REGISTRATION REPORT

Part B
Section 8 Assessment of the relevance of metabolites in groundwater

Detailed summary of the risk assessment

CLOSER (GF-2626)

120 g/L Sulfoxaflor

All Zones

Zonal Rapporteur Member State: France

CORE ASSESSMENT

Applicant: DOW AgroSciences

Date: October 2017

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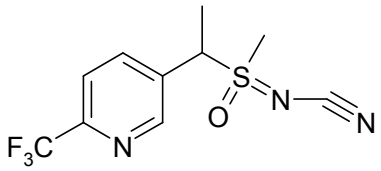
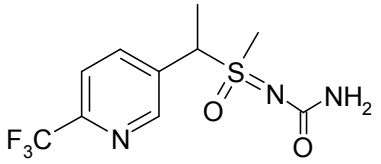
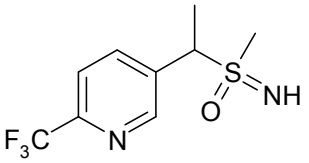
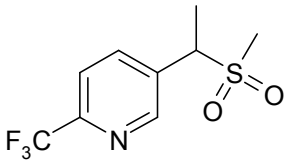
IIIA 12 ASSESSMENT OF THE RELEVANCE OF METABOLITES IN GROUNDWATER

The following metabolites of sulfoxaflor were considered in the risk assessment of groundwater contamination: X11719474, X11579457 and X11519540.

The structures of sulfoxaflor and the three metabolites are shown below:

Metabolite identification	IUPAC Chemical Name	Metabolite Structure
Sulfoxaflor	[methyl(oxo){1-[6-(trifluoromethyl)-3-pyridyl]ethyl}-λ^4-sulfanylidene]cyanamide	
X11719474	N-(methyl(oxido){1-[6-(trifluoromethyl)pyridin-3-yl]ethyl}-λ^4-sulfanylidene)urea	
X11579457	[5-[1-(S-methylsulfonimidoyl)ethyl]-2-(trifluoromethyl)pyridine	
X11519540	5-(1-methylsulfonyl)ethyl)-2-(trifluoromethyl)pyridine	

IIIA 12.1 Exclusion of degradation products of no concern

None of the above metabolite can be considered as a degradation product of no concern.

IIIA 12.2 Quantification of potential groundwater contamination

PECgw calculations for the three groundwater metabolites of sulfoxaflor (X11719474, X11579457 and X11519540) are detailed in Section 5.

The following maximum PECgw values covering all intended uses (risk envelope approach, see section 5 for details) were calculated:

X11719474, max PECgw = 8.38 µg/L

X11519540, max PECgw = 3.74 µg/L

X11579457, max PECgw = 5.92 µg/L

Since PECgw exceed 0.1 µg/L, the three metabolites are further assessed in the following Step 3 procedure.

IIIA 12.3 Hazard Assessment: Identification of relevant metabolites

Step 3 provides a 3-stage assessment involving (i) biological activity screening, (ii) genotoxicity hazard screening, and (iii) toxicity hazard screening. Any metabolite that does not pass all three stages is considered as "relevant" under regulatory aspects and thus unacceptable at groundwater contamination levels exceeding 0.1 µg/L.

IIIA 12.3.1 Screening for biological activity

According to EFSA conclusion on sulfoxaflor (2014), the 3 metabolites X11719474, X11579457 and X11519540 have no pesticidal activity.

IIIA 12.3.2 Screening for genotoxicity

All metabolites that have passed Stage 1 of Step 3 should be screened for their genotoxic activity.

The three metabolites of sulfoxaflor have been screened for genotoxic activity in the following *in vitro* studies as required by the guidance document: Ames test, gene mutation test with mammalian cells, and chromosome aberration test. The three sulfoxaflor metabolites were negative in all of these assays (see table below and refer to EFSA Conclusion, Appendix A – List of End Points). Therefore, these studies demonstrate the three sulfoxaflor metabolites to be non- mutagenic.

Study type	Species/ strain (sex)	Route/ method	Dose levels*	No-observed effect level (NOEL)	Effects at lowest observed effect level (LOEL)	Report ref.
Metabolite X11719474						
Ames	*S. typhimurium E. coli*	In vitro ± S9	0 - 5000 µg per plate	5000	Negative	Mecchi, 2008
Lymphocyte chromosome aberration	Rat/CD (male)	In vitro ± S9	0 - 2953 µg/ml	2953	Negative	Kleinert, 2008
HGPRT	Chinese hamster ovary	In vitro ± S9	0 - 2953 µg/ml	2953	Negative	Schisler, 2008
Metabolite X11579457						
Ames	*S. typhimurium E. coli*	In vitro ± S9	0 - 5000 µg per plate	5000	Negative	Dakoulas, 2010
Lymphocyte chromosome aberration	Rat/CD (male)	In vitro ± S9	0 – 2525 µg/ml	2525	Negative	Schisler, 2010
HGPRT	Chinese hamster ovary	In vitro ± S9	0 – 2525 µg/ml	2525	Negative	Schisler, 2010
Metabolite X11519540						
Ames	S. typhimurium E. coli	In vitro ± S9	0 - 5000 µg per plate	5000	Negative	Dakoulas, 2010
Lymphocyte chromosome aberration	Rat/CD (male)	In vitro ± S9	0 – 2540 µg/ml	2540	Negative	Schisler, 2010
HGPRT	Chinese hamster ovary	In vitro ± S9	0 – 2540 µg/ml	2540	Negative	Schisler, 2010

IIIA 12.3.3 Screening for toxicity

Additional testing of metabolites for toxicity is required if the parent active substance:

is classified as acutely or chronically toxic or very toxic (T followed by R25, R24, R23 or R48, or T+ followed by R28, R27, R26 or R39)

is classified for reproductive toxicity (any category with R60, R61, R62 or R63)

is classified as a category 1 or category 2 carcinogen (Carc. Cat. 1; R45) or (Carc. Cat. 2; R45).

Sulfoxaflor does not trigger any of the classifications listed above: it is not acutely or chronically toxic or very toxic, a reproductive toxin, or a carcinogen to humans.

However, key effects seen in mammalian toxicity testing species were:

- Acute toxicity (‘Harmful’, IIA 5.2)
- Systemic target organ toxicity to the liver, including tumours (IIA 5.3 and 5.5)

- Increased incidence and size of Leydig cell adenomas with secondary effects including preputial gland tumours (IIA 5.5)
- Developmental toxicity in the rat comprising abnormal foetuses and neonatal death (IIA 5.6)

These effects, together with structural similarity to the parent and predicted levels of potential human exposure, guided the toxicity testing for the three sulfoxaflor metabolites, as summarised in the table below:

Study Point	Study type	Species/ strain (sex)	Route/ method	Dose levels*	No-observed effect level (NOEL)	Effects at lowest observed effect level (LOEL)	Report ref.
Metabolite X11719474							
5.8.2.1/1	ADME probe	Rat/F344 (both)	Oral/ gavage	100 mkd	n/a	n/a	[redacted] 2010
5.8.2.2/1	Acute	Rat/F344 (female)	Oral/ gavage	300 mkd	300	n/a	[redacted] 2007
5.8.2.2/2	Acute	Rat/F344 (female)	Oral/ gavage	2000, 5000 mkd	2000	No death LD50 > 5000. Signs for 48 hours	[redacted] 2010
5.8.2.3/1	Acutes (3 studies)	Rat/F344 (female)	Dermal	1000 mkd	No death. Irritation for 48 hours	No death. Irritation for 48 hours	[redacted] 2007
		Rabbit/ NZW (female)	Eye	0.1 ml (0.069 g)	Conjunctivitis for 24 hours	Conjunctivitis for 24 hours	
5.8. 2.4/1	rLLNA	Mouse/ CBA/J (female)	Dermal	50%	Negative	Negative	[redacted] 2008
5.8.2.5/1	7-day palatability toxicity TK	Rat/F344 (both)	Oral/ diet	0, 125, 250, 500, 1000 mkd	250	↓palatability ↓BWG ↓TRIG ↑liver wt. with hypertrophy & eosinophilia	[redacted] 2010a
5.8.2.5/2	28-day	Rat/F344 (both)	Oral/ diet	0, 1000, 2000, 3000, 8000 ppm	2000 (♂167 mkd ♀184 mkd)	Liver hypertrophy & eosinophilia	[redacted] 2010b
5.8.2.5/3	90-day	Rat/F344 (both)	Oral/ diet	0, 500, 1000, 5000 ppm	1000 (♂65.3 mkd ♀71.8 mkd)	↑Cholesterol ↑liver wt. with hypertrophy, eosinophilia, fatty change & SCN	[redacted], 2010c
5.8. 2.5/4	Tolerability	Dog/ Beagle (male)	Oral/ gavage	50, 100 mkd	100	n/a	[redacted] 2009
5.8. 2.5/5	90-day	Dog/ Beagle (male)	Oral/ gavage	0, 10, 25, 50, mkd	50	n/a	[redacted] 2010b
5.8. 2.7/1	Liver mode-of-action	Rat/F344 (male)	Oral/diet	0 or 8000 ppm	n/a	n/a	[redacted] 2010

Study Point	Study type	Species/ strain (sex)	Route/ method	Dose levels*	No-observed effect level (NOEL)	Effects at lowest observed effect level (LOEL)	Report ref.
5.8.2.8/1	Reproduction screen (OECD 421)	Rat/CD (both)	Oral/diet	0, 1000, 2000, 5000 ppm	2000 (Toxicity ♂ 162 mkd ♀ >167 mkd)	↑liver wt. with hypertrophy	[redacted] 2010e
					5000 (Reproduction ♂ 396 mkd ♀ >451 mkd)	n/a	
5.8. 2.8/2	Developmental	Rat/CD (female)	Oral/diet	0, 1000, 2000, 5000 ppm	5000 (368 mkd)	n/a	[redacted] 2010f
Metabolite X11579457							
5.8.5.1/1	Acute	Rat/F344 (female)	Oral/ gavage	2000 mkd	n/a	No death. LD50 > 2000 Piloerection for 24 hours	[redacted] 2010
Metabolite X11519540							
5.5.6.1/1	ADME probe	Rat/F344 (male)	Oral/ gavage	2, 50 mkd	n/a	n/a	[redacted] 2011
5.8.6.2/1	Acute	Rat/F344 (female)	Oral/ gavage	320, 1000, 2000 mkd	n/a	LD50 = 565 No death at 320 Death at 1000 and 2000	[redacted] 2010
5.8.6.3/1	7-day palatability	Rat/F344 (both)	Oral/ diet	250, 500, 1000 mkd	n/a	↓palatability Body weight loss ↑liver wt. with hypertrophy, eosinophilia, very slight multifocal SCN (250 mkd)	[redacted] 2010
5.8.6.3/2	28-day	Rat/F344 (both)	Oral/ diet	0, 100, 300, 1000, 2000 ppm	n/a	↑liver wt. with hypertrophy, eosinophilia. Very slight increased nos. of mitotic figures (♂ only, 100 ppm)	[redacted] 2010 (101104)
5.8.6.3/3	90-day	Rat/F344 (both)	Oral/ diet	0, 5, 25, 100, 500 ppm	5 (males) 25 (females)	↑ liver wt. And histopathologic alterations	[redacted] 2011 (101195)

Study Point	Study type	Species/ strain (sex)	Route/ method	Dose levels*	No-observed effect level (NOEL)	Effects at lowest observed effect level (LOEL)	Report ref.
5.8.6.5/1	Liver mode-of-action	Rat/F344 (male)	Oral/diet	0, 100 ppm	n/a	n/a	[redacted] (2011)
5.8.6.6/1	nAChR agonism	Rat recombinant receptors	In vitro	0-3 mM	n/a	n/a	[redacted] 2011
5.8.6.6/2	Reproduction screen (OECD 421)	Rat/CD (both)	Oral/diet	0, 25, 100, 300 ppm	25 (parental) 300 (reproductive)	↑liver wt. with hypertrophy n/a	[redacted] 2011 (11040)

The comparative toxicity of the primary metabolite X11719474 with parent sulfoxaflor

The studies for X11719474 listed above provided the following key pieces of information for X11719474:

1. X11719474 is always significantly less toxic than the parent molecule, sulfoxaflor
2. It does not exhibit acute oral toxicity
3. It does not exhibit agonism at the fetal rat muscle nAChR (IIA 5.6.12/3) and does not cause developmental toxicity
4. It DOES have the same toxicity profile as sulfoxaflor and key properties for both molecules are: lack of genotoxicity, reduced palatability in feed, associated transient reductions in body weight gain, increased blood cholesterol and liver is the primary/only target organ, with the same liver effects by the same CAR activated mode of action (MoA).

These differences and similarities between X11719474 and the parent molecule are summarized in Table 12.3.3-1. This table compares the lowest observed effect level (LOEL) that caused an effect for both molecules, which was considered the easiest, most transparent and reliable way to determine the difference in relative toxicity (i.e., a relative potency factor (RPF)) for each effect.

In many cases, X11719474 does not produce the effect caused by sulfoxaflor at the highest dose levels tested; examples include absence of acute death even at 5000 mg/kg bw, absence of any effects in dogs, and absence of effects on foetal development and neonatal survival. In all of these cases, the difference in toxicity is at least a factor of 5, and by a significant margin. For example, for acute toxicity, the RPF was >5.0 based on death for sulfoxaflor versus no effects for X11719474; for toxicity in dogs the RPF of >5.0 was based on exceedance of a maximum tolerated dose (MTD) over a few days of treatment for sulfoxaflor versus no effects in 90 days of treatment for X11719474, and for developmental toxicity the RPF was >5.2 based on effects in 100% of sulfoxaflor litters versus 0% of X11719474 litters. Finally, for neonatal survival, even the RPF of >11.4

underestimated the true RPF value as it was based on a clear (~19%) loss of pups for sulfoxaflor versus 0% loss for X11719474. Interestingly, it was already known that X11719474 did not bind to the insect nAChR (Young, 2009) and based on structure it seemed highly unlikely that it would bind to or result in agonism at the rat fetal muscle nAChR (agonism at which had been shown to be responsible for the developmental effects of sulfoxaflor in rats). It was confirmed experimentally that indeed X11719474 did not cause agonism at the fetal rat muscle nAChR (IIA 5.6.12/3).

In all other cases, X11719474 produced the same effects as sulfoxaflor but always at higher dose levels. For the most sensitive (only) clinical chemistry effect, increased blood cholesterol, the difference in toxicity from the 28-day rat study is at least 8.3 but this is a significant underestimate of the true margin of difference because the increase caused by X11719474 is only approximately half of that caused by sulfoxaflor. For liver effects, the 90-day rat study provides the most robust and precise comparison of relatively toxicity as the extent of the effects are very similar for both molecules and the study design comprises larger group sizes (n = 10) than most other studies. For example, the relative liver weight increases are the same with a relative potency factor of 7. This value is also supported by hepatocellular hypertrophy in females, while a slightly higher value would be supported by hypertrophy and other changes more indicative of toxicity per se, comprising fatty change (vacuolation), single cell necrosis and aggregates of macrophages (90-day rat).

In summary, a comprehensive set of toxicology studies has been conducted on the metabolite, X11719474, that was developed as a consequence of the toxicity of the parent molecule, sulfoxaflor. X11719474 was consistently less toxic than sulfoxaflor and does not have acute or developmental toxicity because of its known absence of agonism to the rat muscle nicotinic acetylcholine receptor, which is the mode-of-action (MoA) by which sulfoxaflor causes these effects, as described in detail elsewhere in the sulfoxaflor dossier (IIA 5.6 and 5.11). The most reliable indicators of the relative toxicity of X11719474 are effects on the primary systemic target organ, the liver, in the rat 90-day study. For all liver effects – liver weight increase, hepatocellular hypertrophy, fatty change (vacuolation), single cell necrosis and aggregates of macrophages – the difference in toxicity between X11719474 and sulfoxaflor is consistently a factor of 7, i.e., X11719474 is approximately 7 times less toxic than sulfoxaflor. In conclusion, for use in human health risk assessment, the proposed RPF for X11719474 is 7.

Table 12.3.3-1: Summary of relative toxicity of X11719474 compared to sulfoxaflor

Study Type	Parameter	Sex	Sulfoxaflor X		11719474		Relative Potency Factor (RPF)
			Dose (mkd)	Effect (% of control*)	Dose (mkd)	Effect (% of control*)	
Acute oral rat	LD50	♀	1000	LD50	>5000	NE	> 5.0
	Lowest Lethal Dose	♀	1000	LLD	>5000	NE	> 5.0
28-day rat	Body Wt. Gain	♂	155.0	91.5	662.0	NE	4.3
	Body Wt. Gain	♀	170.0	90.0	734.0	NE	4.3
	Relative Liver Wt.	♂	79.4	128.5	662.0	136.6	8.3
	Relative Liver Wt.	♀	88.3	107.7	734.0	118.0	8.3
	Cholesterol	♂	24.8	145.1	662.0	125.4	26.7
	Cholesterol	♀	88.3	140.5	734.0	121.6	8.3
	Hypertrophy:						
	% Slight	♂	79.4	40.0	662.0	100.0	8.3
	% Moderate	♂	155.0	60.0	662.0	NE	> 4.3
	% Very Slight	♀	88.3	60.0	734.0	100.0	8.3
	Vacuolation:						
	% Very Slight	♂	79.4	40.0	662.0	NE	> 8.3
	% Slight	♂	79.4	60.0	662.0	NE	> 8.3
	% Very Slight	♀	170.0	20.0	734.0	NE	> 4.3
	Single Cell Necrosis:						
	% Very Slight	♂	155.0	20.0	662.0	NE	> 4.3
90-day rat	Body Wt. Gain	♂	47.6	89.5	327.0	NE	6.9
	Body Wt. Gain	♀	101.0	80.5	352.0	NE	3.5
	Relative Liver Wt.	♂	47.6	114.1	327.0	115.6	6.9
	Relative Liver Wt.	♀	51.6	107.5	352.0	106.7	6.8
	Cholesterol	♂	94.9	227.1	327.0	117.5	3.4
	Cholesterol	♀	101.0	183.1	352.0	118.5	3.5
	Hypertrophy:						
	% Slight	♂	47.6	70.0	327.0	100.0	6.9
	% Moderate	♂	47.6	30.0	327.0	NE	> 6.9
	% Very Slight	♀	51.6	90.0	352.0	90.0	6.8
	% Slight	♀	101.0	50.0	352.0	NE	> 3.5
	Vacuolation:						
	% Very Slight	♂	47.6	60.0	327.0	70.0	6.9
	% Slight	♂	47.6	40.0	327.0	NE	> 6.9
	% Moderate	♂	94.9	50.0	327.0	NE	> 3.4
	Single Cell Necrosis:						
	% Very Slight	♂	47.6	80.0	327.0	70.0	6.9
	% Slight	♂	47.6	20.0	327.0	NE	> 6.9
	% Very Slight	♀	51.6	30.0	352.0	NE	> 6.8

Study Type	Parameter	Sex	Sulfoxaflor X		11719474		Relative Potency Factor (RPF)
			Dose (mkd)	Effect (% of control*)	Dose (mkd)	Effect (% of control*)	
	Aggregates of Macrophages:						
	% Slight	♂	47.6	30.0	327.0	NE	> 6.9
	% Slight	♀	101.0	20.0	352.0	NE	> 3.5
90-day dog	Body Wt.	♂	10.0	11.1	50.0	NE	>5
	Body Wt.	♀	10.0	11.8	50.0	NE	>5
	Feed Consumption	♂	10.0	65.3	50.0	NE	>5
	Feed Consumption	♀	10.0	84.9	50.0	NE	>5
Reproduction rat	Pup Survival PND 1 (%)	Both	39.5	94.5	451.0	NE	> 11.4
	Pup Survival PND 4 (%)	Both	39.5	81.2	451.0	NE	> 11.4
	Pup Body Wt. PND 1	♂	78.2	75.0	451.0	NE	> 5.8
	Pup Body Wt. PND 1	♀	78.2	78.0	451.0	NE	> 5.8
Developmental rat	Fetal Body Wt.	Both	70.2	88.4	368.0	NE	> 5.2
	% Abnormal Fetuses	Both					
	External		70.2	75.6	368.0	NE	> 5.2
	Visceral		70.2	12.8	368.0	NE	> 5.2
	Skeletal		70.2	30.1	368.0	NE	> 5.2
	% Abnormal Litters	N/A					
	External		70.2	100.0	368.0	NE	> 5.2
	Visceral		70.2	29.0	368.0	NE	> 5.2
	Skeletal		70.2	70.8	368.0	NE	> 5.2

N/A = Not applicable; NE = No treatment-related effect; * Except when indicated under 'Parameter'

The comparative toxicity of other metabolites with sulfoxaflor and X11719474

X11579457: The second metabolite of interest from a toxicology perspective was X11579457. X11579457 is a soil metabolite and is most closely related to X11719474, with a primary imine as opposed to a urea group attached to the trifluoromethylpyridine side chain.

For X11579457 the strategy was to comprehensively investigate genotoxic potential via three *in vitro* studies (Ames, HGPRT and RLCAT) and to compare acute oral toxicity in the rat. The result of the *in vitro* genotoxicity tests were unequivocally negative. It had no acute oral toxicity at a limit dose of 2000 mg/kg body weight. In these limited respects, it is the same as X11719474.

X11519540: The third metabolite of interest from a toxicology perspective was X11519540. X11519540 was found in soil and animals and is most closely related to X11579457, being a sulfone versus a sulfoximine metabolite of X11719474. Also,

X11519540 is an impurity in the technical grade active ingredient of sulfoxaflor and is therefore present in the toxicology studies conducted using the TGAI. The table below summarises X11519540 concentrations in the TGAI sulfoxaflor toxicology batches (taken from J-IIA):

- from Report FOR-10-25, analyzed concurrently with the pilot-scale samples -

Substance Content	**(g/kg)**			
	TSN105885	TSN106108	TSN003725-0001*	**Method**
Sulfoxaflor	987	971	956	HPLC/UV[3]
(diastereomer ratio -A/B)[1]	(35 /65)	(38 / 62)	(50 / 50)	-
A2	nd	0.4	0.3	HPLC/UV[3]
X11719474	4.4	3.4	nd	HPLC/UV[3]
A5 [2]	0.5	0.2	nd	HPLC/UV[3]
A1	1.3	0.1	nd	HPLC/UV[3]
X11519540 3.2		**20.0**	**33.8**	**HPLC/UV[3]**
B2	0.3	0.2	nd	HPLC/UV[3]
C2	nd	0.3	nd	HPLC/UV[3]
X12082275	nd	0.3	0.6	HPLC/UV[3]
C1	nd	nd	nd	HPLC/UV[3]
D1	nd	nd	nd	HPLC/UV[3]
D3	nd	nd	nd	HPLC/UV[3]
D2	nd	nd	nd	HPLC/UV[3]
E1	nd	nd	nd	HPLC/UV[3]
E5	nd	0.1	nd	HPLC/UV[3]
E2	nd	0.4	nd	HPLC/UV[3]
E3	nd	0.3	nd	HPLC/UV[3]
E4	nd	0.3	nd	HPLC/UV[3]
I1	nd	0.6	1.0	HPLC/UV[3]
Water	0.6	0.2	0.4	Karl Fischer
Isopropanol	<LOQ	<LOD	<LOD	GC/FID[4]
Acetonitrile	<LOD	<LOD	<LOD	GC/FID[4]
Mass Balance %	997	998	992	___

nd = not detected;
LOQ: 0.37 g/Kg isopropanol, 0.38 g/Kg acetonitrile; X11719474, X11519540, and X12082275 = 0.3 g/Kg;
[1]from peak area data in batch analysis report
[2]different component observed by LC/MS/MS in batch TSN003725-0010 (labeled as A5b in MS data)
[3] DAS-AM-G-10-16
[4] DAS-AM-G-10-9
Batch TSN105885 was used in probe toxicology studies. Batches TSN106108 and TSN003725-0001 were used in the studies that determined the key toxicological end-points and the purity and impurity profiles shown below indicate they are very similar to each other
* Tox batch

For X11519540 the strategy was to comprehensively investigate genotoxic potential via three *in vitro* studies (Ames, HGPRT and RLCAT) and to compare acute and repeat dose oral toxicity in the rat. It soon became clear from the 28-day dietary toxicity study in rats that X11519540 was more toxic than sulfoxaflor and X11719474. Because of this, and because at the time the potential for human exposure was still being determined, additional studies were included in the X11519540 toxicology programme. These additions were designed to enable a comparison to be made with the key sulfoxaflor effects (systemic target organ toxicity to the liver, including tumours, developmental toxicity in the rat comprising abnormal foetuses and neonatal death) and were designed to enable a human dietary risk assessment to be conducted, if this was deemed necessary.

These effects helped to determine the studies required for X11519540, which are summarized in Table 12.3.3-2. This table compares the lowest observed effect level (LOEL) that caused an effect for both molecules, which was considered the easiest, most transparent and reliable way to determine the difference in relative toxicity (i.e., a relative potency factor (RPF)) for each effect.

These studies provided the following key pieces of information for X11519540:

1. X11519540 is more toxic than the parent molecule, sulfoxaflor, and X11719474
2. It exhibits similar acute oral toxicity to sulfoxaflor
3. It does not exhibit agonism at the fetal rat muscle nAChR and does not cause developmental toxicity
4. It DOES have the same toxicity profile as sulfoxaflor and key properties for both molecules are: lack of genotoxicity, reduced palatability in feed, associated transient reductions in body weight gain, increased blood cholesterol and liver is the primary target organ, with the same liver effects by the same CAR-mediated mode of action (MoA).

Table 12.3.3-2: Summary of relative toxicity of X11519540 compared to sulfoxaflor

Study Type	Parameter	Sex	Sulfoxaflor X		11519540		Relative Potency Factor (RPF)
			Dose (mkd)	Effect (% of control*)	Dose (mkd)	Effect (% of control*)	
Acute oral rat	LD50	♀	1000	LD50	565	LD50	1.8
	Lowest Lethal Dose	♀	1000	LLD	1000	LLD	1
28-day rat	Body Wt. Gain	♂	155	91.5	74	82.6	2.1
	Body Wt. Gain	♀	170	90	77.2	83	2.2
	Relative Liver Wt.	♂	79.4	128.5	7.72	130.6	10

Relative Liver Wt.	♀	88.3	107.7	8.48	125.7	10
Cholesterol	♂	24.8	145.1	7.72	122	3.2
Cholesterol	♀	88.3	140.5	8.48	126.6	10
Liver Hypertrophy:						
% V. Slt - Slight	♂	79.4	40	7.72	100	10.3
% Moderate	♂	155	60	74	20	2.1
% Very Slight	♀	88.3	60	8.48	100	10.4
% Slight	♀	170	NE	24.9	100	6.8
% Moderate	♀	170	NE	77.2	20	2.2
Liver Vacuolation:						
% Very Slight	♂	79.4	40	140	NE	0.6
% Slight	♂	79.4	60	140	NE	0.6
% Very Slight	♀	170	20	152	NE	1.1
Liver SC Necrosis:						
% Very Slight	♂	155	20	23.1	60	6.7
% Very Slight	♀	170	NE	24.9	80	6.8
Liver Necrosis w/ Inflammation:						
% Very Slight	♂	155	NE	74	40	2.1
% Very Slight	♀	170	NE	77.2	40	2.2
Kidney Tubule Degeneration:						
% Very Slight	♂	155	NE	23.1	80	6.7
% Very Slight	♀	170	NE	152	40	1.1
Thyroid Gland:						
% Very Slight	♂	155	NE	7.72	40	20.1
% Slight	♂	155	NE	74	40	2.1
% Very Slight	♀	170	NE	77.2	80	2.2

	Body Wt. Gain	♂	47.6	89.5	30.2	91.1	1.6
	Body Wt. Gain	♀	101	80.5	32.2	73.5	3.1
	Relative Liver Wt.	♂	47.6	114.1	5.92	126.6	8.0
	Relative Liver Wt.	♀	51.6	107.58	6.68	119.5	7.7
	Cholesterol	♂	94.9	227.1	5.92	132.2	16.0
	Cholesterol	♀	101	193.1	6.68	115.5	15.1
	Liver Hypertrophy:						
	% V. Slt - Slight	♂	47.6	70	1.5	60	31.7
	% Moderate	♂	47.6	30	30.2	100	1.6
	% Very Slight	♀	51.6	90	6.68	100	7.7
	% Slight	♀	101	50	32.2	100	3.1
	Liver Vacuolation:						
	% V. Slt - Slight	♂	47.6	100	1.5	80	31.7
90-day rat	% Moderate	♂	94.9	50	30.2	NE	< 3.1
	% Very Slight	♀	101	NE	6.68	10	15.1
	Liver SC Necrosis:						
	% Very Slight	♂	47.6	80	30.2	NE	< 1.6
	% Slight	♂	47.6	20	30.2	NE	< 1.6
	% Very Slight	♀	51.6	30	6.68	100	7.7
	% Slight	♀	101	NE	32.2	10	3.1
	Aggregates of Macrophages:						
	% Slight	♂	47.6	30	30.2	NE	< 1.6
	% Slight	♀	101	20	32.2	NE	< 3.1
	Adrenal Hypertrophy:						
	% Very Slight	♂	94.9	NE	30.2	100	3.1
	% Very Slight	♀	101	NE	32.2	100	3.1

	Adrenal Vacuolation:						
	% Very Slight	♂	94.9	NE	30.2	40	3.1
	% Slight	♂	94.9	NE	30.2	60	3.1
	% Very Slight	♀	101	NE	6.68	60	15.1
	Kidney - Nephropathy, bilateral						
	% Very Slight	♂	94.9	NE	30.2	40	3.1
	% Slight	♂	94.9	NE	32.2	60	2.9
	Thyroid Gland: Hypertrophy						
	% Very Slight	♂	94.9	NE	5.92	40	16.0
	% Very Slight	♀	101	NE	32.2	40	3.1
Reproduction Rat	Pup Survival PND 1 (%)	Both	39.5	94.5	23.4	NE	>1.7
	Pup Survival PND 4 (%)	Both	39.5	81.2	23.4	NE	>1.7
	Pup Body Wt. PND 1	♂	78.2	75	23.4	NE	>3.3
	Pup Body Wt. PND 1	♀	78.2	78	23.4	NE	>3.3

N/A = Not applicable; NE = No treatment-related effect; * Except when indicated under 'Parameter'

X11519540 had similar acute oral toxicity than sulfoxaflor with an LD50 of 566 mg/kg body weight in female F344 rats (sulfoxaflor LD50 = 1000 mg/kg).

X11519540 did not produce one of the major effects sulfoxaflor did, absence of effects on foetal development and neonatal survival, even at the highest dose levels tested. Interestingly, it is known that X11519540 does not bind to the insect nAChR (Watson and Young, 2010) and based on structure it seemed highly unlikely that it would bind to or result in agonism at the rat fetal muscle nAChR (agonism at which had been shown to be responsible for the developmental effects of sulfoxaflor in rats). It was confirmed experimentally that indeed X11519540 did not cause agonism at the fetal rat muscle nAChR (IIA 5.8.6.6/1).

In all other cases, X11519540 produced the same effects as sulfoxaflor but always at lower dose levels. For the most sensitive (only) clinical chemistry effect, increased blood cholesterol, the difference in toxicity from the 28-day and 90-day rat studies is 3.3- to 16-fold for males and 10- to 15.1-fold for females. For the liver weight increase the difference in toxicity is 7.7- to 10-fold for both sexes. This value is also supported by hepatocellular hypertrophy in females, while lower RPF values would be supported by other changes more indicative of toxicity per se, comprising single cell necrosis (RPF =

1.6 to 7.7) and necrosis with inflammation (RPF = 2.1-2.2, 28-day study only) (Table 12.3.3-2). Because the increased liver weight has been shown to be due partly to hypertrophy (relevant to humans) and partly to hyperplasia (not relevant to humans), it is considered that an RPF of 10 based on the liver weight endpoint alone would be overly conservative. Therefore, it is proposed that an RPF of 7 would be more appropriate for liver effects, based on single cell necrosis.

The other X11519540 treatment-related effects were increased incidence of very slight multifocal degeneration of tubules in the kidneys, which occurred in males and females and very slight or slight diffuse follicular cell hypertrophy of the thyroid gland in males, and very slight diffuse hypertrophy of the thyroid gland in females. The thyroid hypertrophy may have been caused by a treatment-related induction of liver microsomal enzymes responsible for biliary excretion of thyroid hormones, with resultant stimulation of the thyroid by thyroid stimulating hormone through disruption of the hypothalamic – pituitary – thyroid axis. Both of these effects are considered to be a result of the higher potency of X11519540, rather than a different MoA. A likely explanation for X11519540's higher potency compared to parent comes from the 28-day rat study where toxicokinetic data show that X11519540 has a much longer half-life than parent (24-35 hours compared to 4-8 hours, respectively). In the case of kidney tubule degeneration and the thyroid follicular cell hypertrophy, the RPFs generated when compared to parent are not considered relevant because the effects were not evident with parent. This is because both of these effects are considered to be a result of the higher potency of X11519540, rather than a MoA different to the parent.

In summary, a comprehensive set of toxicology studies has been conducted on the metabolite, X11519540, that was developed as a consequence of the toxicity of the parent molecule, sulfoxaflor. X11519540 has approximately 2-fold higher acute oral toxicity than sulfoxaflor. It does not have developmental toxicity because of its known absence of agonism to the rat muscle nicotinic acetylcholine receptor, which is the mode-of-action (MoA) by which sulfoxaflor causes these effects, as described in detail elsewhere in this dossier (IIA 5.6). The primary target organ effects (i.e., liver effects) elicited by sulfoxaflor are present when rats are exposed to X11519540. Additional effects/target organs are likely purely a result of the higher potency of X11519540 rather than representing a different MoA. A likely explanation for the higher potency of X11519540 compared to parent comes from the 28- and 90-day rat studies where toxicokinetic analysis of the plasma levels of X11519540 were included. This shows that X11519540 has a much longer half-life than parent (24-36 hours compared to 4-8 hours, respectively). The most reliable indicators of the relative toxicity of X11519540 are effects on the primary systemic target organ, the liver, in the rat 28-day study. It is therefore proposed that an RPF 7 would be most appropriate for liver effects, i.e., X11519540 is approximately 7 times more toxic than sulfoxaflor.

In conclusion, the extensive tox icity te sting undertaken for these three sulfox aflor metabolites indicate that they would not be classified:

as acutely or chronically toxic or very toxic (T followed by R25, R24, R23 or R48, or T+ followed by R28, R27, R26 or R39)

for reproductive toxicity (any category with R60, R61, R62 or R63)

as a category 1 or category 2 carcinogen (C arc. Cat. 1; R45) or (Carc. Cat. 2; R45).

Therefore, these meta bolites sho uld not be considered relevant. Refer to EFSA Conclusion, Point 6.2 (page 16) fo r confirma tion that the three meta bolites are not toxicologically relevant.

IIIA 12.4 Exposure assessment – threshold of concern approach

The three metabolites of sulfoxaflor will have PECgw's of >0.75 µg/L and therefore required a refined risk assessment, as presented in Section IIIA 12.5 below.

IIIA 12.5 Refined risk assessment for non-relevant metabolites

A refined risk assessment is required for non-relevant metabolites that are predicted to occur between 0.75-10 µg/L in groundwater.

A refined risk assessment has been conducted for X11719474, X11579457 and X11519540 considering that these metabolites can potentially occur up to 8.38 µg/l, 5.92 µg/l and 3.74 µg/l respectively.

1. **X11719474:**

X11719474 is not considered relevant in terms of toxicological properties in accordance with EFSA conclusions (2014). Furthermore, it appears to be less toxic than sulfoxaflor in acute and short term studies. The toxicological reference values of the parent compound can therefore be used for X11719474 (EFSA, 2014) (0.04 mg/kg bw/day).

For the adult (60 kg, consuming 2 L water/day), the predicted intake of X11719474 is as follows:

= 8.38 µg/L PECgw × 2 L water/day ÷ 60 kg body weight

= 0.28 µg/kg bw/day ≡ **0.00028 mg/kg bw /day which correspond to 0.00031 mg/kg bw /day in sulfoxaflor equ ivalent** (considering the molar masses of sulfoxaflor (277.3 g/mol) and metabolite X11719474 (295 g/mol) a factor of 0.94 has been applied to the predicted intake of X11719474).

The predicted intake of 0.00026 mg/kg bw /day for an adult represents 0.65% of the sulfoxaflor ADI (0.04 mg/kg bw/day)

For the child (10 kg consuming 1 L water/day), the predicted intake of X11719474 is as follows:

= 8.38 µg/L PECgw × 1 L water/day ÷ 10 kg body weight

= 0.84 µg/kg bw/day ≡ **0.00084 mg/kg bw /day which correspond to 0.00079 mg/kg bw/day in sulfoxaflor equivalent**

The predicted intake of 0.0079 mg/kg bw /d for a child represen ts 1.98% of the sulfoxaflor ADI (0.04 mg/kg bw/day)

For the infant (5 kg consuming 0.75 L water/day), the predicted intake of X11719474 is as follows:

= 8.38 µg/L PECgw × 0.75 L water/day ÷ 5 kg body weight

= 1.26 µg/kg bw/day ≡ **0.00126 mg/kg bw /day which correspond to 0.0012 mg/kg bw/day in sulfoxaflor equivalent**

The predicted intake of 0.0015 mg/kg bw /d for the infant represents 3% of the sulfoxaflor ADI (0.04 mg/kg bw/day)

The consumer risk assessment demonstrates an acceptable risk. The predicted human intake represents 0.65% of the sulfoxaflor ADI (adult), 1.98% of the sulfoxaflor ADI (child) and 3 % (infant) of the sulfoxaflor ADI.

2. **X11579457:**

X11579457 is not considered relevant in terms of toxicological properties in accordance with EFSA conclusions (2014). Furthermore, it is structurally close related to X11719474, therefore the toxicological reference values of the parent compound can be used for X11579457 (EFSA, 2014) (0.04 mg/kg bw/day).

For the adult (60 kg, consuming 2 L water/day), the predicted intake of X11719457 is as follows:

= 5.92 µg/L PECgw × 2 L water/day ÷ 60 kg body weight

= 0.2 µg/kg bw/day ≡ **0.0002 mg/kg bw /day which correspond to 0.00022 mg/kg bw /day in sulfoxaflor equ ivalent** (considering the molar masses of sulfoxaflor (277.3 g/mol) and metabolite X11579457 (252.25 g/mol) a factor of 1.09 has been applied to the predicted intake of X11579457).

The predicted intake of 0.00022 mg/kg bw /day for an adult represents 0.55% of the sulfoxaflor ADI (0.04 mg/kg bw/day)

For the child (10 kg consuming 1 L water/day), the predicted intake of X11579457 is as follows:

= 5.92 µg/L PECgw × 1 L water/day ÷ 10 kg body weight

= 0.59 µg/kg bw/day ≡ **0.00059 mg/kg bw /day which correspond to 0.00064 mg/kg bw/day in sulfoxaflor equivalent.**

The predicted intake of 0.00064 mg/kg bw /d for a child represen ts 1.6% of the sulfoxaflor ADI (0.04 mg/kg bw/day)

For the infant (5 kg consuming 0.75 L water/day), the predicted intake of X11579457 is as follows:

= 5.92 µg/L PECgw × 0.75 L water/day ÷ 5 kg body weight

= 0.89 µg/kg bw/day = **0.00089 mg/kg bw /day which correspond to 0.00097 mg/kg bw/day in sulfoxaflor equivalent.**

The predicted intake of 0.00097 mg/kg bw /d for an infant represents 2.43% of the sulfoxaflor ADI (0.04 mg/kg bw/day)

The consumer risk assessment demonstrates an acceptable risk for metabolite X11579457. The predicted human intake represents 0.55% of the sulfoxaflor ADI (adult), 1.6% of the sulfoxaflor ADI (child) and 2.43 % (infant) of the sulfoxaflor ADI.

3. **X11519540:**

X11519540 is not considered relevant in terms of toxicological properties but shows higher potency than sulfoxaflor (but no agonistic activity towards the nicotinic AChR) (EFSA 2014). The reference values of sulfoxaflor cannot be applied for X11519540.

Based on the same data package available for the approval of the active substance, the use of a RPF of 10 as proposed by the RMS in the DAR was not discussed at EU level and the conclusion was that the reference values of sulfoxaflor cannot be applied for the metabolite. An ADI can be derived based on the 90-day rat study performed on X11519540. Applying a safety factor of 1000 (to account for the extrapolation from subchronic to chronic) to the NOAEL of 1.5 mg/kg bw/day determined in the 90-d rat study (liver effects), the proposed ADI for X11519540 is 0.0015 mg/kg bw/d. (It is to be noted that this ADI is equal to the TTC value used for non-genotoxic substances).

For the adult (60 kg, consuming 2 L water/day), the predicted intake of X11519540 is as follows:

= 3.74 µg/L PECgw × 2 L water/day ÷ 60 kg body weight

= 0.12 µg/kg bw/day ≡ 0.00012 mg/kg bw/day

The predicted intake of 0.00012 mg/kg bw /d for the adult represents 8 % of the proposed X11519540 ADI (0.0015 mg/kg bw/day)

For the child (10 kg consuming 1 L water/day), the predicted intake of X11519540 is as follows:

= 3.74 µg/L PECgw × 1 L water/day ÷ 10 kg body weight

= 0.374 µg/kg bw/day ≡ **0.000374 mg/kg bw/day**

The predicted intake of 0.000374 mg/kg bw /d for the child represen ts 24.9% of the proposed X11519540 ADI (0.0015 mg/kg bw/day)

For the infant (5 kg consuming 0.75 L water/day), the predicted intake of X11519540 is as follows:

= 3.74 µg/L PECgw × 0.75 L water/day ÷ 5 kg body weight

= 0.56 µg/kg bw/day ≡ **0.00056 mg/kg bw/day**

The predicted intake of 0.00056 mg/kg bw /d for the infant represen ts 37.3% of the proposed ADI for X11519540 (0.0015 mg/kg bw/d)

The consumer risk assessment demonstrates an acceptable risk for metabolite X11519540. The predicted human intake represents 8% of the proposed X11519540 ADI (adult), 24.9% of the X11519540 ADI (child) and 37.3 % of the X11519540 ADI (infant).

Conclusion:

Based on (1) a lack of biological activity of the metabolites, (2) the overall lack of genotoxic potential with these metabolites, and (3) the lack of toxicological properties of the metabolites that would require classification and labeling as toxic or very toxic, a reproductive toxin, or a carcinogen, the metabolites can be considered as not relevant under the Step 3 criteria.

The metabolites are also considered acceptable under the Step 5 criteria.