				

Dossier Submitter's Response
Thank you for your comments, editorial changes will be implemented.
RAC's response
The support is noted.

Date	Country	Organisation	Type of Organisation	Comment number
21.03.2013	Belgium		Member State	16
Comment received				
We support the environmental classification proposed by the IE dossier submitter : According to CLP-criteria : Aquatic Acute 1 H400, Acute M-factor 1 Aquatic Chronic 1 H410, Chronic M-factor 1 According to DSD-criteria : N, R50/53 SCL : N, R50/53 : C≥25% N, R51/53 : 2.5%≤C<25% R52/53 : 0.25%≤C<2.5% Some editorial or/and minor comments: P.270 Study 1 : Acute Daphnia test. The result of the test should be read as 48hEC50 instead of 96hEC50.				
Dossier Submitter's Response				
Thank you for your comments, editorial changes will be implemented.				
RAC's response				
The support is noted.				

ATTACHMENTS RECEIVED:

The Developmental Toxicity of Sulfoxaflor in Rats and its Non-Relevance to Humans (File name: DAS comments on CLH report_March 2013), submitted on 22/03/2013 by Dow AgroSciences (*ECHA's comment: additional information provided in the document copied under Toxicity to Reproduction)*