Committee for Risk Assessment

RAC

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: -
CAS number: 946578-00-3

CLH-O-0000004794-65-01/F

Adopted

5 December 2013

5 December 2013

CLH-O-0000004794-65-01/F

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

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

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

EC number: -

CAS number: 946578-00-3

The proposal was submitted by **Ireland** and received by the RAC on **6 February 2013.**

In this opinion, all classifications are given firstly in the form of CLP hazard classes and/or categories, the majority of which are consistent with the Globally Harmonised System (GHS) and secondly, according to the notation of 67/548/EEC, the Dangerous Substances Directive (DSD).

PROCESS FOR ADOPTION OF THE OPINION

Germany has submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/harmonised-classification-and-labelling-consultation*** on **6 June 2013**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **6 February 2013**.

ADOPTION OF THE OPINION OF THE RAC

Rapporteur, appointed by RAC: **Marja Pronk**

Co- rapporteur, appointed by RAC: **Jolanta Staško**

The opinion takes into account the comments provided by MSCAs and concerned parties in accordance with Article 37(4) of the CLP Regulation.

The RAC opinion on the proposed harmonised classification and labelling was reached on **5 December 2013** and the comments received are compiled in Annex 2.

The RAC Opinion was adopted by **consensus**.

OPINION OF THE RAC

The RAC adopted the opinion that **Sulfoxaflor (ISO)** should be classified and labelled as follows:

Classification and labelling in accordance with the CLP Regulation

	Index No	**International Chemical Identification**	**EC No**	**CAS No**	**Classification**		**Labelling**			**Specific Conc. Limits, M-factors**	**Notes**
					Hazard Class and Category Code(s)	**Hazard statement Code(s)**	**Pictogram, Signal Word Code(s)**	**Hazard state-ment Code(s)**	**Suppl. Hazard statement Code(s)**		
Current Annex VI entry	No current Annex VI entry										
Dossier submitters proposal	616-217-00-4	sulfoxaflor (ISO); [methyl(oxo){1-[6-(trifluoromethyl)-3-pyridyl]ethyl}-λ6-sulfanylidene]cyanamide	-	946578-00-3	Acute Tox. 4 Aquatic Acute 1 Aquatic Chronic 1	H302 H400 H410	GHS07 GHS09 Wng	H302 H410		 M=1 M=1	
RAC opinion			-	946578-00-3	Acute Tox. 4 Aquatic Acute 1 Aquatic Chronic 1	H302 H400 H410	GHS07 GHS09 Wng	H302 H410		 M=1 M=1	
Resulting Annex VI entry if agreed by COM			-	946578-00-3	Acute Tox. 4 Aquatic Acute 1 Aquatic Chronic 1	H302 H400 H410	GHS07 GHS09 Wng	H302 H410		 M=1 M=1	

Classification and labelling in accordance with the DSD

	Index No	International Chemical Identification	EC No	CAS No	Classification	Labelling	Concentration Limits	Notes
Current Annex VI entry	No current Annex VI entry							
Dossier submitters proposal	616-217-00-4	sulfoxaflor (ISO); [methyl(oxo){1-[6-(trifluoromethyl)-3-pyridyl]ethyl}-λ6-sulfanylidene] cyanamide	-	946578-00-3	Xn; R22 N; R50-53	Xn; N R: 22-50/53 S: (2-)13-36/37-46-60-61	N; R50-53: C≥ 25%; N; R51-53: 2.5% ≤C < 25%; R52-53: 0.25% ≤C< 2.5%	
RAC opinion			-	946578-00-3	Xn; R22 N; R50-53	Xn; N R: 22-50/53 S: (2-)13-36/37-46-60-61	N; R50-53: C≥ 25%; N; R51-53: 2.5% ≤C< 25%; R52-53: 0.25% ≤C< 2.5%	
Resulting Annex VI entry if agreed by COM			-	946578-00-3	Xn; R22 N; R50-53	Xn; N R: 22-50/53 S: (2-)13-36/37-46-60-61	N; R50-53: C≥ 25%; N; R51-53: 2.5% ≤C< 25%; R52-53: 0.25% ≤C< 2.5%	

SCIENTIFIC GROUNDS FOR THE OPINION

RAC general comment

Sulfoxaflor is a mixture of two diastereomers, each composed of two (racemic) enantiomers. It has a minimum purity of 95% w/w, and the ratio of the two diastereomers is typically in the range of 40:60 to 60:40, but can vary due to epimerization. One batch of sulfoxaflor complying with these specifications was used for testing in by far the majority of studies presented in the CLH dossier.

Sulfoxaflor is (as yet) the only member of a new class of insecticides, the sulfoximines. It is an agonist at insect nicotinic acetylcholine receptors (nAChR), but exhibits structure activity relationships that are different from other nAChR agonists such as the neonicotinoids and nicotine. The unique mode of action is due to a unique chemical moiety, the sulfoximine group. The structure of sulfoxaflor makes it stable to (oxidative) metabolism, unlike the neonicotinoids, most of which are extensively metabolised.

RAC evaluation of physical hazards

Summary of the Dossier submitter's proposal

Sulfoxaflor is not explosive, oxidising, flammable or auto-flammable and does not fulfil the classification criteria for physico-chemical properties. Therefore, no classification is required.

Comments received during public consultation

Physical hazards were not specifically commented on.

Assessment and comparison with the classification criteria

Since sulfoxaflor does not have explosive or oxidising properties and is not (auto-)flammable, RAC supported the non-classification for physical hazards, as proposed by the dossier submitter.

HUMAN HEALTH HAZARD ASSESSMENT

RAC evaluation of acute toxicity

Summary of the Dossier submitter's proposal

Two acute oral toxicity studies were available (one in rat, one in mouse), as well as one acute inhalation study and one acute dermal study (both in rat). The lowest LD_{50} values of sulfoxaflor were 1000 mg/kg bw (female rat) and 750 mg/kg bw (male mice) via the oral route. Sulfoxaflor is not considered acutely toxic via dermal (LD_{50} >5000 mg/kg bw) and inhalation routes (LC_{50} >2.09 mg/l, the highest attainable concentration). According to the CLP Regulation, sulfoxaflor should be classified as Acute Tox. Cat. 4 with the hazard statement H302 "Harmful if swallowed", because the LD_{50} is within the acute toxicity estimate (ATE) limits defining that category (300 < ATE ≤ 2000; oral, mg/kg bw). The classification according to DSD is Xn; R22 "harmful if swallowed", because the LD_{50} is within the ATE limits, 200 < LD_{50} ≤ 2000 mg/kg bw.

Comments received during public consultation

Two Member State competent authorities (MSCAs) supported the classification proposal for acute oral toxicity. No comments opposing the proposal were received.

Assessment and comparison with the classification criteria

Following a comparison of the available oral LD_{50} values in rats and mice with the criteria, RAC supported the conclusion of the dossier submitter that sulfoxaflor should be classified for acute oral toxicity (with **Acute Tox. 4 – H302** (CLP) and **Xn; R22** (DSD)).

RAC also supported the proposal not to classify for acute dermal and inhalation toxicity, given that the available dermal LD_{50} value in rats is above the threshold value for classification (2000 mg/kg bw, under both CLP and DSD) and that no mortalities were observed at the highest attainable aerosol concentration of 2.09 mg/l.

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

Summary of the Dossier submitter's proposal

Clinical signs in the rat oral acute study included muscle tremors, twitches and/or tonoclonic convulsions, decreased activity, decreased reactivity, decreased faeces, eyelids partially closed, hair standing up, laboured respiration, various types of soiling, increased salivation, increased lacrimation, abnormal gait, inability to walk, increased reactivity to stimuli, decreased resistance to removal, and/or decreased responsiveness to touch. These signs were observed from day 1, and all were resolved by day 6 in the surviving animals.

In the mouse oral acute study, clinical signs on day 1 consisted of laboured respiration, muscle twitches, tremors, and/or convulsions, decreased activity, decreased resistance to removal, decreased responsiveness to touch and/or increased reactivity to stimuli. The signs had resolved by day 2 in the surviving animals.

No signs of gross toxicity, dermal irritation, adverse pharmacological effects, or abnormal behaviour were observed in the rat acute dermal study, and in the rat acute inhalation study clinical effects noted were limited to (perineal) soiling.

Muscle tremors, twitches and convulsions were also seen in a rat oral acute neurotoxicity study, as well as (a.o.) decreased motor activity, splayed hindlimbs, soiling, increased lacrimation and salivation. These effects were observed on the day of dosing, but not on days 8 or 15. In this study no gross or histopathological findings in the central or peripheral nervous system were seen.

It was concluded that the results from the standard acute and acute neurotoxicity studies do not indicate that there is specific organ toxicity following a single exposure. The effects observed in these studies were generalised and systemic in nature, occurred at high doses of sulfoxaflor, involved small number of animals, were transitory in nature without significant functional change in any organ system and are not considered to support STOT SE classification.

Comments received during public consultation

This endpoint was not specifically commented on.

Assessment and comparison with the classification criteria

Substances that have produced significant toxicity in humans or that, on the basis of evidence from studies in experimental animals, can be presumed to have the potential to produce significant toxicity in humans following single exposure should be classified as STOT SE 1 or 2 according to the CLP Regulation. Classification should be supported by evidence associating single exposure to the substance with a consistent and identifiable toxic effect that clearly impacts health. Classification in STOT SE 3 is reserved for transient target organ effects and is limited to substances that have narcotic effects or cause respiratory tract irritation.

No clinical signs were observed in the acute dermal study, and in the acute inhalation study only a general sign of toxicity (soiling) was seen. When looking at the clinical signs found in the acute oral studies, however, severe effects like muscle tremors, twitches and convulsions were seen consistently (in all animals that died, and in most surviving animals), while some animals also showed less severe signs (e.g. decreased (re-)activity, increased reactivity to stimuli). The effects were only seen on the day of dosing, and resolved thereafter (the animals that died, all died on day 1). They were observed at lethal and near lethal doses, but as sulfoxaflor is already proposed to be classified for lethality following oral dosing, there is no need to additionally classify for STOT SE for these effects.

The more severe effects described above were also seen in the acute neurotoxicity study, but again at a dose within the lethal dose range. In addition, a decrease in motor activity was observed also at a non-lethal dose of 75 mg/kg bw, only on the day of dosing but not thereafter. This indicates that it probably is a time-of-peak effect and thus transient. Although the dose level of 75 mg/kg bw is within the guidance value range for STOT SE 1 (C ≤ 300 mg/kg bw), the decrease in motor activity was observed without any further impact on health and was not considered to be "more than transient in nature" (CLP Regulation 3.8.2.1.7.3 (b)).

RAC concluded that classification for STOT SE 1 (or 2) is not warranted and furthermore that decreased motor activity was not sufficient evidence for classification for narcotic effects as STOT SE 3.

RAC therefore supported the non-classification for specific target organ toxicity – single exposure.

RAC evaluation of skin corrosion/irritation

Summary of the Dossier submitter's proposal

One study in rabbits (New Zealand albino, 3 females, 4 hour exposure) was available, showing individual mean skin irritation scores (over 24-72 hours) of 0.33, 0.67 and 0.33 for erythema and 0, 0 and 0.33 for oedema. Scores did not exceed 1 for any animal and were completely resolved within 72 hours post-patch removal. It was concluded that no classification for skin irritation or corrosion was required according to either the CLP Regulation or DSD.

Comments received during public consultation

This endpoint was not specifically commented on.

Assessment and comparison with the classification criteria

In the one study available for skin irritation, only slight, transient irritation was observed, with mean scores for erythema and oedema below the threshold value of 2.3 for Skin Irrit. 2 – H315 (CLP) or 2 for Xi; R38 (DSD) in all three animals. RAC therefore supported the conclusion of the dossier submitter that sulfoxaflor should not be classified for skin irritation.

RAC evaluation of eye corrosion/irritation

Summary of the Dossier submitter's proposal

One study in rabbits (New Zealand albino, 3 males) was available. No corneal opacity was seen. Conjunctivitis (in 3 animals) and iritis (in 2 animals) were observed from 1 hour after instillation, but overall incidence and severity decreased with time. All animals were free of ocular irritation within 72 hours. Individual mean eye irritation scores (over 24-72 hours) were 0, 0.33 and 0 for iritis, 0.33, 1 and 1 for conjunctival redness, and 0, 0.33 and 0 for conjunctival chemosis. It was concluded that the irritation scores did not fulfil the criteria for classification according to the CLP Regulation or DSD and no classification for eye irritation or corrosion was proposed.

Comments received during public consultation

This endpoint was not specifically commented on.

Assessment and comparison with the classification criteria

In the one study available for eye irritation, only slight, transient effects on the iris and conjunctivae were observed. The mean scores for iritis and conjuctival redness/chemosis were below the threshold values for classification (1 resp. 2 for Eye Irrit. 2 – H319 (CLP) or 1 resp. 2.5 (redness)/2 (chemosis) for Xi; R36 (DSD)) in all three animals. RAC therefore supported the conclusion of the dossier submitter that sulfoxaflor should not be classified for eye irritation.

RAC evaluation of respiratory tract irritation

Summary of the Dossier submitter's proposal

There was no direct evidence for respiratory tract irritation. There was no evidence of respiratory tract involvement during the rat acute inhalation study, nor is there indirect evidence from acute dermal irritation and eye irritation studies in rabbits or from the 28-day rat repeat dose dermal toxicity study. No recommendation for classification with respect to respiratory tract irritation was made.

Comments received during public consultation

This endpoint was not specifically commented on.

Assessment and comparison with the classification criteria

Given the absence of irritation in an acute inhalation study with rats and the fact that the effects in the skin and eye irritation studies in rabbits were only very slight and transient in nature, RAC concluded that classification of sufloxaflor for respiratory tract irritation was not warranted.

RAC evaluation of skin sensitisation

Summary of the Dossier submitter's proposal

One Local Lymph Node Assay (LLNA) in mice (6 females/group) was available. Erythema was absent. Mice treated with 5%, 25% and 50% sulfoxaflor displayed no proliferative response with Stimulation Indices (SI) that were: 1.0, 1.1, and 1.0, respectively, in comparison to vehicle-treated mice. As these SI were below the value triggering classification (SI ≥3), it was concluded that no classification for skin sensitisation is warranted according to either the CLP Regulation or DSD.

Comments received during public consultation

This endpoint was not specifically commented on.

Assessment and comparison with the classification criteria

A substance is classified as a skin sensitiser if, in an LLNA, the SI is 3 or more. In the LLNA available for sulfoxaflor, the maximum SI found was 1.1. RAC therefore supported the conclusion of the dossier submitter that sulfoxaflor should not be classified for skin sensitisation.

RAC evaluation of respiratory sensitisation

Summary of the Dossier submitter's proposal

No information was available.

Comments received during public consultation

This endpoint was not specifically commented on.

Assessment and comparison with the classification criteria

In the absence of data, no conclusion could be drawn by RAC on the classification for respiratory sensitisation.

RAC evaluation of repeated dose toxicity (DSD) and specific target organ toxicity (CLP) – repeated exposure (STOT RE)

Summary of the Dossier submitter's proposal

No classification for Specific Target Organ Toxicity (STOT) after repeated exposure (STOT RE) or for repeated dose toxicity (DSD) was proposed by the dossier submitter.

No effects were seen by the dermal route (28-day dermal study in rats with a NOAEL at the highest dose tested of 1000 mg/kg bw/day) whereas no repeated-dose toxicity study was conducted by inhalation. Significant palatability issues influenced the dose levels used in sub-chronic oral studies in mice, rats and especially dogs. The dietary route was used for mice and rats while gavage was the only route feasible in the dog. In the latter species, gavage administration at the highest achievable dose (6 mg/kg bw/day for 1 year) resulted in transient decreases in feed consumption and body weight gain only. In short-term (sub-chronic) and long-term dietary toxicity studies in rats and mice, the main target organ identified was the liver. In mice at high doses also hypertrophy/vacuolisation of the *zona fasciculata* of the adrenal gland (both sexes) and altered haematological parameters and some extramedullary haematopoiesis (females only) were noted, all very slight in nature. Additionally, in the long-term mouse study, an exacerbation in the cumulative incidence of spontaneous dermatitis was seen in high dose males. This lesion is common in CD-1 mice and was interpreted to be secondary to general unthriftiness and stress induced by liver tumours in the high dose group. Also in the long-term rat study, effects on the testis were observed but these were considered secondary to the testicular tumours found (see section on carcinogenicity). Any alterations to other organs/tissues were within historical controls, lacked a dose response and were interpreted to be of no or doubtful toxicological

significance. There was no evidence of immunotoxicity or neurotoxicity seen in specific rat studies to examine these endpoints.

Liver
In most studies with rats and mice, liver effects in males were more pronounced than in females which may, at least in part, be related to an initial longer half-life of elimination and a higher plasma AUC_{24h} (area under the curve at 24 hours) in males. In sub-chronic toxicity studies (28- and 90-days), the main effects observed in rats and mice at the LOAEL comprised a consistent pattern of increased liver weight with histopathologic effects (very slight to moderate in nature) including hepatocellular hypertrophy with altered tinctorial properties. In rats, single cell necrosis was detected at 90 days, with vacuolisation (fatty change) in males. In mice, hepatocellular necrosis was seen at 28 days in males, together with mitotic figures. Cholesterol levels were increased in rats but not in mice, which had elevated triglyceride in females. The lowest dose causing adverse liver effects in these sub-chronic toxicity studies was 750 ppm in the 90-day rat study (equal to 47.6/51.6 mg/kg bw/day in males and females, respectively), with the male rat being most sensitive. Following the 28-day recovery period in this study (for 1500 ppm (equal to 94.9/101 mg/kg bw/day) group only), the liver was normal in the females. Some hepatocellular hypertrophy and vacuolisation were still present males, though greatly reduced. According to the dossier submitter it is reasonable to assume that the lesser effects seen at ≈ 50 mg/kg bw/day would also have recovered.

In the 1-year toxicity study in rats (i.e., the interim kill of the 2-year carcinogenicity study), adverse liver effects were limited to the high dose level of 500 ppm (21.3 mg/kg bw/day) in males and 750 ppm (39.0 mg/kg bw/day) in females. They comprised increased blood cholesterol and liver effects such as increased weight, hepatocellular hypertrophy, fatty change, single cell necrosis and increased aggregates of macrophages. The histopathological changes were mostly very slight to slight in nature.

In the 2-year rat and 18-month mouse studies, sulfoxaflor induced an increase in liver tumours (see section on Carcinogenicity). There is mechanistic evidence for a phenobarbital-type, CAR-mediated mechanism to explain the liver responses and enzyme induction profiles that occured with sulfoxaflor treatment. Thus, the liver tumours were considered to be species specific by the dossier submitter, whereas the non-neoplastic liver effects appeared to be potentially relevant effects for humans. In these carcinogenicity studies, adverse (non-neoplastic) effects on the liver were limited to the highest doses tested in these studies (500 ppm (21.3 mg/kg bw/day)/750 ppm (39.0 mg/kg bw/day) in male/female rats, and 750 ppm (79.6 mg/kg bw/day)/1250 ppm (176 mg/kg bw/day) in male/female mice). As compared to the 1-year rat study, increases in blood cholesterol and liver weight were no longer observed in the 2-year rat study, but hepatocellular hypertrophy, fatty change, single cell necrosis and increased aggregates of macrophages (mostly very slight to slight in nature) were evident. In the 18-month mouse study, the effects included massively increased liver weights, increased incidences of liver nodules and liver histopathology, including hypertrophy with altered tinctorial properties (slight to moderate), necrosis and fatty change (both very slight). The dossier submitter concluded that the liver effects in these two studies do not trigger classification as the LOAELs were above the extrapolated guidance values for a 2-year study (12.5 and 6.25 mg/kg bw/day under CLP and DSD, respectively).

Taking the 90-day rat study as the key study, the dossier submitter concluded that the evidence in the short-term toxicity studies indicated that the effects on the liver at approximately 50 mg/kg bw/day consisted of slight to moderate adaptive changes with some toxicity. There was significant recovery form these effects following withdrawal of exposure to the higher dose level of 94.9 mg/kg bw/day.

Under CLP, the cut-off value for classification with STOT RE 2 is $10 < C \leq 100$ mg/kg bw/day. STOT RE is assigned on the basis of 'significant' or 'severe' toxicity which causes functional disturbance or morphological change which are toxicologically relevant. The increased liver size at ≈ 50 mg/kg bw/day is not associated with evidence of functional change at this dose. While there is evidence of liver toxicity (necrosis/fatty change without clinical chemistry), this is shown to recover in the higher dose level and is not considered severe. Thus, the dossier submitter concluded that classification is not required under CLP.

Under DSD, the cut-off value for classification with R48 is ≤ 50 mg/kg bw/day, for effects associated with major functional change and/or major organ damage which the evidence suggests to be irreversible. According to the dossier submitter, the available data on liver toxicity do not meet the criteria for classification under DSD.

Comments received during public consultation

Two MSCAs commented on this endpoint. One supported the 'no classification' proposal, the other wondered whether the necrosis observed in the mouse repeated dose studies might qualify for STOT RE 2. The dossier submitter responded that the necrosis seen in the 28-day and 90-day mouse studies below or at the cut-offs of 300 and 100 mg/kg bw/day, respectively, was characterised as 'very slight' to 'slight' only and could therefore not be considered to be significant morphological changes (in the liver) that are toxicologically relevant. In the 18-month mouse study, an increase in necrosis was only observed at a dose level above the cut-off of 12.5 mg/kg bw/day for classification, and this necrosis was also characterised as 'very slight' only.

Assessment and comparison with the classification criteria

The repeated dose toxicity of sulfoxaflor was investigated in six short-term studies: two 28-day oral studies (one in rat and one in mouse), three 90-day oral studies (in rat, mouse and dog), and one 28-day dermal study in rat. Furthermore, a 1-year study in dogs and two long-term studies (a 2-year study in rats with interim sacrifice after 1 year, and an 18-months study in mice) were available. The 90-day oral rat study included special investigations on neurotoxicity and immunotoxicity, and was followed by a 4-wk recovery period.

Dermal

The 28-day dermal study in rats showed no local toxicity at doses up to and including 1000 mg/kg bw/day, the highest dose tested. Systemically, only marginal effects indicative of liver damage were observed at 1000 mg/kg bw/day. In effect, no severe effects were observed at dose levels relevant for classification, neither under CLP (extrapolated guidance value 600 mg/kg bw/day) nor DSD (extrapolated guidance value 300 mg/kg bw/day).

Oral

In the available short- and longer term studies the liver (rat, mouse), adrenals (mouse) and testis (rat) were identified as target organs. In the rat, effects on the testis were only observed at the end of the 2-year rat study, and included testicular and epididymal weight changes associated with significant pathology. These effects are all considered secondary to the formation of testicular adenomas (Leydig cell tumours), and are further discussed in the section on Carcinogenicity below. In dogs, the only administration route feasible was gavage, due to significant palatability issues. Even via gavage the highest achievable dose (6-10 mg/kg bw/day in the 90-day and 1-year study) was relatively low, and this dose only resulted in (transient) decreases in feed consumption and body weight gain.

Liver effects in the rat consisted of increases in absolute and relative liver weight and in cholesterol level, as well as hypertrophy (with altered tinctorial properties; very slight to moderate), vacuolisation (consistent with fatty change; very slight to moderate) and necrosis (very slight to slight) of hepatocytes and very slight to slight multifocal aggregates of macrophages (histiocytes). The effects occurred at dose levels from 79.4/88.3 and 47.6/51.6 mg/kg bw/day in the 28- and 90-day study, respectively, and at 21.3/39 mg/kg bw/day in the 1-/2-yr study. Male rats were more affected than female rats. The effective dose levels are (mostly) below the (extrapolated) guidance values for classification as STOT RE 2 (300 mg/kg bw/day for a 28-day study, 100 mg/kg bw/day for a 90-day study, 25 mg/kg bw/day for a 1-year study, 12.5 mg/kg bw/day for a 2-year study). There was no clear evidence of organ dysfunction though, and the severity of the histopathological lesions (mostly only very slight to slight, sometimes increasing to moderate) was never marked or severe. Besides, recovery of liver toxicity was shown upon withdrawal of treatment (complete recovery in females, and either completely recovered or greatly reduced effects in males). In mice, very similar liver effects were observed, of comparable severity, but at higher dose levels (from 230/273 and 98/247 mg/kg bw/day in the 28- and 90-day study, respectively, and at 79.6/176 mg/kg bw/day in the 18-month study). Given the above, RAC concluded that the liver effects in rodents do not meet the criteria for "significant" or "severe" toxicity under CLP (i.e. clearly indicating functional

disturbance or a significant impact on health), nor for "serious damage" under DSD. Hence, they do not warrant classification under CLP or DSD.

In mice, effects on the adrenals were observed in the 28-day, 90-day and 18-month studies at or from 524, 98 and 176 mg/kg bw/day, respectively. Absolute and relative adrenal weights were increased, accompanied by very slight hypertrophy of the zona fasciculata in the 28- and 90-day study. In the 18-month study there were no histopathological changes. Aside from the grading being only very slight, the effects occurred at dose levels at or above the (extrapolated) guidance values for classification (under CLP 300 mg/kg bw/day for a 28-day study, 100 mg/kg bw/day for a 90-day study and 17 mg/kg bw/day for an 18-month study; under DSD two-fold lower). Thus, the adrenal effects do not warrant classification.

Next to effects on liver and adrenals, in some studies (28-day rat and 90-day mouse) also very slight to slight splenic haematopoiesis was observed (in rats from 79.4/88.3 mg/kg bw/day, in (female) mice at 247 mg/kg bw/day), possibly related to small changes observed in some haematological parameters like haemoglobin and haematocrit. Given the minor nature of these effects they do not warrant classification. Besides, in female mice the effective dose level was above the guidance level for classification (100 and 50 mg/kg bw/day under CLP and DSD, respectively).

Overall, it can be concluded that in the available short- and longer term studies no biologically relevant effects warranting classification under CLP/DSD have been observed. Sulfoxaflor further provided no evidence of neurotoxicity or immunotoxicity in the 90-day oral rat study. RAC therefore concluded that sulfoxaflor should not be classified for toxicity upon repeated exposure.

RAC evaluation of germ cell mutagenicity

Summary of the Dossier submitter's proposal

In a battery of *in vitro* genotoxicity studies – Ames test, lymphocyte chromosome aberration test, and CHO-HGPRT test - sulfoxaflor did not cause gene mutations or chromosome aberrations. In addition, Sulfoxaflor did not induce micronuclei in somatic cells in an *in vivo* mouse micronucleus test. It was concluded that no classification for mutagenicity is required according to either the CLP Regulation or Directive 67/548/EEC.

Comments received during public consultation

One MSCA supported the 'no classification' proposal for mutagenicity. No comments opposing the proposal were received.

Assessment and comparison with the classification criteria

Sulfoxaflor tested negative in three *in vitro* assays (see above) and in one *in vivo* micronucleus assay with mice. RAC supported the conclusion of the dossier submitter that sulfoxaflor should not be classified for mutagenicity.

RAC evaluation of carcinogenicity

Summary of the Dossier submitter's proposal

No classification for carcinogenicity was proposed by the dossier submitter. Two carcinogenicity studies are summarised in the CLH report: a 2-year combined toxicity and carcinogenicity dietary study in rats (OECD TG 453) and an 18-month carcinogenicity dietary study in mice (OECD TG 451). In addition, the CLH report contains several mode of action (MoA) studies and a Human Relevance Framework (HRF) analysis for each tumour type found, in order to investigate the mechanisms behind the observed carcinogenic effects and to discuss the human relevance of the observed tumours.

Dietary administration of sulfoxaflor to F344 rats resulted in tumours of the liver, testes and the preputial gland in male rats at the highest dose only (500 ppm/21.3 mg/kg bw/day). There was no evidence of carcinogenicity in female rats.

(1) Liver tumours: statistically significantly increased tumour incidences were observed for both adenomas (33% vs. 8% in controls) and combined adenomas/carcinomas (33% vs 14% in controls). These incidences exceeded the testing laboratories historical control ranges for these tumours (2-12% for adenomas, 2-14% for combined adenomas/carcinomas). The liver tumours in male rats were corroborated by the presence of non-neoplastic liver lesions, such as hepatocellular hypertrophy, fatty change, single cell necrosis and increased aggregates of macrophages.

(2) Testicular tumours: the incidence of bilateral Leydig cell (interstitial cell) adenomas was statistically significantly increased when compared to both concurrent and historical controls (88% vs. 64% and 71 (64-76)%, respectively). At the mid dose of 100 ppm (4.24 mg/kg bw/day), the incidence of this tumour type (80%) was also increased above the historical control range, but was not statistically significant. In contrast to the bilateral adenomas, the incidences of the unilateral adenomas were decreased, so that when combining unilateral with bilateral incidences, the combined incidence at 500 ppm (92%) was not increased over either concurrent (88%) or historical (76–92%) controls. The larger Leydig cell adenomas resulted in higher testicular weights (with absolute weights at 100 and 500 ppm 46% and 62% higher than controls, respectively), indicative of tumour load. F344 rats are known to have high background rates for Leydig cell tumours (LCT) but it is considered that the increase is treatment-related given the exacerbation of the tumour load, concomitantly resulting in effects on secondary sexual organs as a side effect. The secondary effects consisted of severe atrophy of testicular seminiferous tubules (100 and 500 ppm), decreased amount of sperm in the epididymides (100 and 500 ppm), decreased secretory material in the accessory sex glands (500 ppm), and an increase in the incidence of preputial gland carcinomas (500 ppm).

(3) Preputial gland tumours: an increased incidence of carcinomas was observed at 500 ppm. These carcinomas could potentially be treatment-related, but since the preputial glands were not histopathologically examined in all animals (only when triggered by the presence of a gross lesion), it is difficult to ascertain the exact incidence of this tumour and whether or not there is a dose response.

Dietary administration of sulfoxaflor to CD-1 mice resulted in tumours of the liver only, and only at the highest doses tested (750 ppm/79.6 mg/kg bw/day in males, 1250 ppm/176 mg/kw bw/day in females), with male mice much more susceptible than female mice.
(1) Liver tumours: In male mice, statistically significantly increased tumour incidences were observed for adenomas (48% vs 24% in controls), carcinomas (34% vs 4% in controls) and combined adenomas/carcinomas (60% vs 26% in controls). These incidences exceeded the testing laboratories historical control ranges for these tumours (10-18% for adenomas, 0-2% for carcinomas, and 10-20% for combined adenomas/carcinomas). In female mice, none of the liver tumour incidences statistically significantly differed between treated animals and controls, but there was a positive trend for the carcinomas, of which the incidence at 1250 ppm (9%) was outside the historical control range (0%). At 500/1250 ppm, massively increased liver weights and increased incidences of liver nodules were observed, as well as non-neoplastic liver lesions (hypertrophy with altered tinctorial properties, necrosis, fatty change and, in males, also eosinophilic and vacuolated foci and mitotic alteration).

Liver tumours
Regarding the liver tumour formation, five additional studies on sulfoxaflor in rats and mice studying events associated with a phenobarbital(PB)-like MoA were provided (such as constitutive androstane receptor (CAR) and pregnane X receptor (PXR)-associated events, changes in cholesterol synthesis and hepatocellular proliferation), including an experiment with humanized and knockout PXR/CAR mice. Also studies regarding the activity of other nuclear receptors, aryl hydrocarbon receptor (AhR) and peroxisome proliferator-activated receptor alpha (PPARα), were provided as well as a HRF analysis on liver tumour formation. For details, see the background document (section 4.10.3.1)).

Several possible MoAs for the hepatocellular carcinogenesis observed (like e.g. peroxisome proliferation, AhR agonism, cytotoxicity, genotoxicity) could be dismissed, because the results of the available studies indicated lack of plausibility and/or coherence. The most plausible MoA for liver tumour formation in both rats and mice was considered to occur via:

- activation of the CAR nuclear receptor (key event #1), followed by
- liver enzyme induction,
- increased liver weight, hepatocyte hypertrophy, and
- hepatocyte proliferation (key event #2), leading to
- pre-neoplastic, altered hepatic foci and
- formation of adenomas and carcinomas (key event #3).

This MoA is similar to the MoA established for PB. Key event #1 in this MoA is activation of the CAR nuclear receptor, and was demonstrated by the observed increase in Cyp2b enzyme expression and activation upon sulfoxaflor treatment. PXR nuclear receptor-mediated Cyp3a cytochrome induction was also observed, but to a lesser degree. Supportive, associative key events to #1 included increased liver weight and microscopic hepatocellular hypertrophy. Key event #2 is an increase in hepatocellular proliferation and was identified in both mice and rats following sulfoxaflor treatment. Male mice further showed an increase in hepatocellular foci (eosinophilic and clear cells). The key events for sulfoxaflor showed clear, threshold, dose-responsive alterations and provided informative, time-related characterisation of sulfoxaflor-induced liver effects. The pivotal role of CAR was confirmed in an experiment with humanized and knockout PXR/CAR mice. Knockout PXR/CAR mice failed to induce liver effects upon sulfoxaflor treatment, whereas humanized PXR/CAR mice showed similar (albeit weaker) liver effects as wild type mice, with one notable exception: hepatocellular proliferation was totally absent.

It was concluded that the key events are consistent with a CAR-mediated, PB-like MoA, for which there is a high level of confidence. This PB-like MoA for liver tumour formation is considered not relevant to humans due to differences in rodent and human responses to CAR activation, in particular as to hepatocellular proliferation, a critical event in the development of liver tumours. Next to indications that human hepatocytes are refractory to the hyperplastic effects of PB, there is epidemiological data showing that in humans receiving PB for many years, at levels comparable to those in rodent bioassays, there is no evidence of a hepato-carcinogenic effect. PB is thus not a hepatocarcinogen in humans. Using the HRF, Holsapple *et al*. (2006) have concluded that for compounds which have robust data to support a PB-like MoA it can be concluded that the hepatocarcinogenic response is not relevant to humans.

On the basis of robust data showing the MoA for sulfoxaflor-mediated liver effects to be PB-like, plus the absence of hepatocellular proliferation in sulfoxaflor-treated humanized (and knockout) PXR/CAR mice, the dossier submitter concluded that the rodent liver tumours associated with administration of high dose levels of sulfoxaflor would not pose a cancer hazard to humans.

<u>Leydig cell tumours</u>
The CLH report included three additional studies investigating possible MoAs behind the LCT formation, as well as a HRF analysis of the LCT (for details, see background document, section 4.10.3.2 and Annex I). These studies focused on three known MoAs that were considered most likely to be operative, i.e. reduced testosterone biosynthesis, increased testosterone biliary elimination, and dopamine agonism/enhancement. Other known MoAs for LCT in rats (i.e. mutagenicity, androgen receptor antagonism, oestrogen receptor agonism/antagonism, 5-alpha-reductase inhibition, aromatase induction and GnRH (LHRH) agonism) were all not considered as plausible because the available genotoxicity, carcinogenicity and reproductive toxicity studies provided evidence that sulfoxaflor is not genotoxic and did not show effects on endpoints that would normally have been affected with these MoAs (such as male anogenital distance, accessory sex gland weights, mating or fertility indices, vaginal patency, or pituitary effects). In one of the additional studies it was indeed shown that sulfoxaflor did not interact with the oestrogen receptors, had no agonism or antagonism on androgen receptors and did not inhibit aromatase activity.

Direct data obtained with sulfoxafor showed no evidence of increased biliary excretion of testosterone or any measured gene in the steroidogenic pathway, whereas 8-week exposure up to 500 ppm sulfoxaflor resulted in decreased serum prolactin (Prl) and increased serum luteinizing hormone (LH) and testosterone levels and in decreased testis LH and Prl receptor gene expression at week 4, but not at week 2 or week 8. The most likely MoA responsible for LCT in the Fischer 344 rat was therefore proposed to operate through the following key events:

1) increased neuronal dopamine release via specific dopaminergic neuron-based nicotinic acetylcholine receptor (nAChR) agonism, leading to
2) decreased serum Prl levels, leading to
3) downregulation of LH receptor gene expression in Leydig cells, leading to
4) transient decreases in serum testosterone, leading to
5) increased serum LH levels, leading to
6) promotion of Leydig cell tumourigenesis.

On the basis of all study results it was concluded that the LCT promotion was caused by a subtle, but chronic, dopamine enhancement in a uniquely susceptible animal model, the Fischer 344 rat. The data for sulfoxaflor were judged with a moderate degree of confidence to adequately explain the promotion of F344 rat LCT following chronic dietary administration of sulfoxaflor, and judged with a very high degree of confidence to support a hormonally-mediated, threshold-based, nonlinear MoA. The moderate degree of confidence is due to some data gaps: direct evidence was mainly available for the early and not so much for the late key events in this MoA (e.g. there were no hormone measurement data that confirm a decrease in serum testosterone level, and Leydig cell proliferation has not been demonstrated), and dose-response and temporal relationships have not been demonstrated in full. Yet, it was recognized that these latter relationships are difficult to ascertain, due to the subtle nature and long latency for the apical endpoint effect of Leydig cell hyperplasia and tumours, in combination with inherent variability in hormone data and feedback compensation by the hypothalamic-pituitary-gonadal (HPG) axis.

It is known that the background incidence of Fischer rat LCT is very much higher than in other rat strains, mice and humans. These interspecies differences in background incidence are well understood, and result from quantitative and qualitative differences of Leydig cell response to hormonal stimuli. Rat Leydig cells contain >10-fold more LH receptors (LHR) than humans, which confers greater sensitivity to slight changes in LH levels. In addition to this quantitative difference, rat, but not human, Leydig cells have both Prl receptors (PrlR) and gonadotropin releasing hormone receptors (GnRHR) on their surface. Stimulation of rat Leydig cells through both PrlR and GnRHR are a rat-specific mechanism by which LCT formation can occur. For PrlR involvement in LCT, dopamine agonists (e.g., muselergine) reduce Prl release by the anterior pituitary gland. This results in decreased binding of Prl to PrlR on Leydig cells, leading to downregulation of the LHR. Decreased LHR gene expression results in slight but transitory decreases in testosterone production, which via the HPG-axis feeds back to a compensatory increase in circulating LH, leading to Leydig cell stimulation and hyperplasia over time. As a consequence of the species differences between rat and human Leydig cells, it is generally considered that the dopamine enhancement MoA behind the LCT has no relevance to humans.

All in all, the dossier submitter concluded that the sulfoxaflor-induced promotion of Fischer rat LCT has a MoA that is hormonally-mediated and threshold-based, and would be considered to have no relevance to humans due to qualitative and quantitative differences between rat and human Leydig cells. On that basis, the Fischer rat Leydig cell tumours associated with administration of high dose level of sulfoxaflor would not pose a cancer hazard to humans.

<u>Preputial gland tumours</u>
There was no direct experimental investigation into the effects causing the preputial gland tumours. The CLH report provided a HRF analysis though, for preputial gland carcinoma (for details, see background document, section 4.10.3.3 and Annex I), in which the following key events are proposed:
1) agonism, via nicotinic acetylcholine receptors (nAChR), to dopaminergic neurons in the hypothalamus, resulting in increased dopamine release;
2) dopamine-mediated inhibition of prolactin (Prl) release from the anterior pituitary resulting in reduced serum prolactin levels;
3) reduced stimulation of Prl receptors on Leydig cells resulting in reduced LH receptor density on Leydig cells (human Leydig cells do not have functional Prl receptors and hence the sequence of events beyond this step cannot occur in humans);
4) reduced LH receptor density leads to transiently reduced testosterone production by Leydig cells;
5) reduced serum testosterone levels stimulates increased production of LH from the pituitary;

6) the continuous drive of increased dopamine release leads to a 'resetting' of the HPG axis to a slightly higher level of activity and hence higher testosterone production;
7) the slightly higher testosterone level stimulates preputial gland proliferation which, over a lifetime, promotes normal spontaneous tumourigenesis in the rat preputial gland.

It was concluded that dopamine enhancement is the most plausible MoA behind the preputial gland carcinoma formation that sulfoxaflor seemed to promote (albeit that the exact incidence of this tumour and whether or not there is a dose response cannot be ascertained). This MoA, which is also considered responsible for the Leydig cell tumours and associated effects on the epididymides and accessory sex glands, is not relevant to humans (see under LCT above). Furthermore, humans do not have an anatomic equivalent to rodent preputial glands, there were no effects in the female rat correlate of the preputial gland (clitoral gland), there were no effects in mice on the preputial/clitoral glands, and there were no effects in rats and mice on other sebaceous glands (skin, Zymbal's gland).
Taking into account that the preputial gland tumour promotion by sulfoxaflor is most likely secondary to the LCT, the dossier submitter concluded these tumours to be of little human relevance. It was further concluded that, since humans do not have an anatomical correlate to the preputial gland, the observed carcinomas in rats may have no relevance to humans, *per se*.

Conclusion
The dossier submitter concluded that, in the absence of human data on sulfoxaflor exposure and carcinogenicity, Carc. 1A is precluded. The observed increased tumour incidences in male rats and in male and female mice may warrant Carc. 1B or Carc. 2, "*…unless there is strong evidence that the mechanism of tumour formation is not relevant for humans*". In view of increased tumour incidences having been seen in two animal species, the dossier submitter argued that in principle a case for Carc. 1B could be made. However, neither Carc. 1B nor Carc. 2 was considered appropriate, taking the following additional considerations into account: sulfoxaflor has no structural relationship with other known carcinogens, there is a complete lack of genotoxicity seen with sulfoxaflor in *in vitro* and *in vivo* studies, increased tumour incidences were only observed at the highest doses tested, there is a very high background incidence of Leydig cell tumours in F344 rats, there is mechanistic evidence that the MoAs behind the observed tumours (a PB-like, CAR-mediated mechanism for the liver tumours, and a weak but chronic dopamine agonism/enhancement behind the Leydig cell and preputial gland tumours) are not relevant for humans, and humans do not have functional homologues to preputial glands. Hence, the dossier submitter concluded that, overall, the weight of evidence supports the non-relevance to humans of the rodent-specific tumorigenic effects and that hence no classification is required under CLP.

Based on a similar reasoning, classification under DSD with category 1, 2 or 3 was considered inappropriate, so 'no classification' was proposed under DSD.

Comments received during public consultation
One MSCA was in support of the 'no classification' proposal, agreeing that the MoA studies support the non-relevance to humans. Another MSCA asked for a specification of which tumours in rats and mice are not relevant for classification. This MSCA further noted that phenobarbital was not included as reference compound in the mechanistic studies which, when included, would have made the claim for a PB-like MoA more convincing. In response to this, the dossier submitter remarked that many mechanistic studies concentrated on looking at what intracellular receptors were involved in the initial response to treatment, and that 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. On the basis thereof the dossier submitter concluded that 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.

Assessment and comparison with the classification criteria
There are no human data on sulfoxaflor exposure and carcinogenicity. In animal experiments (a 2-year rat study and an 18-month mouse study), administration of sulfoxaflor via the diet resulted in increased incidences of liver, testes and preputial gland tumours in high dose (500 ppm) male rats and of liver tumours in high dose male (750 ppm) and female (1250 ppm) mice

(see table below). Sulfoxaflor was not carcinogenic in female rats. The mechanisms behind the carcinogenicity and the human relevance of the observed tumours were investigated/evaluated in several mechanistic studies and a HRF analysis for each tumour type.

Tumour incidence rates in rat and mouse bioassay

	Dose (ppm)							HC
RAT 2-year study	**0**	**25**	**100**		**500**			
♂ Hepatocellular								
adenoma	8%	4%	10%		**33%**			2-12%
carcinoma	6%	2%	2%		0%			0-2%
combined	14%	6%	12%		□**3%**			2-14%
♂ Leydig cell								
adenoma (unilateral)	24%	16%	10%		4%[#]			12-16%
adenoma (bilateral)	64%	76%	80%		**88%**			64-76%
combined	88%	92%	90%		92%			76-92%
♂ Preputial gland								
carcinoma[$]	63% (5/8)	88% (7/8)	100% (7/7)		100% (10/10)			(0-100%)
MOUSE 18-month study	**0**	**25**	**100**	**250**		**750**	**1250**	
♂ Hepatocellular								
adenoma	24%	13%	20%			**48%**		10-18%
carcinoma	4%	0%	8%			**34%**		0-2%
combined	26%	13%	24%			**60%**		10-20%
♀ Hepatocellular								
adenoma	2%	2%		0%			4%	0-6%
carcinoma	0%	2%		0%			9%[*]	0%
combined	2%	4%		0%			11%[*]	0-6%

Values in bold: statistically significantly different from control; [#] negative trend; [*] positive trend; [$] not all animals investigated (only those with mass/nodule/abcess)
HC, historic control data from the testing laboratory

Liver

In high dose male rats, tumour incidences of both adenomas and combined adenomas/carcinomas were statistically significantly increased above concurrent and historical control incidences. The same was true for high dose male mice, for adenomas, carcinomas, as well as combined adenomas/carcinomas. In high dose female mice, the tumour incidences did not statistically significantly differ from controls, but there was a positive trend for the carcinomas and for combined adenomas/carcinomas, with incidences outside the historical control range. There was no effect of treatment on the liver tumour incidences in female rats.

Other liver lesions observed in the 2-year rat study (see section *Supplemental information* in Annex 1) included increased incidences of hepatocellular hypertrophy, of individual cell necrosis, and of aggregates of macrophages in high dose males and females. High dose females additionally showed an increased incidence of multifocal vacuolisation of hepatocytes (consistent with fatty change), as well as a decreased incidence of rats with the highest number of basophilic foci of altered hepatocytes. At the 1-year interim sacrifice the absolute/relative liver weights were slightly increased at the high dose (17/13% and 3/6% in males and females, respectively), whereas at terminal sacrifice the overall liver weights were not affected.

In the 18-month mouse study, other liver lesions observed in high dose mice also included hepatocellular hypertrophy, necrosis and fatty change, while males also showed eosinophilic and vacuolated foci and mitotic alteration (see section *Supplemental information* in Annex 1). The number of mice with one or more mass nodules in the liver was also higher at the high dose, and the high dose animals had considerably increased absolute and relative liver weights (87 resp.

79% in males, 51 resp. 47% in females). For most effects, male mice were more severely affected than female mice.

RAC concluded that DNA-reactivity/genotoxicity is not a potential MoA for sulfoxaflor, and that also cytotoxicity is unlikely to be involved given the absence of consistent and significant necrosis. Looking at the results of the MoA and short- and long-term toxicity studies for both mice and rats, RAC, in line with JMPR (2012) and US EPA (2012-2013) supported the conclusions drawn in the HRF analysis that:
- the studies "clearly demonstrate a sulfoxaflor-induced, robust, dose-related increase in the Cyp2b/CAR-associated transcript and associated increase in specific Cyp2b protein (Cyp2b10 in mice and Cyp2b1 in rats) and enzymatic activity (PROD/BROD). These results are consistent with the direct activation of the CAR nuclear receptor. In addition, analysis of hepatocellular proliferation indicates a clear, thresholded, dose-related induction of S-phase DNA synthesis. Both of these key events were demonstrated to be directly tied to the activity of the CAR nuclear receptor by the use of genetically modified mouse models (i.e., CAR/PXR-null, knockout, CARKO/PXRKO), where no CAR activity (gene or protein expression of Cyp2b10) or increase in hepatocellular proliferation was noted at a carcinogenic dose level of 750 ppm. Furthermore, the gross and microscopic hypertrophic effects of sulfoxaflor on the liver were reversible upon removal of the test material. Lastly, the Cyp2b/CAR-associated gene expression and protein data from these MoA experiments in both mice and rats define a very specific sulfoxaflor MoA while, simultaneously, rule out other nuclear receptor-mediated MoAs for rodent hepatic carcinogens such as PPAR-α or AhR agonism"; and that:
- "the specificity for the MoA was demonstrated for sulfoxaflor using genetically engineered mouse models. As previously described, the CARKO/PXRKO mice were refractory to the CAR-mediated hepatic effects demonstrated for sulfoxaflor in wild type mice. Moreover, and most importantly, humanised CAR/PXR (hCAR/hPXR) mice demonstrated a similar, although quantitatively less, response for most endpoints directly associated with CAR activation, but no increase in hepatocellular proliferation was noted."

Overall, RAC found the MoA behind the liver tumour formation to be well investigated for sulfoxaflor, and concluded that the data provided support for a non-genotoxic, threshold-based, CAR-mediated, mitogenic MoA, with key events in this MoA having been demonstrated to occur in mice and rats. However, as to the critical event late in this MoA, i.e. hepatocellular proliferation, experiments with sulfoxaflor in humanized CAR/PXR mice seem to indicate that there is a difference in response between rodents and humans: at a carcinogenic dose level, human receptors were able to support the sulfoxaflor-induced hypertrophic response but not the hyperplastic response. Consequently, in the absence of cell proliferation, liver tumours are not expected to occur in humans. Hence, RAC supported the conclusion of the dossier submitter that the rodent liver tumours associated with administration of high dose levels of sulfoxaflor would not pose a cancer hazard to humans, and therefore do not warrant classification. It is to be noted that this conclusion cannot be interpreted to mean that liver tumour formation via a CAR-mediated MoA is not relevant to humans in all cases: when such a MoA is claimed for a substance, RAC will judge on a case-by-case basis whether sufficient and good quality data have been provided showing the presence or absence of key events supporting the MoA and the (non-)relevance to humans.

<u>Male reproductive tract</u>
Testicular and epididymal weights of mid and high dose male rats showed clear treatment-related responses and were associated with significant pathology (see section *Supplemental information* in Annex 1). Absolute/relative testes weights were approximately 46/50% and 62/68% higher than controls for the mid and high dose groups, respectively, and similarly for absolute/relative epididymal weights which were reduced by 23/19% and 26/22%, respectively). The higher testes weights were due to the presence of interstitial (Leydig) cell adenomas in the testes. The latter concerned an increase in bilateral adenomas outside the historical control range at the mid and high dose. The overall incidence (i.e. unilateral and bilateral adenoma combined) was however not affected (no dose-response, within historical control range). There was a clear link between animals with higher testicular weight and severe atrophy of seminiferous tubules, decreased amounts of sperm in the epididymides, and decreased secretory material in the coagulating glands, prostate, and seminal vesicles; all presumably secondary to the testicular adenomas. Another effect (possibly also secondary) was an increase in the incidence of preputial gland

carcinomas, as observed in high dose male rats. Whether or not these carcinomas were treatment-related is difficult to establish, given that only animals having a gross lesion in this organ were histopathologically examined, so that the exact incidence is unknown. Given this, RAC concluded that the preputial gland tumours in themselves form insufficient evidence to warrant classification.

Recognising that male Fischer F344 rats are particularly susceptible for developing LCT (given the background incidence of 75-100%) and that the overall incidence for LCT in the 2-year rat study was not affected and within the background range, RAC concluded that the LCT are not related to sulfoxaflor-treatment and therefore do not warrant classification.

Conclusion
RAC concluded that, overall, the weight of evidence supports the conclusion of the dossier submitter that the liver tumours observed in male rats and male and female mice upon sulfoxaflor treatment are unlikely to be relevant to humans, and that hence no classification is required for these tumours under CLP and DSD. RAC further concluded that the LCT and preputial gland tumours do not require classification under CLP and DSD, given that there is no (LCT) or an uncertain (preputial gland tumours) relationship to treatment with sulfoxaflor.

RAC evaluation of reproductive toxicity

Summary of the Dossier submitter's proposal

The dossier submitter did not propose classification for reproductive toxicity, based on the results of eight reproductive toxicity studies: a reproduction/developmental toxicity screening study and a 2-generation reproduction study in rats, a developmental toxicity probe study, a definitive developmental toxicity study and a developmental neurotoxicity study in rats, a gavage probe developmental toxicity study, a dietary probe developmental toxicity study and a definitive dietary developmental toxicity study in rabbits. In these studies, there were four relevant findings with respect to reproductive toxicity, according to the dossier submitter. Seven mechanistic/MoA-studies and a HRF analysis were provided to further explain the primary findings.

The first, primary, finding according to the dossier submitter was post-natal pup mortality and specific limb abnormalities (forelimb flexure, bent clavicle and hindlimb rotation) observed in the 2-generation study and developmental neurotoxicity studies in rats, but not in the rabbit studies. It has been hypothesized that these effects have a single MoA mediated via the rat foetal-type muscle nicotinic acetylcholine receptor (nAChR) through the following key events: (1) binding to the receptor, (2) agonism (activation) at the receptor, causing (3) sustained muscle contracture in the near-term foetus and neonatal offspring. This sustained muscle contracture results in limb contractures, bent clavicles, and reduced function of the diaphragm, which compromises respiration in offspring at birth and reduces neonatal survival. The hypothesis has been supported by a series of studies investigating the findings in the rat which have demonstrated that:

- the effect of sulfoxaflor on pup survival was due to *in utero*, not lactational, exposure;
- sulfoxaflor was not developmentally toxic in the rabbit, despite the achievement of similar maternal and foetal systemic concentrations of sulfoxaflor in both rat and rabbit;
- sulfoxaflor has been shown to be a partial agonist of the rat foetal muscle nAChR. In contrast, sulfoxaflor has no detectable agonist activity on the human foetal muscle nAChR or on the adult forms of skeletal muscle nAChR (from either human or rat);
- the critical period of developmental susceptibility to sulfoxaflor-induced foetal abnormalities and reduced neonatal survival is between GD 16 and GD-22), and that the foetal structural abnormalities are rapidly reversible after birth in surviving pups.

According to the dossier submitter, the extensive data presented have gone a significant way towards identifying the MoA of the observed foetal mortalities and morphological alterations and have provided significant evidence that the MoA may not be relevant to humans. The dossier submitter noted some inconsistencies and data gaps though in the evidence: rabbit muscle nAChRs have not been investigated in functional receptor studies; the possibility of interaction with other cholinergic receptors (neuronal/nicotinic and muscarinic) has only been considered in an indirect way; the foetal morphological findings have not been reported in all (probe) reproductive toxicity and mechanistic rat studies with sulfoxaflor; and the specific binding to rat

foetal nAChR with associated post-natal mortality and structural alterations has not been found for other structurally related neonicotinoids. Although this raised some doubts whether the 'significant support' could be considered 'sufficient proof' for the non-relevance to humans, the dossier submitter was of the opinion that a case for non-classification could be supported on the basis of the data presented for the pharmacologically mediated effects.

The second relevant finding was a reduction in mean rat pup body weight on PND 1. This effect was observed in a number of studies including the reproduction screening study at 1000 ppm, the developmental neurotoxicity study at 400 ppm (associated with a statistically significant delay in surface righting response for pups), and a cross-fostering study (being one of the mechanistic studies) at 1000 ppm. It was noted that these doses also impaired neonatal survival, and that pup weights were not different from controls immediately after birth in the cross-fostering study. The dossier submitter therefore concluded that the reduced pup weight on PND 1 (and in some studies also on PND 4) is likely to be a consequence of breathing difficulties and an inability to move and nurse normally, due to the pharmacological action of sulfoxaflor which is rat-specific. As such, it is not considered relevant to humans, and therefore does not warrant classification.

The third relevant finding according to the dossier submitter was an increased post-implantation loss and decreased foetal weights in the main rat developmental toxicity study at 1000 ppm. These foetotoxic effects were observed at a dose level that caused significant maternal toxicity (decrease in maternal weight (by 9% at GD 21) and in maternal weight gain (by 22% over GD 6-21)). Also, the increase in post-implantation loss was not dose-related and was within recent historical control data range, with data for the concurrent control at the lower end of this range. The increased post-implantation loss was therefore considered unlikely to be treatment-related, and thus not supporting classification. In contrast, the clear reduction in foetal weight (11.6%) was considered likely to be treatment-related. However, given the significant maternal toxicity at the same dose, it was considered a borderline effect that may be 'a secondary consequence of other toxic effects'. This does not justify classification, as supported by the absence of this effect in a second species, the rabbit.

The fourth relevant finding was a statistically significant delay in preputial separation (2.4 days) in the rat 2-generation study at 400 ppm in F1 males. This effect is androgen dependent, but the underlying reason for induction of this finding by sulfoxaflor is not known. However, there were no other indications of androgenic or anti-androgenic effects of sulfoxaflor: there were no effects on male/female anogenital distance and reproductive function/organs in the 2-generation study and on balanopreputial separation and vaginal patency at the same dose level in the developmental neurotoxicity study, and in specific MoA studies (see section on Carcinogenicity above) sulfoxaflor was shown not to interact with the oestrogen and androgen receptors nor to inhibit aromatase activity. Taken together, the dossier submitter considered the effect on preputial separation not supportive of classification.

Conclusion

The dossier submitter concluded that, in the absence of human evidence, Repr. 1A (CLP)/Repr. Cat. 1 (DSD) is precluded. The adverse effects on pup survival and structural alteration in the rat (and, secondary, on early post-natal pup body weight) would normally warrant Repr. 1B or Repr. 2 (CLP)/Repr. Cat. 2 or 3 (DSD). Where the DSD criteria state *'…Even when clear effects have been demonstrated in animal studies the relevance to humans may be doubtful because of the doses administered, for example, where effects have been demonstrated only at high doses, or where marked toxicokinetic differences exist, or the route of administration is inappropriate. For these or similar reasons it may be that classification in category 3, or even no classification, will be warranted*', the CLP criteria emphasise the issue of mechanistic data stating *'...However, when there is mechanistic information that raises doubt about the relevance of the effect for humans, classification in Category 2 may be more appropriate.'*

According to the dossier submitter, extensive mechanistic evidence has been presented providing significant support that the observed neonatal mortalities and morphological alterations are related to the pharmacological action of sulfoxaflor and that these pharmacological effects are not relevant to humans, due to specific differences in the subunit structure of the muscle nAChR between humans and rats (and rabbits) and differences therefore in the binding and functional activation of the receptor complex by sulfoxaflor. Although the dossier submitter recognised some inconsistencies and data gaps in the evidence (one of which was later on addressed by IND with

an additional mechanistic study; see section *Additional key elements* in Annex 1, pp. 249-250), on balance it was concluded that the good quality mechanistic data go beyond the criterium for 'raising doubt'. Hence, it was considered that the significant support for the non-relevance to humans of the effects on post-natal survival and skeletal alterations (and, secondary, early post-natal pup body weight) provided a strong argument for non-classification and thus no classification under CLP and DSD was proposed by the dossier submitter.

Comments received during public consultation

One MSCA was in support of the 'no classification' proposal, agreeing that the mode of action studies support the non-relevance to humans. Another MSCA wondered whether the high post-natal mortality in rats (as induced by pre-natal exposure and not by exposure via milk) might qualify for classification for developmental toxicity. The dossier submitter responded that 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. Post-natally, 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.
IND supported the 'no classification' proposal by the dossier submitter, but considered the non-relevance to humans of the two primary developmental effects of sulfoxaflor in rats (foetal abnormalities and reduced neonatal survival) to have been proven beyond any reasonable doubt. Adding further weight to this conclusion, IND in their comments provided a detailed response to the alleged inconsistencies/data gaps in the evidence (see Annex 2 to this opinion for the Response to comments document (RCOM)). IND further announced that they will provide an additional mechanistic study once finalised, and also an updated HRF, incorporating the results of the additional mechanistic study that is aimed at characterising the agonist effects of sulfoxaflor on mammalian (rat and human) muscle nAChRs expressed in human embryonic kidney (HEK) cells. According to IND, the preliminary results of this new study confirm specific agonism of the rat foetal receptor only. RAC could confirm this conclusion when the final study results (Millar, 2012; see section *Additional key elements* in Annex 1) were provided to RAC.

Assessment and comparison with the classification criteria

Fertility

No effects on reproductive organs have been described for the repeated dose toxicity studies presented in the CLH dossier, except for the 2-year rat study. At the end of that study, testicular and epididymal weights were increased, associated with significant pathological findings. However, these effects are all considered secondary to the observed formation of Leydig cell tumours (see section on Carcinogenicity above). In the rat 2-generation study, levels up to and including the highest dose of 400 ppm sulfoxaflor (24.6-34.4 mg/kg bw/day) did not have an effect on mating, conception, fertility or gestation indices, time to mating, or gestation length, in the first or second generation. Histopathological examination of the reproductive organs of P1 and P2 did not reveal any treatment-related effects either. Higher doses of sulfoxaflor (up to 1000 ppm) were tested in a reproduction/developmental toxicity screening study, but also in these studies no effect on the reproductive performance/parameters were seen. RAC therefore concluded that there is no need to classify sulfoxaflor for effects on sexual function and fertility.

Developmental toxicity

Rat
In the rat 2-generation study, developmental effects were limited to the high dose of 400 ppm (24.6-34.4 mg/kg bw/day), a dose at which there was only little evidence of parental toxicity. The developmental effects comprised slightly decreased neonatal survival in both generations from immediately after birth until PND 4; this in turn led to a lower percentage of live pups up to culling on PND 4. This finding is consistent with the reduced neonatal survival observed on PND 1 and 4 in the reproduction/developmental toxicity screening study at 500 and 1000 ppm. In both studies, pup survival was normal for the remainder of the pre-weaning period. A cross-fostering study in rats demonstrated that the effect on pup survival is due to *in utero*, not lactational, exposure.

In addition to neonatal mortality, there was an apparent treatment-related delay (2.4 days) in preputial separation in F1 (but not F2) males in the 2-generation study. There was however no effect on anogenital distance or on other parameters consistent with altered androgenicity.

Dietary administration at GD 6-21 of 1000 ppm sulfoxaflor (70.2 mg/kg bw/day), but not 150 and 25 ppm, resulted in some maternal toxicity and in developmental toxicity. Maternal toxicity was evidenced by decreases in body weight (7-9%) and body weight gain (22%), with concomitant decreased feed consumption, throughout the treatment period. In addition, slightly increased relative liver weight (6.1%) was noted. Developmental toxicity was evidenced by decreases in foetal body weight (11.6%) and gravid uterine weight (13.2%) and by a lower number of viable foetuses per litter due to an apparent increase in post-implantation loss. In addition, clear increases in several foetal abnormalities (forelimb flexure, hindlimb rotation, convoluted ureter, hydroureter, bent clavicle and fused sternebrae) occurred. In two critical window of exposure studies it was shown that the critical period of developmental susceptibility to sulfoxaflor-induced foetal abnormalities and reduced neonatal survival occurs shortly before birth (GD 20-22), and that the foetal abnormalities are rapidly reversible after birth.

In a dietary rat developmental neurotoxicity study, administration up to and including 400 ppm sulfoxaflor (~29 mg/kg bw/day) from GD 6 to LD 21 did not result in maternal toxicity. The high dose of 400 ppm, however, did result in effects on the offspring: postnatal survival from birth to PND 4 was reduced (viability index 76.5% as compared to 93% for controls), pup body weights were decreased (on PND 1 and 4 by 11.8 and 6.5%, respectively) resulting in a delay in surface righting response (6.3 days as compared to 5.3 days for controls). Other developmental landmarks were not affected, and survival and body weights were not different from controls throughout the remainder of the pre-weaning period (PND 4-21). No treatment-related effects were observed with respect to detailed clinical observations, locomotor activity, auditory startle response, and learning and memory. Furthermore, there were no treatment-related effects on brain weights, measurements, and morphometric parameters or histopathology of the brain and/or central and peripheral nervous systems for offspring on PND 21 and 72.

Rabbit
Administration of 30, 150 or 750 ppm sulfoxaflor in the diet on GD 7-28 did not result in developmental toxicity in any dose group. At the high dose of 750 ppm (31.9 mg/kg bw/day) there was maternal toxicity in the form of decreased faeces in 7 out of 26 animals, decreased mean body weight gain (~50%) from GD 7-13, overall decreased mean body weight gain (12%) throughout treatment (GD 7-28), and decreased mean feed consumption (8-21%) from GD 7-17. A subsequent neonatal survival study confirmed the absence of neonatal deaths in rabbits following sulfoxaflor treatment.

In summary, no developmental effects were observed in rabbits whereas in rats there were various effects that might be relevant for classification. These are:

1. Reduced early postnatal survival (PND 1-4), shown to be the result of *in utero* exposure, with the critical window shortly before birth.
2. Foetal abnormalities (in particular foetal limb contractions and bent clavicles), also shown to be the result of *in utero* exposure, and shown to be reversible by PND 2-4.
3. Reduced pup weight on PND 1(-4).
4. Increased post-implantation loss and decreased foetal weight.
5. Slight delay in preputial separation.

Effects no. 1 and 2 did not occur in rabbits, despite similar maternal and foetal blood data, showing that the interspecies difference was most likely due to toxicodynamics, not toxicokinetics. Sulfoxaflor's insecticidal MoA is agonism of the insect nAChR. Given this, plus the fact that the rat limb skeletal structures were normal in foetuses with limb contracture abnormalities, suggesting that the effects on limbs and clavicles resulted from an action on neonatal offspring muscle, the mammalian muscle nAChR was investigated as a biologically plausible target responsible for these effects in the rat. The muscle nAChRs are found in the intramuscular junctions of skeletal muscles. Disregulation of these receptors can result in muscle contraction, difficulties in breathing, and ultimately death. There are two subtypes of muscle nAChRs, a foetal form and an adult form. In rats, functional expression of the foetal form occurs between GD 15 and 17, resulting in

synchronized foetal limb movements and diaphragmatic responsiveness (critical for transition to extrauterine respiration) between GD 16 and 17. The switch from foetal to adult form occurs shortly after birth in rats (starting late during the first postnatal week), whereas in humans this occurs already several weeks before birth (starting in the 2nd trimester).

The available mechanistic studies (including the new study by Millar, 2012) and the further explanations provided by IND during public consultation, addendum and post-report analysis adequately show that the foetal abnormalities and reduced postnatal survival in rats occur via a single MoA: sulfoxaflor's sustained agonism at the foetal-type muscle nAChR and subsequent sustained muscle contracture of the limb, shoulder girdle and diaphragm, resulting in limb contracture, bent clavicles, and abnormal neonatal respiration after birth, resulting in neonatal offspring death. Several alternative MoAs were addressed in the HRF (including e.g. agonism at neuronal nAChRs or muscarinic AChRs, acetylcholinesterase inhibition), but the evidence presented (albeit mostly indirect) together with the detailed response by IND during public consultation make it plausible that these alternative MoAs are not likely to be involved.

RAC considered the proposed MoA not relevant to humans: it has been shown that sulfoxaflor can bind to human (and rabbit) foetal muscle nAChR, but there is no agonist effect of sulfoxaflor on human foetal (and adult) muscle nAChR, nor when expressed in *Xenopus* oocytes, nor when expressed in HEK cells. 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. Further, the effects of concern in rats are linked to the foetal-type muscle nAChR, not the adult-type, and have a critical window shortly before birth. In humans, however, at that time point foetal-type muscle nAChR is no longer present, as transition to the adult-type has then already been completed for several weeks. Based on the above, classification for these effects is therefore not warranted. Support for this is the finding that although sulfoxaflor binds to rabbit foetal muscle nAChR, it does not induce any developmental effects in rabbits despite similar systemic exposure to that in rats.

As to effects 3-5, RAC supported the conclusion of the dossier submitter that these effects do no warrant classification, for the reasons given by the dossier submitter.

Overall, RAC concluded that there is no need to classify sulfoxaflor for developmental toxicity.

ENVIRONMENTAL HAZARD ASSESSMENT

RAC evaluation of environmental hazards

Summary of the Dossier submitter's proposal

The dossier submitter proposed environmental hazard classifications for Aquatic Acute 1 with an M-factor 1 and for Aquatic Chronic 1 with an M-factor 1 (N; R50/53 DSD, including specific concentration limits).

Degradation

One study on hydrolysis (OECD TG 111), two on photodegradation (OECD TG 316) and one on ready biodegradation (OECD 310) of sulfoxaflor are summarised in the CLH report. Also, a study on aerobic transformation in aquatic sediment system is included (OECD 308).

The hydrolysis study was performed at three pHs (5, 7 and 9) in which sulfoxaflor remained unchanged (DT_{50} > 1000 days for the whole pH range). The dossier submitter concluded that sulfoxaflor is hydrolytically stable.

Both reported photo-transformation studies showed slow photodegradation for sulfoxaflor and for a separately tested major metabolite (X11719474). The dossier submitter concluded that direct and indirect aqueous photolysis should not be considered as a relevant mechanism for degradation in water bodies.

The headspace test (OECD TG 310) on ready biodegradation showed less than 3% degradation of sulfoxaflor after 28 days study period. No significant deviations to the OECD TG 310 were noted

and the study was considered reliable. The dossier submitter concluded that based on this study sulfoxaflor shall be regarded as not readily biodegradable.

The water/sediment simulation test was performed in two pond systems, one with coarse (sand) sediment and one with fine (silt loam) sediment. The study did not have significant deviations to the OECD TG 308 and was considered reliable by the dossier submitter. The maximum level of mineralisation (i.e. the measured $^{14}CO_2$) was 1.6% in the fine sediment system and 0.55% in the coarse sediment system. The total (water and sediment combined) concentration of sulfoxaflor in the system decreased slowly throughout the study and was 16.6% in the silt loam sediment and 46.1% in the sandy sediment at 103 days after treatment (DAT). Only one degradation product (X11719474) was formed reaching its maximum of 65.6% at DAT 88 in the silt loam sediment and 71% at DAT 76 in the sandy sediment and the dossier submitter stated that the metabolite was more persistent than the applied parent compound. The DT_{50} (whole system) for sulfoxaflor was 89 days in the sand sediment system and 37 days in the silt loam sediment system, with a geometric mean DT_{50} of 57.08 days for the two systems.

The dossier submitter concluded that sulfoxaflor is neither readily nor rapidly degradable in the environment.

Bioaccumulation
Estimation of bioaccumulation potential was based on measured partition coefficients. The log P_{ow} was 0.806 at pH 5, 0.802 at pH 7 and 0.799 at pH 9. No details on the used method were given in the report and the reliability of the measured values is not known. The dossier submitter concluded that based on the measured log Pow value the bioaccumulation potential of sulfoxaflor is low.

Aquatic acute toxicity
Acute aquatic toxicity of sulfoxaflor was tested in several studies covering all three trophic levels relevant for environmental hazard assessment. In three out of four acute toxicity studies in fish an exact LC_{50} value was not determined, since mortality was not observed at any exposure concentration (> 316 mg/l). An LC_{50} of 266 mg/l was defined for sheepshead minnow (*Cyprinodon varieagatus*) based on measured sulfoxaflor concentrations in water (OECD TG 203).

Four species of invertebrates were tested for acute toxicity of sulfoxaflor. For the water flea (*Daphnia magna*), the EC_{50} (48 h) value was concluded to be higher than the highest exposure concentration (>399 mg/l) since no treatment related effect was observed in any applied concentration. Mysid shrimp (*Americamysis bahia*) was more sensitive to sulfoxaflor and the LC_{50} value based on measured test concentrations after 96-hour exposure was 0.643 mg/l (EPA TG 72-1). Acute toxicity of sulfoxaflor for the Eastern oyster (*Crassostrea virginica*) was 86.5 mg/l (mean measured). Two separate acute tests with a non-biting midge (*Chironomus dilutus*) were reported. The LC_{50} value in a 96-hour study where the non-biting midge was exposed to sulfoxaflor via water was 0.622 mg/l (mean measured). In another study where the non-biting midge was exposed to sulfoxaflor spiked sediment for 10 days an LC_{50} of 0.119 mg/l was found based on the measured concentrations.

Sulfoxaflor showed low toxicity to aquatic algae (4 species tested) and plants (Duckweed; *Lemna gibba*) with $L(E)C_{50}$ values > 100 mg/l. A freshwater diatom *Navicula pelliculosa* was the only species for which an exact acute toxicity value was defined and the EC_{50} for it was 85.7 mg/l (mean measured).

The most sensitive species in the reported reliable acute aquatic tests was the non-biting midge (*C. dilutus*) having a 96-h LC_{50} of 0.622 mg/l. Based on this the dossier submitter concluded that Aquatic Acute 1 with an M-factor 1 is warranted.

Aquatic chronic toxicity
Chronic toxicity studies covered also all the three trophic levels relevant for environmental classification. An early-life stage (ELS) study (OECD TG 210) in fathead minnow (*Pimephales promelas*) resulted in a NOEC-value of 5.05 mg/lmg/l based on measured sulfoxaflor concentrations. Another ELS study (OPPTS TG 850.1400) in sheepshead minnow (*C. variegatus*) resulted in a lower NOEC value of 1.21 mg/l (measured concentrations).

Chronic toxicity of sulfoxaflor was tested in three species of aquatic invertebrates. A 21–day study (OECD TG 211) in water flea (*D. magna*) resulted in a NOEC value of 50 mg/l based on nominal concentrations. In mysid shrimp (*A. bahia*) the NOEC value in a 28 day-study was 0.114 mg/l (measured concentrations). Chronic toxicity in the non-biting midge (*Chironomus riparius*) was studied in a system where the larvae were exposed to overlying water which was spiked with radiolabelled sulfoxaflor. The dossier submitter reported a NOEC value of 0.0384 mg/l based on the initial measured concentrations in the overlying water. This value was a result of calculation where the initial sulfoxaflor concentration 0.0526 mg/l was multiplied by 0.73 (a value derived from the mean measured recovery of sulfoxaflor (73%) in the 0.1 mg/l nominal treatment level).

The same algae and plant studies as for acute toxicity were used for deriving NOEC values for chronic toxicity of sulfoxaflor. Only two studies had NOEC values below the highest test concentrations. The reported NOEC for freshwater cyanobacteria (*Anabaena flos-aquae*) was 13 mg/l and for the freshwater diatom (*N. pelliculosa*) 3.7 mg/l, both values based on measured concentrations.

The dossier submitter concluded that *C. riparius* is the most sensitive tested aquatic organism having a NOEC ≤ 1 mg/l. Since sulfoxaflor is not rapidly biodegradable, classification for Aquatic Chronic 1 with an M-factor of 1 according to CLP is warranted as the NOEC is between 0.01 mg/l and 0.1 mg/l. The DSD classification for chronic effects is based on the surrogate approach and the acute study in *C. dilutus* (LC_{50} 0.622 mg/l) as the decisive study. Since the substance is not readily degradable, the dossier submitter concluded that R53 is warranted.

Comments received during public consultation

Five MSCAs supported the classification proposal for aquatic acute and chronic toxicity. No comments opposing the proposal were received.

Assessment and comparison with the classification criteria

Degradation

The results of simulation tests indicate that sulfoxaflor does not fulfill the criteria for rapid degradability of >70% degradation in 28 days (under both CLP and DSD).

The level of biodegradation reached by sulfoxaflor during a ready biodegradation experiment was 2.5% after 28 days.

The degradation of sulfoxaflor was assessed using media from two natural water/sediment systems. The geometric mean DT_{50} in the aquatic system was 57.08 days (36.67 to 88.86). Mineralisation of sulfoxaflor was relatively low, reaching a maximum of 1.6% in fine sediment and 0.55% in coarse systems after 103 days. The only metabolite found in both systems within the study was X11719474, which is also a major soil metabolite. It was formed in both systems at the maximum amount of ~65-71% and displayed greater persistence than the parent compound according to the CLH report. The DT_{50} for the metabolite was not determined as the distinct decline phase was not reached for X11719474 by the end of the study.

Abiotic studies also support the overall evidence that the substance does not rapidly degrade. In the aquatic environment, sulfoxaflor was shown to be hydrolytically and photolytically stable in the whole range of the environmentally relevant pH (5-9). For the aqueous hydrolysis DT_{50} was > 1000 days, for direct photolysis DT_{50} = 489 days and for indirect aqueous photolysis DT_{50} = 224 days.

Based on this information, RAC agreed with the dossier submitter that sulfoxaflor is not considered to undergo fast primary degradation or ultimate degradation under environmental conditions and can be considered not rapidly/readily degradable for the purpose of classification and labelling.

Bioaccumulation

The dossier submitter considers sulfoxaflor to have a low bioaccumulation potential based on measured log K_{ow} range of 0.799 – 0.806 at 20°C in the whole range of environmentally relevant pH (5-9). This log Kow range is below the trigger of log ≥ 4 (CLP) and log ≥ 3 (DSD), so RAC

agreed with the conclusion of the dossier submitter that sulfoxaflor has no significant bioaccumulation potential.

RAC noted that the reported surface tension of 57.5 mN/m for sulfoxaflor is below the cut-off value of 60 mN/m for surface active substances.

Aquatic Acute Toxicity
The submitted toxicity studies indicate that sulfoxaflor exhibits low acute toxicity to fish (96h-LC_{50} values of >100 mg/l), freshwater crustaceans (*Daphnia magna,* 48h-EC_{50} value of >100 mg/l), algae and aquatic vascular plants (96h- or 7d- EC50 values of >100 mg/l) and the Eastern oyster (*Crassostrea virginica*; 96h-EC_{50} = 86.5 mg/l). Of higher sensitivity to the toxicity of sulfoxaflor were the midge larvae *Chironomus dilutes* (a sediment dwelling insect) and the mysid shrimp *Americamysis bahia* (a saltwater free-swimming crustacean), with 96-h EC_{50}s of 0.622 mg/l and 0.643 mg/l, respectively.

Aquatic Chronic Toxicity
The lowest chronic toxicity value (a NOEC of 0.0384 mg/l) was produced in a 28-day emergence test with *Chironomus riparius* in artificial sediment, exposed via the overlying water. Laboratory chronic toxicity studies indicate sulfoxaflor to be slightly toxic to *Daphnia* (21-d NOEC 50 mg/l) and to exhibit slight effects on growth in a long-term early life stage toxicity study in fathead minnow (*Pimphales promelas*; 30-d NOEC 5.05 mg/l). Stronger effects on growth were found in an early life stage toxicity study with sheephead minnow (*Cyrpinodon variegatus*; 38-d NOEC 1.21 mg/l). Chronic studies also indicate that sulfoxaflor is highly toxic to the mysid shrimp (*A. bahia*; 28-d NOEC of 0.114 mg/l).

In summary, in both acute and chronic studies sulfoxaflor is of highest toxicity to midge larvae (*Chironomus*) and to the mysid shrimp (*Americamysis bahia*). An overview of the results of the available toxicity studies is presented in the section on *Supplemental information* in Annex 1.

Sediment organisms
In terms of aquatic acute and chronic hazard, the most sensitive species tested was *Chironomus dilutus* and *Chironomus riparius,* respectively, results upon which the dossier submitter chose to base the classification proposal.

The CLP guidance does not provide information on how to use sediment data for classification and labelling purposes. It is stated in the REACH guidance (Guidance on Information Requirements and Chemical Safety Assessment, Chapter R.7B, section R.7.8.11.1) that "whole sediment tests with benthic organism are not standard tests for classification and labelling, as only exposure via the water phase is normally considered for this purpose." The tests using *Chironomus* are described in more detail below.

Method	**Test organism**	**Test Design**	**Results mg/l**			**Remarks/Reference**
			Endpoints	**NOEC**	**LC50/ EC50**	
OECD 202, OPPTS 850.1010	*Chironomus dilutus*	Acute, 96h, spiked water, static	immobility	-	0.622	Mean measured Gerke, 2008d
OECD 219	*Chironomus riparius*	Chronic, 28d, spiked water, static	survival, emergence	0.0384 (overlying water)	-	Corrected for degradant formation Gerke, 2009

Study 1 (Gerke, 2008d): Midges (*C. dilutes*) were exposed to nominal concentrations of (control), 0.13, 0.25, 0.5, 1.0, 2.0, 4.0, 8.0, 16 and 32 mg/l sulfoxaflor (purity 95.6%) in water in a static system over a period of 96h. Measured concentrations remained between 80 and 120% of the nominal concentrations. Because of the slight difference between the nominal and measured concentrations, the author of the study based the toxicity endpoints on mean measured

concentration. A 96-h LC_{50} value of 0.622 mg/l (or 0.656 mg/l nominal concentration) was determined for sulfoxaflor. Sublethal effects (organisms that were lethargic or displaying erratic movement) were noted at all tested concentrations.

Study 2 (Gerke, 2009): The chronic toxicity of sulfoxaflor to midge larvae (*C. riparius*) in whole sediment was determined by dosing the water phase. Midges were exposed to sulfoxaflor (non-radiolabelled mixed with ^{14}C-sulfoxaflor radiolabelled) to the overlying water in a static system over a period of 28 days to nominal concentrations of 0.00157, 0.00313, 0.00625, 0.0125, 0.0250, 0.0500 and 0.100 mg/l. Emergence, development rate and survival rate were used to determine toxicity endpoints. The total radioactive residues (TRR) in overlying water were 105-113% of the nominal concentrations on day 0 and decreased to about 72-81% of the nominal concentrations after 28 days. Data on the parent compound sulfoxaflor indicate a more rapid decrease of the sulfoxaflor concentration (108% of nominal at 0.100 mg/l level (= 0.108 mg/l) at day 0 to 50% of nominal (= 0.0502 mg/l) at day 28) than that of the TRR in the overlying water. This suggests that the test substance may be incorporated into the pore water or sediments as well as degraded to X11719474 over the 28 day-period. Therefore, in the study, all biological response evaluations were calculated based on initial and mean measured ^{14}C-labelled *sulfoxaflor* concentrations in the overlying water, rather than on *TRR* concentrations, by applying a correction factor of 73% (mean sulfoxaflor concentration over 0-28 days is 0.079 mg/l, which is 73% of the initial 0.108 mg/l). Based on survival and emergence and corrected initial mean measured concentrations, the 28-day NOEC was 0.0384 mg sulfoxaflor/l and the LOEC was 0.081 mg/l.

The data from the chronic toxicity study was recalculated because RAC did not agree with the method used to derive the correction for degradation. The assumption that the degradant will be present from the start of the test is not accurate as the concentration of the degradant gradually builds up over time and the correction therefore needs to take this into account. Furthermore, using the arithmetic mean in the analysis of overlying water samples for sulfoxaflor and degradant X11719474 may not be the most appropriate approach for calculating the concentrations (for more details see the section on *Supplemental information* in Annex 1). Using the calculation methods described therein, the 28-day NOEC for survival and emergence based on corrected TWA-concentrations for the parent compound was determined to be 0.0374 mg/l**.** This value is similar to the NOEC of 0.0384 mg/l in the CLH dossier, but as it has been calculated by a more appropriate method; RAC decided to use the NOEC of 0.0374 mg/l.

In the view of RAC, given that exposure via the water phase is normally considered for classification and labelling purposes, sediment studies that use spiked water test design can in principle be taken into account. RAC concluded that the two *Chironomus* studies are performed according to OECD guidelines and are GLP compliant. Furthermore, the test results are in compliance with the guideline's validity criteria. Sulfoxaflor is a new active ingredient developed as an insecticide. Therefore it is not surprising that the *Chironomus* species is particularly sensitive to the test substance. Whereas the acute *Chironomus* study did not involve sediment, the chronic *Chironomus* toxicity study is a water-sediment study, and therefore exposure via (ingestion of) sediment cannot be ruled out. However, sulfoxaflor sorbs weakly to soil (K_d 0.26-1.29 ml/g, K_{dOC} 13-83 mL/g, K_f 0.16-1.28 ml/g and K_{fOC} 12-71 ml/g, average 1/n is 0.96) although desorption experiments show that absorption of sulfoxaflor is only partly reversible (K_f^{des} 1.20-7.4 ml/g, K_{fOC}^{des} 55-613 ml/g, average $1/n^{des}$ is 0.98). Based on the low sorption potential and test design using water-spiking, exposure to sulfoxaflor is considered to occur primarily via the water. Also, the *Chironomus* spend their most sensitive larval stage (first instar) free swimming in the water phase and will therefore only be exposed to sulfoxaflor via the water in this stage. Based on the above, RAC considered the submitted toxicity studies with *Chironomus* acceptable for use in the classification of sulfoxaflor.

RAC noted that US EPA (2012-2013) in their assessment of sulfoxaflor accepted the acute study for risk assessment purposes. EPA accepted the chronic 28-day study only as a supplemental study, because the effects on midge larvae reproduction were not quantified per US EPA Agency wide guidelines for chronic sediment toxicity testing. This is not the case with the OECD guidelines.

Conclusion of environmental classification according to Regulation (EC) No 1272/2008

In aquatic toxicity studies, the lowest acute LC_{50} value of 0.622 mg/l sulfoxaflor was obtained for *Chironomus dilutes*. Sulfoxaflor is neither rapidly degradable nor bioaccumulative. As the lowest acute LC_{50} is ≤ 1mg/l, sulfoxaflor fulfills the criteria for **Aquatic Acute 1 – H400**. The assignment of an **M-factor of 1** is applicable, based on $0.1 < L(E)C_{50} \leq 1$ mg/l.

In chronic toxicity studies, the lowest chronic NOEC value of 0.0374 mg/l sulfoxaflor is obtained for *Chironomus riparius*. In addition, sulfoxaflor is not rapidly degradable. A NOEC value ≤ 0.1 mg/l and the substance being not rapidly degradable warrants hazard category 1 for aquatic chronic toxicity. Sulfoxaflor fulfills the criteria for **Aquatic Chronic 1 – H410**. The assignment of an **M-factor 1** is applicable, based on $0.01 < NOEC \leq 0.1$ mg/l. Support for this classification can be found in the surrogate approach, where use of the acute LC_{50} value of 0.622 mg/l for the most sensitive organism tested (*Chironomus dilutes*) and the fact that sulfoxaflor is not rapidly degradable would also lead to a classification for sulfoxaflor as Aquatic Chronic 1 – H410 with M-factor 1.

Conclusion of environmental of classification according to DSD

In aquatic toxicity studies, the lowest acute LC_{50} value of 0.622 mg/l sulfoxaflor is obtained for *Chironomus dilutes*. Sulfoxaflor is neither readily degradable nor bioaccumulative. With an $LC_{50} \leq$ 1mg/l plus the fact that the substance is not readily degradable, sulfoxaflor fulfills the criteria for classification with **N; R50-53**. The following specific concentration limits apply:

N; R50/53: C≥ 25%;
N; R51/53: 2.5% ≤C< 25%;
R52/53: 0.25% ≤C< 2.5%

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ANNEXES:

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

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