SULFOXAFLOR

First draft prepared by
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Explanation

Sulfoxaflor is the International Organization for Standardization (ISO)–approved name for [methyl(oxo){1-[6-(trifluoromethyl)-3-pyridyl]ethyl}-λ^6-sulfanylidene]cyanamide (International Union of Pure and Applied Chemistry [IUPAC]) (Chemical Abstracts Service No. 946578-00-3), a novel insecticide from the sulfoximine class. Sulfoxaflor contains two chiral centres (the sulfur atom and the

carbon atom attached to position 3 of the pyridine ring) and is a mixture of the four possible stereoisomers. Both (*E*)- and (*Z*)-isomers (involving the S=N double bond and the cyano group) exist, but they rapidly interconvert at ambient temperatures. Sulfoxaflor is effective against a wide range of sap-feeding insects and exerts its insecticidal activity as an agonist at the insect nicotinic acetylcholine receptor (nAChR), which plays a central role in the mediation of fast excitatory synaptic transmission in the insect central nervous system. Sulfoxaflor has not been evaluated previously by the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) and was reviewed at the present Meeting at the request of the Codex Committee on Pesticide Residues.

All pivotal studies were certified as complying with good laboratory practice (GLP) or an approved quality assurance programme.

Evaluation for acceptable daily intake

Unless otherwise stated, the studies evaluated in this monograph were performed by laboratories that were certified for GLP and complied with the relevant Organisation for Economic Co-operation and Development (OECD) test guideline(s) or similar guidelines of the European Union or United States Environmental Protection Agency. As these guidelines specify the clinical pathology tests normally performed and the tissues normally examined, only significant exceptions to these guidelines are reported here, to avoid repetitive listing of study parameters.

The purity of the test substance used in the majority of toxicological studies was determined to be at least 95.6% weight per weight (w/w), comprising the (*E*)- and (*Z*)-diastereomers in a 50.5:49.5 ratio, by high-performance liquid chromatography.

1. Biochemical aspects

1.1 Absorption, distribution and excretion

In a pilot study conducted to determine the absorption, distribution, metabolism and elimination of sulfoxaflor in F344/DuCrl rats and Crl:CD1(ICR) mice, one male and one female rat and two male and two female mice received ^{14}C-labelled sulfoxaflor (purity 98.3%; radiochemical purity > 97%) as a 0.5% methylcellulose suspension by oral gavage at a single dose of 100 mg/kg body weight (bw); the dose volume was 5 ml/kg bw. Time-course blood samples (at 0.25, 0.5, 1, 2, 4, 8, 12, 24, 48 and 72 hours post-dosing) and excreta were collected from rats, whereas only excreta were collected from mice up to 72 hours post-administration. The urine samples collected during the first 12 hours post-dosing were analysed for parent compound and metabolites. Additionally, two rats (one male, one female) and four mice (two males, two females) were orally dosed with ^{14}C-labelled sulfoxaflor, and plasma was collected at the time of peak concentration, or C_{max} (T_{max}: 2 hours post-dosing for rats and 1.5 hours post-dosing for mice), and analysed for parent and metabolites.

The orally administered ^{14}C-labelled sulfoxaflor was rapidly absorbed from the gastrointestinal tract in both rats and mice. The plasma C_{max} of radiolabelled material in male and female rats was 64 and 66 µg/g, respectively, which was reached 2 hours after the administration of the dose (Table 1). The C_{max} for red blood cells was 57 and 60 µg/g in male and female rats, which was reached in 1 and 2 hours, respectively. Elimination of radioactivity from plasma and red blood cells was slightly slower from male rats than from female rats, corresponding to a half-life during the terminal β phase ($t_{½β}$) of 9 and 11 hours (male) and 7 and 8 hours (female) for plasma and red blood cells, respectively. Faster elimination of the radioactivity by female rats was also reflected in 1.2-fold greater total body clearance (Cl), which was 129 and 141 ml/kg bw per hour from male plasma and red blood cells and 159 and 175 ml/kg bw per hour from female plasma and red blood cells, respectively. Similarly, the mean

Table 1. Summary of toxicokinetic parameters in the plasma of rats following oral administration of ^{14}C-labelled sulfoxaflor at 100 mg/kg bw

Parameter	Males	Females
T_{max} (h)	2	2
C_{max} (µg/g)	63.6	66.1
$t_{½β}$ (h)	8.8	7.2
$AUC_{0→t}$ (µg·h/g)	817	611
$AUC_{0→∞}$ (µg·h/g)	819	663
Cl (ml/kg bw per hour)	129	159
MRT (h)	12.7	10.4

From Saghir, Clark & McClymont (2008)
AUC, area under the curve; Cl, clearance; C_{max}, peak concentration; MRT, mean residence time; $t_{½β}$, half-life during the terminal β phase; T_{max}, time to reach C_{max}

Table 2. Recovery of radioactivity in excreta and blood in rats and mice 72 hours post-dosing with ^{14}C-labelled sulfoxaflor at 100 mg/kg bw

	Recovery of radioactivity (% of administered dose)			
	Rats		Mice	
	Males	Females	Males	Females
Expired carbon dioxide	0.02	0.04	ND	ND
Plasma/red blood cells	0	0	0	0
Urine/rinse	98.05	87.24	84.69	79.80
Faeces	4.62	4.89	12.60	13.00
Total	102.69	92.16	97.29	92.80

From Saghir, Clark & McClymont (2008)
ND, not determined

residence time (MRT) of ^{14}C-labelled sulfoxaflor in the male rat was between 2 and 5 hours longer than in the female rat. The slower elimination and longer MRT of ^{14}C-labelled sulfoxaflor in the male rat resulted in 1.2-fold greater areas under the curve ($AUC_{0→∞}$: 819 and 663 µg·h/g for plasma and 751 and 603 µg·h/g for red blood cells).

Urinary elimination was 57–68% and 27–50% within 12 hours after dosing and increased to 77–83% and 66–70% of the administered dose within 24 hours for rats and mice, respectively. In total, 87–98% and 80–85% of the administered ^{14}C-labelled sulfoxaflor were recovered in the urine of rats and mice, respectively, within 72 hours post-dosing (Table 2). Faecal elimination accounted for only 5% in rats and 13% in mice, most likely representing unabsorbed sulfoxaflor, due to its recovery in faeces within the gastrointestinal transit time of 24 hours.

No metabolites of the test material were found in plasma following a single oral dose of ^{14}C-labelled sulfoxaflor to rats or mice, whereas trace levels of the urea metabolite (X11719474) were found in urine samples at levels comparable with its concentration as an impurity in the test material (≤ 0.44% of parent) (Saghir, Clark & McClymont, 2008).

In a study on toxicokinetics and metabolism conducted according to OECD test guideline 417, groups of four male and four female F344/DuCrl rats received sulfoxaflor-2-^{14}C-pyridine (purity 95.6%; radiochemical purity 99.7%) as a 0.5% methylcellulose suspension by oral gavage at a single dose of 5 or 100 mg/kg bw (groups 1 and 2) or repeated doses of 5 mg/kg bw (group 3; 14 daily doses

Table 3. Summary of toxicokinetic parameters in the plasma of rats following oral or intravenous administration of ^{14}C-labelled sulfoxaflor

Parameter	5 mg/kg bw, oral		100 mg/kg bw, oral		5 mg/kg bw, intravenous	
	Males	Females	Males	Females	Males	Females
T_{max} (h)	1.6	0.5	2.3	1.3	—	—
C_{max} (µg/g)	4.7	5.34	84.3	89.8	—	—
Absorption $t_{½}$ (h)	0.38	0.18	0.47	0.69	—	—
Elimination $t_{½α}$ (h)	5.1	4.6	5.9	4.2	5.3	4.6
Elimination $t_{½β}$ (h)	39.1	39.6	39.4	45.0	42.3	44.1
$AUC_{0→t}$ (µg·h/g)	48.7	44.7	1007	888	39.6	35.7
Cl (ml/kg bw per hour)	—	—	—	—	102	107

From Hansen et al. (2009)
AUC, area under the curve; Cl, clearance; C_{max}, peak concentration; $t_{½α}$, half-life during the α elimination phase; $t_{½β}$, half-life during the terminal β phase; T_{max}, time to reach C_{max}

of non-radiolabelled sulfoxaflor followed by a single dose of ^{14}C-labelled sulfoxaflor on day 15); the dose volume was 5 ml/kg bw. In addition, four rats of each sex received ^{14}C-labelled sulfoxaflor as Intralipid 10% IV Fat Emulsion at a single intravenous dose of 5 mg/kg bw (group 4); the dose volume was about 2.5 ml/kg bw. The study continued for 168 hours post-dosing. Excreta and tissues were collected for groups 1–4, whereas time-course blood samples (at 0.25, 0.5, 1, 2, 4, 8, 12 and 24 hours and every 24 hours thereafter) were collected from animals of groups 1, 2 and 4.

Orally administered ^{14}C-labelled sulfoxaflor was rapidly absorbed without any apparent lag time, and C_{max} was reached within 1.6 hours or 2.3 hours for the low- or high-dose group, respectively (Table 3). Elimination of the radioactivity from plasma was rapid during the α elimination phase ($t_{½α}$ = 4–6 hours), followed by relatively slow elimination during the terminal β phase ($t_{½β}$ = 39–45 hours). The AUC and C_{max} of radioactivity in plasma were essentially dose proportional between the low- and high-dose groups, suggesting unsaturated kinetic behaviour of sulfoxaflor up to 100 mg/kg bw. The kinetics (absorption, elimination half-life, AUC, clearance) of radioactivity in red blood cells were similar to those in plasma, except that detectable levels of radioactivity were found in red blood cells for an extended period of time when compared with plasma.

The per cent absorption of the orally administered dose in all three oral groups (single 5 mg/kg bw, multiple 5 mg/kg bw or single 100 mg/kg bw) was at least 92–96%, based on recovery in urine, non-gastrointestinal tissues and expired air (Table 4). Total recovery of radioactivity from all the animals averaged 102% and 108% in the oral and intravenous dose groups, respectively. The orally absorbed dose was rapidly excreted in urine (92–97%) without any sex difference. The majority of the urinary elimination (89–94%) occurred within the first 24 hours post-dosing. Only a small percentage (5–8%) of the oral dose was eliminated in faeces. The intravenously administered test material was also rapidly excreted in urine (97–101%). The majority of the urinary elimination (91–95%) occurred in the first 24 hours post-dosing. Only a small percentage (6–9%) of the intravenous dose was eliminated in faeces. Systemic bioavailability, calculated from the dose-corrected plasma AUC data for the low oral and intravenous dose groups, was 94% for both male and female rats.

Only 0.2–1.2% of the administered ^{14}C-labelled sulfoxaflor (oral administration: low single, high single or multiple dose) remained in the tissues after 168 hours (7 days) post-dosing. An average of 0.6–1.3% of the intravenous dose of ^{14}C-labelled sulfoxaflor remained in the tissues of the animals 168 hours post-dosing (Table 4).

The concentrations of radioactivity in tissues at termination were below 0.02 µg equivalent (eq) per gram for the low single oral dose group and for the intravenous dose group (except for skin,

Table 4. Recovery of radioactivity in tissues and excreta in rats 168 hours post-dosing with ^{14}C-labelled sulfoxaflor

	Recovery of radioactivity (% of administered dose)							
	5 mg/kg bw		100 mg/kg bw		5 mg/kg bw		5 mg/kg bw	
	Oral/single dose		Oral/single dose		Oral/multiple dose		Intravenous/single dose	
	Males	Females	Males	Females	Males	Females	Males	Females
Tissues	0.18	0.19	1.17	0.19	0.94	0.30	1.29	0.57
Final cage wash	0.44	0.62	1.20	0.91	9.92	0.42	0.21	0.39
Faeces	6.73	6.46	7.98	5.23	6.75	7.11	8.80	6.13
Urine/rinse	92.37	91.75	93.83	93.75	96.82	94.56	97.07	100.62
Expired volatiles, carbon dioxide	0	0	ND	ND	ND	ND	ND	ND
Total	100.2	99.7	104.4	100.4	103.4	102.4	107.7	108.0

From Hansen et al. (2009)
ND, not determined

0.08–0.19 µg eq/g). In the high single oral dose group, the highest concentrations were in skin (4.6 µg eq/g), red blood cells (0.57 µg eq/g), kidneys (0.36 µg eq/g), gastrointestinal tract (0.29 µg eq/g), liver (0.27 µg eq/g) and bladder (0.25 µg eq/g). The tissue concentrations of radioactivity from the multiple-dosed animals followed a similar trend to that observed in animals given a single low dose. Except for skin (0.05–0.18 µg eq/g), concentrations in all tissues for both male and female rats were below 0.03 µg eq/g (Table 5).

In summary, ^{14}C-labelled sulfoxaflor administered orally was rapidly and extensively absorbed, widely distributed throughout the body and readily eliminated, mainly via the urine, with low tissue residues (Hansen et al., 2009).

In a study on tissue distribution and metabolism of sulfoxaflor conducted according to OECD test guideline 417, groups of eight male and eight female F344/DuCrl rats received sulfoxaflor-2-^{14}C-pyridine (purity 95.6%; radiochemical purity 99.3%) as a 0.5% methylcellulose suspension by oral gavage at a single dose of 5 or 100 mg/kg bw; the dose volume was 5 ml/kg bw. Four rats of each sex per dose level were killed at predetermined times of either C_{max} plasma radioactivity (i.e. 1 and 0.5 hour post-dosing for males and females, respectively, at 5 mg/kg bw; 2 hours post-dosing for both males and females at 100 mg/kg bw) or ½ C_{max} plasma radioactivity (i.e. 7 and 6 hours post-dosing for males and females, respectively, at 5 mg/kg bw; 8 and 7 hours post-dosing for males and females, respectively, at 100 mg/kg bw). Urine, faeces, blood and tissues were collected and analysed for radioactivity. Additionally, the profile of sulfoxaflor-derived metabolites was investigated in pooled liver and kidney homogenates and pooled plasma samples.

Orally administered sulfoxaflor was rapidly absorbed without any apparent lag time. Total recovery of radioactivity in animals terminated at ½ C_{max} was greater than 98% for both dose levels (Table 6).

Test material–derived radioactivity (as micrograms ^{14}C-labelled sulfoxaflor equivalent per gram) was highest in the gastrointestinal tract, liver, kidney and urinary bladder, consistent with portal of entry and primary tissues of excretion. Plasma levels were approximately 50–60% of those found in the kidney and liver in both groups (Table 7).

In summary, administered sulfoxaflor was rapidly absorbed and widely distributed throughout the body, with the highest levels in portal of entry and excretory tissues. Test material–derived radioactivity in tissues (other than portal of entry and excretory tissues) tracks that of systemic blood, showing no potential for bioaccumulation (Rick et al., 2010).

Table 5. Recovery of radioactivity in tissues of rats 168 hours post-dosing with ^{14}C-labelled sulfoxaflor

	Recovery of radioactivity (µg eq/g)							
	5 mg/kg bw		100 mg/kg bw		5 mg/kg bw		5 mg/kg bw	
	Oral/single dose		Oral/single dose		Oral/multiple dose		Intravenous/single dose	
	Males	Females	Males	Females	Males	Females	Males	Females
Adrenals	0.011	0.009	0.235	0.195	0.012	NQ	0.011	0.009
Bladder	0.006	0.010	0.254	0.138	0.011	0.007	0.010	NQ
Blood	0.009	0.008	0.206	0.200	0.018	0.013	0.010	0.008
Bone	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ
Bone marrow	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ
Brain	0.007	0.005	0.199	0.125	0.007	NQ	0.007	0.006
Carcass	0.007	0.007	0.163	0.145	0.011	0.008	0.007	0.005
Fat	NQ	0.013	0.170	0.194	0.012	0.010	0.008	0.007
Gastrointestinal tract	0.010	0.015	0.184	0.292	0.011	0.006	0.013	0.006
Heart	0.007	0.008	0.185	0.167	0.006	0.007	0.008	0.006
Kidneys	0.012	0.014	0.303	0.358	0.017	0.012	0.014	0.014
Liver	0.010	0.009	0.269	0.209	0.018	0.011	0.013	0.008
Lungs	0.007	0.012	0.174	0.170	0.011	0.010	0.008	0.007
Lymph nodes	NQ	0.006	0.141	0.139	0.009	NQ	0.006	0.005
Muscle	0.006	0.006	0.149	0.124	0.009	0.006	0.007	0.005
Pancreas	0.007	0.008	0.172	0.161	0.010	0.007	NQ	0.005
Pituitary	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ
Plasma	0.005	0.004	0.182	0.139	0.009	0.008	0.005	NQ
Red blood cells	0.009	0.009	0.570	0.327	0.024	0.022	0.014	0.013
Skin	0.010	0.011	4.608	0.304	0.183	0.049	0.187	0.080
Spleen	0.007	0.007	0.149	0.149	0.010	0.007	0.007	0.008
Testes/ovaries	0.007	0.006	0.165	0.116	0.008	0.006	0.007	0.005
Thymus	0.005	0.005	0.145	0.112	0.012	0.005	0.004	0.003
Thyroid	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ
Uterus	—	0.008	—	0.118	—	0.006	—	0.005

From Hansen et al. (2009)
NQ, not quantifiable

1.2 Biotransformation

In the study on the toxicokinetics and metabolism of sulfoxaflor-2-^{14}C-pyridine in F344/DuCrl rats described above (Hansen et al., 2009), excreta from the animals were analysed for metabolites of sulfoxaflor.

Seven radiolabelled components (peaks A–H) were identified in urine and/or faecal samples. Parent sulfoxaflor was the primary component in both urine and faecal samples, accounting for a total of more than 93% of the administered dose.

In urine samples, six radioactive peaks (peaks B, C, D, E, F and G) were detected. Two of these peaks (peaks F and G) were consistently greater than 5% of the administered dose and were

Table 6. Recovery of radioactivity in tissues and excreta of rats at different time points after oral administration of ^{14}C-labelled sulfoxaflor

	Recovery of radioactivity (% of administered dose)							
	5 mg/kg bw				100 mg/kg bw			
	Males	Females	Males	Females	Males	Females	Males	Females
	Termination (h post-dosing)							
	1	0.5	7	6	2	2	8	7
Blood	2.81	2.55	1.60	1.55	2.04	2.06	1.22	1.11
Kidneys	1.07	0.96	0.65	0.69	0.77	0.78	0.57	0.49
Liver	6.58	5.74	3.57	3.39	4.07	3.39	2.88	2.51
Tissues, total	91.67	91.78	52.87	57.06	86.06	81.74	49.30	42.12
Final cage wash	5.32	2.98	16.36	10.77	11.01	17.07	11.19	17.98
Faeces	0	0	0.21	ND	0	0	0.11	0.05
Urine	0	0	29.04	30.23	0	0	41.00	40.25
Total	97.0	94.8	98.5	98.1	97.1	98.8	101.6	100.4

From Rick et al. (2010)
ND, not determined

Table 7. Recovery of radioactivity in tissues of rats at different time points after oral administration of ^{14}C-labelled sulfoxaflor

	Recovery of radioactivity (µg eq/g)							
	5 mg/kg bw				100 mg/kg bw			
	Males	Females	Males	Females	Males	Females	Males	Females
	Termination (h post-dosing)							
	1	0.5	7	6	2	2	8	7
Adrenals	8.09	8.73	4.50	6.10	130.3	161.23	66.00	96.25
Bladder	11.47	6.25	13.44	5.37	280.9	272.0	438.0	165.2
Blood	4.28	5.13	2.42	3.51	66.85	84.72	40.59	51.72
Bone	2.65	1.73	1.11	1.31	29.93	35.35	28.01	35.28
Bone marrow	4.77	5.25	2.65	3.71	73.42	87.00	49.44	57.95
Brain	2.98	2.84	1.69	2.46	37.39	44.63	27.37	35.22
Carcass	4.45	4.12	2.43	3.44	68.71	80.19	45.08	48.11
Fat	2.29	2.24	1.46	1.62	31.07	40.21	24.97	26.01
Gastrointestinal tract	12.31	24.30	5.05	8.11	380.1	481.4	141.0	146.6
Heart	5.13	6.62	3.20	4.69	90.47	111.5	56.92	68.77
Kidneys	8.04	8.34	4.70	6.10	109.2	136.2	83.17	89.99
Liver	10.93	11.94	5.68	7.77	129.9	143.7	82.40	101.1
Lungs	5.59	6.30	3.08	4.43	87.17	107.2	53.61	63.95
Lymph nodes	4.33	5.36	2.53	3.80	67.33	87.37	40.68	45.72
Muscle	5.00	5.16	2.93	4.07	77.10	97.84	49.35	58.77
Pancreas	6.47	7.59	3.72	5.46	101.2	124.9	63.69	81.58
Pituitary	5.57	6.45	3.35	4.82	84.80	107.3	57.70	66.25
Plasma	4.92	5.62	2.65	3.90	72.02	93.23	46.81	57.65
Red blood cells	3.95	4.95	2.32	3.45	63.54	82.50	41.49	49.56
Skin	3.62	3.62	2.15	2.72	49.95	63.32	25.66	38.97
Spleen	5.03	5.82	2.89	4.14	78.00	95.45	54.44	61.82
Testes/ovaries	4.07	5.86	2.93	4.27	65.15	100.43	48.85	61.38
Thymus	4.38	5.42	2.78	3.88	74.52	92.40	46.08	56.77
Thyroid	5.92	6.75	9.33	5.08	95.27	121.4	60.05	74.19
Uterus	—	5.35	—	3.94	—	92.71	—	55.18

From Rick et al. (2010)

identified as diastereomers of parent sulfoxaflor. Peak F accounted for 48.0–54.4% and peak G for 33.7–40.5% of the administered dose in all groups. Peak B (a glucuronide conjugate of the metabolite X11721061) accounted for 2.66–4.07% of the administered dose in all groups. Each of the remaining three metabolites (peaks C, D and E) accounted for less than 1% of the administered dose and was found only in pooled urine samples (Table 8).

In faecal samples, four metabolite peaks (peaks A, F, G and H) were consistently detected in all dose groups; none of these radioactive peaks was greater than 5% of the administered dose. Peaks F and G were identified as diastereomers of parent sulfoxaflor. Two minor components (peaks A and H), both less than 1% of the administered dose, were present only in extracts of faecal samples (Hansen et al., 2009).

In the study of the tissue distribution and metabolism of sulfoxaflor-2-^{14}C-pyridine in F344/DuCrl rats described above (Rick et al., 2010), pooled liver and kidney homogenates and pooled plasma samples were analysed for metabolites of sulfoxaflor.

Parent sulfoxaflor, identified as its two diastereomers, was the only component detected in the kidney, liver and plasma samples above the limits of detection of 0.286, 0.563 and 0.146 µg eq/g tissue, respectively, indicating that the metabolism of sulfoxaflor was minimal, at most (Rick et al., 2010).

The proposed metabolic scheme for sulfoxaflor in rats is illustrated in Figure 1.

2. Toxicological studies

2.1 Acute toxicity

(a) Lethal doses

Results of studies of the acute toxicity of sulfoxaflor are summarized in Table 9.

In an acute oral toxicity study conducted according to OECD test guideline 425 (2001, "up and down procedure"), male and female F344/DuCrl rats received sulfoxaflor (purity 95.6%) in 0.5% aqueous methylcellulose at a single dose of 630, 1000, 1580 or 2000 mg/kg bw by gavage in a volume of 10 ml/kg bw. Single animals were treated sequentially. Depending on the survival or death of the treated animal, the dose level administered to the next animal was increased or decreased, respectively. The rats were monitored daily for mortality and clinical signs for a 2-week period, and body weights were recorded on days 1, 2, 8 and 15. On day 15, surviving animals were killed, necropsied and examined for gross pathological changes.

One of two males and one of three females dosed with 1000 mg/kg bw died; additional mortalities were observed at a dose level of 1580 mg/kg bw (1/1 female; 1/1 male survived) and 2000 mg/kg bw (1/1 male; females not treated). In one animal of each sex treated with 630 mg/kg bw, muscle tremors and decreased activity were observed on day 1; these resolved by day 2. One female had decreased faeces on days 2 and 3. Animals treated with 1000, 1580 or 2000 mg/kg bw exhibited muscle tremors, twitches and/or tonoclonic convulsions. Further clinical signs were noted in some animals at these dose levels. Time of peak effect was approximately 2 hours post-dosing for most animals.

The estimated acute oral median lethal dose (LD_{50}) in rats was 1405 mg/kg bw in males and 1000 mg/kg bw in females (Brooks et al., 2008).

Table 8. Radioactive peaks detected in urine and faeces of rats dosed with ^{14}C-labelled sulfoxaflor

	% of administered dose							
	5 mg/kg bw		100 mg/kg bw		5 mg/kg bw		5 mg/kg bw	
	Oral/single dose		Oral/single dose		Oral/multiple dose		Intravenous/single dose	
	Males	Females	Males	Females	Males	Females	Males	Females
Peak A, faeces only	0.935	0.723	0.812	0.701	0.896	0.736	0.982	0.511
Peak B, urine only	2.88	2.66	3.42	3.52	3.26	3.25	3.80	4.07
Peak C, urine only	0.417	0.266	0.950	0.154	0.248	0.280	0.340	0.280
Peak D, urine only	0.750	0.659	0.621	0.600	0.660	0.817	0.758	0.716
Peak E, urine only	0.349	0.403	0.782	0.585	NQ	0.416	0.491	0.648
Peak F, urine and faeces	54.1	54.3	55.9	54.4	56.8	55.6	57.9	58.2
Peak G, urine and faeces	37.9	37.6	38.1	38.1	39.7	38.8	41.2	42.0
Peak H, faeces only	0.700	0.569	0.466	0.430	0.452	0.568	0.477	0.320
Total	98.1	97.1	101	98.5	102	100	106	107

From Hansen et al. (2009)
NQ, non-quantifiable

Figure 1. Proposed metabolic scheme for sulfoxaflor (XDE-208) in rats

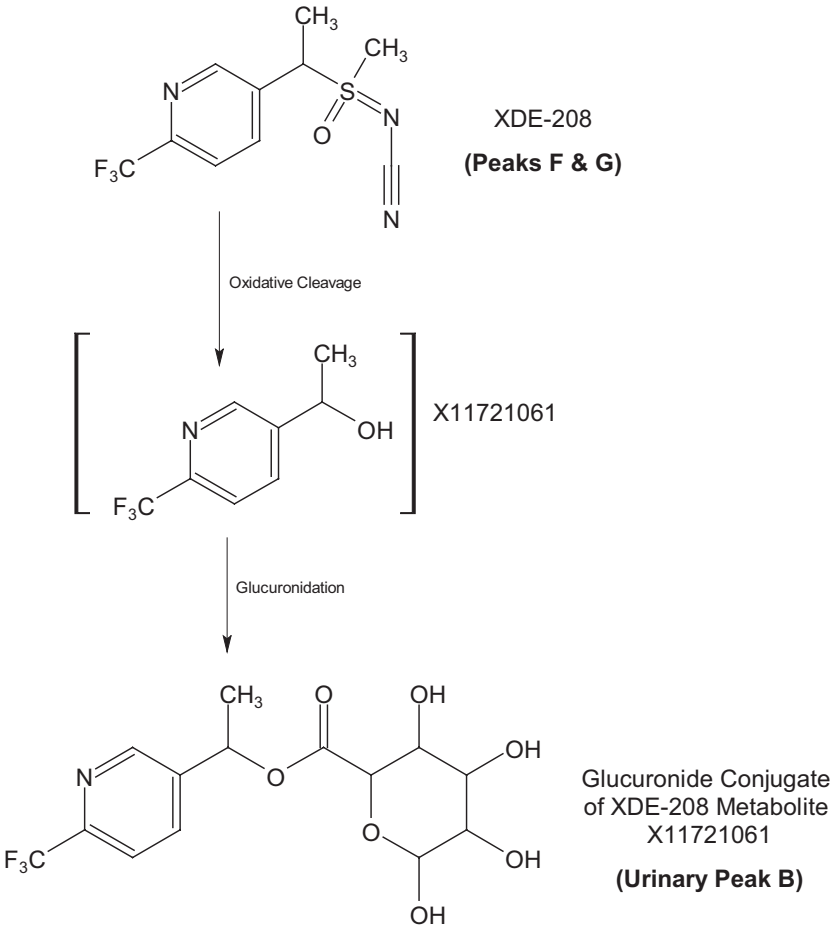


Table 9. Summary of acute oral, dermal and inhalation toxicity of sulfoxaflor

Species	Strain	Sex	Route	Purity (%)	LD_{50} (mg/kg bw) or LC_{50} (mg/l)	Reference
Rat	F344/DuCrl	M & F	Oral	95.6	M: 1405 F: 1000	Brooks et al. (2008)
Mouse	Crl:CD1(ICR)	M	Oral	95.6	750	Brooks, Wiescinski & Golden (2008)
Rat	Fischer 344	M & F	Dermal	95.6	> 5000	Durando (2008a)
Rat	F344/DuCrl	M & F	Inhalation	95.6	> 2.09	Krieger & Radtke (2009)

F, female; LC_{50}, median lethal concentration; LD_{50}, median lethal dose; M, male

In an acute oral toxicity study conducted according to OECD test guideline 425 (2001, "up and down procedure"), male (Crl:CD1(ICR)) mice received sulfoxaflor (purity 95.6%) in 0.5% aqueous methylcellulose at a single dose of 560, 750 or 1000 mg/kg bw by gavage in a volume of 10 ml/kg bw. Single animals were treated sequentially. Depending on the survival or death of the treated animal, the dose level administered to the next animal was increased or decreased, respectively. The animals were monitored daily for mortality and clinical signs for a 2-week period, and body weights were recorded on days 1, 2, 8 and 15. On day 15, surviving animals were killed, necropsied and examined for gross pathological changes.

One of three males dosed with 750 mg/kg bw died; additionally, the animal that received 1000 mg/kg bw died. The animal treated with 560 mg/kg bw showed laboured respiration, muscle convulsions, decreased activity and decreased resistance to removal on day 1, which resolved by day 2. Prior to death, one animal dosed at 750 mg/kg bw exhibited clinical signs that included muscle convulsions and increased activity. The surviving two animals exhibited decreased activity, muscle twitches, tremors and/or convulsions, decreased responsiveness to touch or increased reactivity to stimuli on test day 1, which resolved by test day 2. Prior to death, the animal dosed at 1000 mg/kg bw exhibited clinical signs that consisted of muscle twitches, tremors, convulsions, increased reactivity to stimuli and increased responsiveness to touch. The surviving animals in the 750 mg/kg bw group lost body weight on test day 2 and then gained weight on later measurements.

The estimated acute oral LD_{50} in male mice was 750 mg/kg bw (Brooks, Wiescinski & Golden, 2008).

In an acute dermal toxicity study conducted according to OECD test guideline 402 (1987), groups of five Fischer 344 rats of each sex were administered sulfoxaflor (purity 95.6%) at a single dose of 5000 mg/kg bw. The pure solid test substance was moistened with distilled water to achieve a dry paste by preparing a 50% (w/w) mixture, which was transferred to a gauze layer (5.1 cm × 7.6 cm). The gauze strip was placed on the rat's dorsal area and trunk and secured in place using adhesive tape. After 24 hours, the dressing was removed and the area cleansed with soap solution and tap water, followed by gentle drying of the area with a paper towel. The rats were observed for clinical signs and mortality for at least 14 days, and body weight was checked weekly. On day 15, surviving animals were terminated, necropsied and examined for gross pathological changes.

No clinical signs were observed, and no mortalities occurred at 5000 mg/kg bw, the only dose tested. No abnormalities were observed at gross necropsy. The dermal LD_{50} was greater than 5000 mg/kg bw for male and female rats (Durando, 2008a).

In an acute inhalation toxicity study conducted according to OECD test guideline 403 (1981), groups of five (F344/DuCrl) rats of each sex were exposed (nose only) to a dust aerosol of sulfoxaflor (purity 95.6%) at a concentration of 2.09 mg/l for 4 hours. The observation period lasted for 14 days.

On day 15, all animals were terminated, necropsied and examined for gross pathological changes. Measurements of particle size distribution showed a mass median aerodynamic diameter (MMAD) of 3.6 µm (geometric standard deviation [GSD] ± 1.33 µm); 12% of the total particulate mass had an aerodynamic diameter below 1.3 µm, and 96% of the particulate mass was present in size fractions with an aerodynamic diameter below 6.1 µm.

No mortality occurred at the tested concentration. All rats tolerated the exposure with some evidence of reversible clinical signs in two female rats (soiling of the haircoat during the exposure period, perineal soiling during the post-exposure period). Body weight loss was observed on test day 2, but by day 4, the pre-exposure body weights were exceeded. No abnormalities were observed at gross necropsy.

Under the conditions of the study, the median lethal concentration (LC_{50}) for male and female rats after dust inhalation was greater than 2.09 mg/l, which was the highest attainable concentration (Krieger & Radtke, 2009).

(b) Dermal and ocular irritation and dermal sensitization

Results of the studies on dermal and eye irritation and dermal sensitization are summarized in Table 10.

In a study of skin irritation potential conducted according to OECD test guideline 404 (2002), 0.5 g of sulfoxaflor (purity 95.6%) moistened with distilled water was applied to the shorn dorsal skin of three female New Zealand White rabbits under a semi-occluded dressing for 4 hours. Skin reactions were scored at 1, 24, 48 and 72 hours post-treatment.

No signs of systemic toxicity or mortality were observed during the study period. Very slight erythema was seen in all animals 1 and 24 hours after the treatment and in one animal 48 hours after the treatment. Very slight oedema was observed in all animals 1 hour after the treatment. Seventy-two hours after removal of the patch, scores for erythema and oedema were 0 for all three animals. It was concluded that sulfoxaflor is non-irritating to rabbit skin according to the European Union (EU) classification criteria (Classification, Labelling and Packaging Regulation, Dangerous Substances Directive) (Durando, 2008b).

In a study of eye irritation potential conducted according to OECD test guideline 405 (2002), 0.1 ml of sulfoxaflor (purity 95.6%) was placed into the conjunctival sac of one eye of each of three male New Zealand White rabbits. Ocular lesions were scored at 1, 24, 48 and 72 hours post-instillation.

No signs of systemic toxicity were observed during the study period. No signs of corneal opacity were observed in any of the test animals during the study. One hour after test substance instillation, all three treated eyes exhibited positive conjunctivitis, and iritis was evident in two eyes. The overall incidence and severity of irritation decreased with time. All animals were free of ocular irritation within 72 hours. The individual averages of the scores for the 24-, 48- and 72-hour observation periods for conjunctival redness (which was the most responsive parameter) were 0.3, 1 and 1 for all three test animals. It was concluded that sulfoxaflor is not irritating to the eyes according to the EU classification criteria (Classification, Labelling and Packaging Regulation, Dangerous Substances Directive) (Durando, 2008c).

In a study of skin sensitization potential conducted according to OECD test guideline 429 (2002, local lymph node assay), sulfoxaflor (purity 95.6%) was topically applied to the dorsal surface of each ear of female CBA/J mice. Groups of six mice per dose were dosed once daily for 3 consecutive days with 25 µl of a formulation of the test compound at concentrations of 5%, 25% and 50% in dimethylsulfoxide (DMSO). These dose levels were chosen on the basis of preliminary

Table 10. Summary of irritation and skin sensitization potential of sulfoxaflor

Species	Strain	Sex	End-point	Purity (%)	Result	Reference
Rabbit	New Zealand White	F	Skin irritation	95.6	Not irritating	Durando (2008b)
Rabbit	New Zealand White	M	Eye irritation	95.6	Not irritating	Durando (2008c)
Mouse	CBA/J	F	Skin sensitization (LLNA)	95.6	Not sensitizing	Wiescinski & Sosinski (2008)

F, female; LLNA, local lymph node assay; M, male

results showing that concentrations of 1%, 5%, 10%, 20%, 40% and 50% caused no effects on body weight, and erythema was not induced. Two additional groups received the vehicle (DMSO) or α-hexylcinnamaldehyde (30%) to serve as negative and positive controls, respectively. Animals were checked for mortality and clinical signs at least daily during the study. In particular, the site of application was examined for signs of local irritation. Individual body weights were measured at study start and at scheduled termination.

No mortality or clinical signs were observed during the study. In particular, no cutaneous reactions were observed at the application site of animals treated with the test compound. Body weight changes were comparable between control and treated groups. Negative responses were observed at all tested dose levels (stimulation indices: 1.0, 1.1 and 1.0 for 5%, 25% and 50% concentrations, respectively). The results of the positive control demonstrated the validity of the assay (stimulation index: 12.0). On the basis of this study, sulfoxaflor did not show any sensitization potential and does not warrant classification or labelling as a skin sensitizer (Wiescinski & Sosinski, 2008).

2.2 Short-term studies of toxicity

Mice

In a palatability study, groups of 4–5 female Crl:CD1(ICR) mice were fed diets containing sulfoxaflor (purity 98.3%) at concentrations of 0, 2000, 3000, 4500 or 6000 parts per million (ppm), equal to 0, 317, 418, 345 and 462 mg/kg bw per day, for 3–7 days. Initially, groups of five female mice were given a diet containing 0 or 2000 ppm daily for 7 days. No significant effects were noted, and the mice were necropsied on study day 8 along with a group of three control mice, which were initially excluded from the study following randomization. The original five control mice remained on study for comparison with subsequent treatment groups. As no significant effects were observed in mice given 2000 ppm, an additional group of five female mice received diets containing 3000 ppm daily for up to 7 days. No significant effects were noted; therefore, two additional groups of four female mice received diets containing 4500 or 6000 ppm and were terminated after 3 days on study because of decreases in feed consumption and body weights. The five female mice in the control group given untreated feed throughout the study were necropsied with the mice in the 4500 and 6000 ppm dose groups. Animals were evaluated by daily body weights, feed consumption and cage-side examinations. A complete necropsy was conducted on all animals, and liver weights were recorded at necropsy. Samples of liver tissue from animals in the control, 4500 ppm and 6000 ppm groups were collected after 3 days of treatment for possible future enzyme analyses. Blood samples were collected from all animals (treated and controls) at necropsy and stored at −80 ºC for possible toxicokinetic analysis. Histological evaluation of the liver from all animals was conducted.

The 4500 and 6000 ppm dose groups were terminated after 3 days of treatment for humane reasons based on decreased feed consumption (reduced to 46% and 41% of control, respectively), excessive feed scratching and body weight decrements (10% and 16%, respectively, compared with day 1 values). The poor palatability of sulfoxaflor in the diet resulted in decreased feed consumption

and a lower test material intake value at 4500 ppm (345 mg/kg bw per day) than at 3000 ppm (418 mg/kg bw per day). Absolute liver weights were increased by 34% and 27% and relative liver weights were increased by 40% and 44%, relative to concurrent controls, for mice given 4500 and 6000 ppm, respectively. These liver weight increases were considered related to treatment.

Mice given 3000 ppm were observed with scratched feed (four of five animals) on test day 2, and the available feed consumption value was decreased by 39% compared with controls. Mean body weights for these animals were decreased by approximately 4% relative to controls, and one individual body weight had decreased by 7% when compared with its value on day 1. However, at termination, feed consumption and body weight values were comparable to those of controls. Liver weights (absolute and relative) of animals given 3000 ppm were increased by 40% and 53.4%, respectively, when compared with the nearest available historical control averages (values for concurrent controls were not available during this study phase), and hence the increases were interpreted to be treatment related.

Animals given 2000 ppm had decreases in mean body weights and feed consumption of 5% and 31%, respectively, on study day 2 when compared with controls. These animals quickly adjusted to the feed, and, by study day 4, body weights and feed consumption values were comparable to those of controls. At necropsy (day 8), absolute and relative liver weights were increased by 17% and 21%, respectively, relative to concurrent controls, and the increases were considered to be treatment related.

Treatment-related histopathological changes in the liver consisted of hepatocellular hypertrophy (with altered tinctorial properties) in the majority of animals given 2000 ppm and above. A very slight overall increase in the numbers of mitotic figures was present in the majority of the livers from animals given 2000 or 3000 ppm and from some given 4500 or 6000 ppm, compared with the controls, indicating increased hepatocyte proliferation. Occasional, scattered, individual necrotic hepatocytes were observed in some animals given 4500 or 6000 ppm. Some animals given 4500 or 6000 ppm had very slight or slight hepatic fatty change consistent with reduced feed consumption (Table 11).

Under the conditions of this study, the highest palatable dietary concentration of sulfoxaflor for female CD-1 mice was 3000 ppm. At concentrations of 4500 ppm and above, sulfoxaflor was not sufficiently palatable to maintain acceptable growth and to be used in subsequent studies (Thomas & Dryzga, 2010).

In a study of toxicity conducted according to OECD test guideline 407, groups of five male and five female CD-1 mice were fed diets containing sulfoxaflor (purity 98.3%) at a concentration of 0, 300, 1500 or 3500 ppm, equal to 0, 43.9, 230 and 524 mg/kg bw per day for males and 0, 53, 273 and 638 mg/kg bw per day for females, for 28 days. Satellite groups of three male and three female CD-1 mice were fed diets supplying 0 or 1500 ppm sulfoxaflor for 3 days (termination on day 4). The livers of mice in the satellite group were collected and stored for possible future mechanistic studies. Parameters evaluated were daily cage-side observations, weekly detailed clinical observations, ophthalmic examinations, body weights, feed consumption, haematology, clinical chemistry, selected organ weights, and gross pathological and histopathological examinations.

Feed consumption for males and females given 1500 or 3500 ppm was lower than for the controls during days 1–2, whereas feed consumption was comparable to that of the controls after day 4. Slight treatment-related decreases in body weights and body weight gains were observed in males and females given 1500 or 3500 ppm during the 1st week of the study. Body weight gains of males given 3500 ppm were only 50% of the control values on day 8, but subsequently improved and were comparable to those of controls throughout the rest of the study. There was a loss of body weight in high-dose females through day 4. However, body weight and body weight gains were comparable to those of controls from day 5 through the rest of the study. Body weight gains of males and females

Table 11. Summary of selected histopathological findings in hepatocytes of mice fed diets containing sulfoxaflor for 3–7 days

	Incidence of finding				
	Dietary concentration (ppm)				
	0	2000	3000	4500	6000
Number of mice examined	*8*	*5*	*5*	*4*	*4*
Altered tinctorial properties					
- very slight	0	1	0	1	0
Hypertrophy, with altered tinctorial properties					
- very slight	0	0	0	3	2
- slight	0	4	5	0	2
Mitotic alteration, increased, multifocal					
- very slight	0	3	5	2	1
Necrosis, individual cells, multifocal					
- very slight	0	0	0	2	3
Vacuolation, consistent with fatty change					
- very slight	0	0	0	2	2
- slight	0	0	0	2	1

From Thomas & Dryzga (2010)

given 1500 ppm were comparable to those of controls from day 4 and day 3 through the rest of the study, respectively.

At termination on day 30, males and females given 1500 or 3500 ppm had treatment-related elevations in mean serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities (only changes in ALT were statistically significant). Triglyceride levels and serum alkaline phosphatase (ALP) activity were also elevated in males and females given 3500 ppm (Table 12).

At necropsy, there were treatment-related increases in the mean absolute and relative liver weights of males and females given 1500 ppm (28% and 30% in males and 16% and 19% in females, respectively) or 3500 ppm (93% and 93% in males and 44% and 48% in females, respectively) compared with controls (Table 12). Increases in liver weights were associated with treatment-related centrilobular/midzonal hepatocyte hypertrophy in the livers of males and females given 1500 or 3500 ppm. Males given 300 ppm had marginal treatment-related increases in mean absolute (15%) and relative (11%) liver weights over the respective control values, but these were not statistically significant and were not associated with detectable hepatocyte hypertrophy or clinical chemistry changes and were therefore considered a non-adverse effect. Other treatment-related histopathological changes consisted of a very slight or slight increase in hepatocyte mitoses and very slight hepatocyte fatty change in the livers of males given 1500 or 3500 ppm. Treatment-related multifocal necrosis of scattered individual hepatocytes was present in the livers of males given 1500 or 3500 ppm and in females given 3500 ppm. Males given 3500 ppm had elevations in absolute and relative adrenal gland weights. Although these were not statistically significant, they exceeded the historical control range and were associated with hypertrophy of the zona fasciculata of the adrenal cortex. They were therefore considered to be treatment related. Males given 3500 ppm also had very slight or slight treatment-related atrophy of the mesenteric adipose tissue.

Toxicokinetic analysis of the plasma from the above animals showed that dose-proportional intake of sulfoxaflor was translated into a dose-proportional increase in plasma concentrations of sulfoxaflor (Table 12). The systemic exposure of sulfoxaflor was approximately 40% higher in males

Table 12. Summary of selected findings in mice fed diets containing sulfoxaflor for 28 days

	Males				Females			
	Dietary concentration (ppm)							
	0	300	1500	3500	0	300	1500	3500
Plasma concentration (µg/g), day 26 or 30	0	23.7[a]	49.3	104	0	7.11	35.32	73.3
ALT (U/l)	38	55	258*	193*	37	45	68*	77*
ALP (U/l)	80	60	96	127*	87	105	106	128*
AST (U/l)	82	79	186	148	87	90	126	109
Triglycerides (mg/dl)	95	98	110	221*	74	62	89	123*
Calcium (mg/dl)	9.6	9.7	10.0	10.1*	10.1	10.2	10.4	10.4*
Liver weight, absolute (g)	1.73	1.98	2.20	3.33*	1.37	1.35	1.59	1.97*
Liver weight, relative (% of body weight)	4.87	5.42	6.32*	9.42*	4.90	4.92	5.84*	7.26*
Kidney weight, absolute (g)	0.63	0.67	0.54	0.54*	0.39	0.35	0.35	0.35*
Kidney weight, relative (% of body weight)	1.77	1.83	1.57	1.53*	1.41	1.29	1.27	1.30*
Hepatocytes (no.)								
- hypertrophy	0	0	5	5	0	0	5	5
- mitotic alteration	0	0	1	4	0	0	0	0
- single-cell necrosis, focal	1	1	0	0	1	0	0	0
- single-cell necrosis, multifocal	0	0	4	5	0	0	0	4
- vacuolation/fatty change	0	0	1	4	0	0	0	0
Adrenals, hypertrophy zona fasciculata (no.)	0	0	0	5	0	0	0	0

From Thomas et al. (2008)
ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; U, units; * $P < 0.05$
[a] Mean = 10.48 after removing one outlier.

than in females. The urinary elimination of sulfoxaflor in males was between 33% and 44% and in females was between 23% and 65% of what they consumed during a 24-hour period. Only parent sulfoxaflor was observed in the chromatograms of the plasma samples from the sulfoxaflor-dosed mice. In addition to parent sulfoxaflor, urine samples contained one potential metabolite, which was present at less than 2% of the parent sulfoxaflor concentration, based on equimolar mass spectroscopic response, indicating that the majority of the dietary administered sulfoxaflor was excreted essentially unchanged in the urine.

The no-observed-adverse-effect level (NOAEL) was 300 ppm (equal to 43.9 mg/kg bw per day), based on liver toxicity (increased serum ALT, multifocal single-cell necrosis and vacuolation/fatty change of hepatocytes) in males at 1500 ppm (equal to 230 mg/kg bw per day) and above (Thomas et al., 2008).

In a study of toxicity conducted according to OECD test guideline 408, groups of 10 male and 10 female CD-1 mice were fed diets containing sulfoxaflor (purity 96.6%) at a concentration of 0, 100, 750 or 1250 ppm for males and at a concentration of 0, 100, 1500 or 3000 ppm for females, for at least 90 days. These concentrations corresponded to time-weighted average doses of 0, 12.8, 98.0 and 166 mg/kg bw per day for males and 0, 16.2, 247 and 489 mg/kg bw per day for females. Parameters evaluated were daily cage-side observations, weekly detailed clinical observations, ophthalmic examinations, body weights, feed consumption, haematology, clinical chemistry, selected organ weights, and gross pathological and histopathological examinations. In addition, toxicokinetic analyses were conducted on urine (day 80) and terminal blood plasma.

There were no treatment-related effects on clinical signs, ophthalmic parameters, body weights or feed consumption.

There was a minor reduction in haematocrit and haemoglobin concentration in females given 1500 or 3000 ppm. Males given 1250 ppm had statistically significant increases in the activities of serum ALT by 200%, AST by 43% and ALP by 142%. Serum total cholesterol and total bilirubin concentrations were statistically significantly decreased in males given 750 and 1250 ppm, to below the respective historical control range. Females given 1500 or 3000 ppm had elevations in the activities of serum ALT (125% and 171% increase, respectively) and AST (44% and 31% increase, respectively) and decreased ALP activity (3000 ppm only) compared with respective controls, but these only occasionally reached statistical significance, although for AST and ALP, values were outside the respective historical control range. Serum triglycerides were elevated in females given 1500 or 3000 ppm, and serum cholesterol concentration was elevated in females given 3000 ppm (Table 13).

Males given 750 or 1250 ppm and females given 1500 or 3000 ppm had treatment-related increases in absolute (20% and 74% in males and 36% and 54% in females, respectively) and relative liver weights (26% and 85% in males and 40% and 50% in females, respectively) compared with the respective controls. Other treatment-related organ weight changes consisted of increased absolute and relative adrenal weights in males given 1250 ppm and lower absolute kidney weights in males given 750 or 1250 ppm. The lower kidney weights were, however, considered non-adverse because of the lack of any treatment-related histopathological changes in the kidneys of the high-dose males. Absolute and relative thymus weights were decreased in high-dose females.

Males given 750 or 1250 ppm and females given 1500 or 3000 ppm had slight or moderate, treatment-related, centrilobular to midzonal hepatocyte hypertrophy, with altered tinctorial properties (increased cytoplasmic eosinophilia). Other treatment-related histological liver effects consisted of an overall, very slight or slight increase in the numbers of mitotic figures (hepatocytes in mitosis) in the liver of males given 1250 ppm and very slight fatty change in hepatocytes of males given 750 or 1250 ppm. Males given 750 or 1250 ppm had treatment-related, very slight necrosis of scattered, individual hepatocytes, whereas this change in females given 1500 or 3000 ppm was infrequent or minimal (Table 13). Males given 750 or 1250 ppm and females given 1500 or 3000 ppm had treatment-related, very slight hypertrophy of the zona fasciculata of the adrenal cortex. A very slight, treatment-related fatty change was also present in the zona fasciculata of the adrenal cortex in some females given 1500 or 3000 ppm. Four out of 10 females given 3000 ppm had a very slight, treatment-related increase in extramedullary erythrocytic haematopoiesis in the spleen.

Toxicokinetic analysis of the plasma (based on the daily test material intake during the last 7 days of the study) showed that dose-proportional intake of sulfoxaflor translated into a dose-proportional increase in plasma concentrations of sulfoxaflor only up to the middle dose for both male (750 ppm, 92.3 mg/kg bw per day) and female (1500 ppm, 227 mg/kg bw per day) mice. In males, the systemic exposure, as measured by the plasma concentration of sulfoxaflor, became supra-linear between the middle (92.3 mg/kg bw per day) and high (152 mg/kg bw per day) doses (3.9-fold increase instead of 1.6-fold expected from the difference in the test material intake between the middle and high doses). Conversely, in females, plasma concentrations of sulfoxaflor reached a plateau, remaining almost unchanged between the middle (227 mg/kg bw per day) and the high (467 mg/kg bw per day) doses (Table 13). Total elimination of sulfoxaflor in 24-hour urine remained dose proportional only up to the middle dose and showed less than a dose-proportional increase at the highest dose, in both male and female mice. These data are consistent with a saturation of elimination of sulfoxaflor in male mice at the highest dose and a saturation of absorption of sulfoxaflor from the gastrointestinal tract in female mice at the highest dose. On the basis of these results, the kinetically derived maximum dose (i.e. the dose above which kinetics become non-linear) was considered to be 92.3 and 227 mg/kg bw per day (750 and 1500 ppm) for male and female mice, respectively.

Table 13. Summary of selected findings in mice fed diets containing sulfoxaflor for 90 days

	Males				Females			
	Dietary concentration (ppm)							
	0	100	750	1250	0	100	1500	3000
Substance intake (mg/kg bw per day), week 13	0	10.7	92.3	152	0	14.7	227	467
Plasma concentration (µg/g), week 13	0	2.1	16.8	65.7	0	1.4	18.1	17.9
Haemoglobin (g/dl)	15.0	14.8	15.1	14.5	15.7	15.3	14.9*	14.8*
Haematocrit (%)	49.4	48.8	49.2	48.3	50.9	49.7	48.7*	48.2*
ALT (U/l)	41	44	53	123*	28	33	63*	76
AST (U/l)	72	76	73	103*	97	104	140	127
ALP (U/l)	45	42	50	109*	83	85	72	61*
Cholesterol (mg/dl)	157	150	124*	89*	80	86	84	124*
Triglycerides (mg/dl)	101	77	106	127	70	72	105*	131*
Liver weight, absolute (g)	1.79	1.87	2.16*	3.13*	1.41	1.37	1.91*	2.17*
Liver weight, relative (% of body weight)	4.22	4.45	5.30*	7.73*	4.28	4.21	6.00*	6.43*
Adrenal weight, absolute (mg)	7.8	8.7	8.6	10.3	10.0	10.4	11.4	11.2
Adrenal weight, relative (% of body weight)	0.018	0.021	0.021	0.026*	0.031	0.032	0.036	0.034
Hepatocytes, hypertrophy (no.)								
- very slight	9	9	0	0	0	0	1	0
- slight	0	0	9	1	0	0	9	3
- moderate	0	0	1	9	0	0	0	7
Hepatocytes, mitotic alteration (no.)	0	0	0	10	0	1	0	0
Hepatocytes, necrosis, focal/multifocal (no.)	0	0	8	10	0	0	5	4
Hepatocytes, vacuolation/fatty change (no.)	0	0	6	10	0	0	0	1
Adrenals/zona fasciculata, hypertrophy (no.)	2	1	4	10	3	2	7	10
Adrenals/zona fasciculata, vacuolation/fatty change (no.)	0	0	0	0	0	0	5	2

From Thomas et al. (2010a)
ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; U, units; * $P < 0.05$

The NOAEL was 100 ppm (equal to 12.8 mg/kg bw per day), based on effects in the liver (vacuolation/fatty change of hepatocytes) and the adrenals (hypertrophy and/or vacuolation of the zona fasciculata) observed in males at 750 ppm (equal to 98.0 mg/kg bw per day) and above and in females at 1500 ppm (equal to 247 mg/kg bw per day) and above (Thomas et al., 2010a).

Rats

In a palatability study, groups of five female F344/DuCrl rats were fed diets containing sulfoxaflor (purity 98.1%) at a concentration of 0, 2748, 5495 or 10 990 ppm (equal to 0, 198, 256 and 291 mg/kg bw per day) for up to 8 days to evaluate palatability and perform toxicokinetic analysis in blood plasma. Parameters evaluated were daily cage-side observations, body weights, feed consumption and gross examinations.

Feed consumption was reduced by 59%, 74% or 84% in animals given 2748, 5495 or 10 990 ppm, respectively, relative to controls following 1 day of administration. This was attributed to decreased palatability of sulfoxaflor in rodent feed. The reduction in feed consumption was associated with decreases in body weight at all dose levels. All animals fed 5495 or 10 990 ppm had marked body weight loss by days 5 and 4, respectively, and were humanely euthanized on that day. Animals

fed 2748 ppm lost up to 6.1 g body weight by day 4; however, there was a slight recovery in feed consumption (31% reduction) and body weights (18% reduction) by day 8, relative to controls. Sulfoxaflor was present in the plasma of rats fed 2748 ppm at a mean concentration of 72.8 µg eq/g plasma on day 8. No metabolites were detected in the plasma.

Therefore, under the conditions of this study, sulfoxaflor was not sufficiently palatable at concentrations greater than 2748 ppm (198 mg/kg bw per day) for these dose levels to be used in future dietary toxicity studies. Sulfoxaflor was quantifiable in rat plasma, and there were no metabolites detected (Yano et al., 2009a).

In a study of toxicity conducted according to OECD test guideline 407, groups of five male and five female F344/DuCrl rats were fed diets containing sulfoxaflor (purity 98.1%) at a concentration of 0, 300, 1000, 2000 or 3000 ppm (equal to 0, 24.8, 79.4, 155 and 205 mg/kg bw per day for males and 0, 26.5, 88.3, 170 and 192 mg/kg bw per day for females) for 28 days. Animals were observed daily for mortality and clinical signs. A detailed physical examination was performed weekly. Body weight and feed consumption were recorded frequently throughout the study. Blood samples for toxicokinetic analysis were taken on day 27 and for haematology and clinical chemistry determinations on day 29 (before final necropsy), whereas urine samples were collected during the last week of the study. At study termination, all animals were necropsied, selected organs were weighed and a range of tissues were taken, fixed and examined microscopically.

Administration of sulfoxaflor to male and female rats at 3000 ppm resulted in excessive reductions in feed consumption (by 31% and 36%) and marked body weight loss (by 21% and 19%) compared with controls after 9 days of administration, respectively; therefore, all animals in the 3000 ppm dose group were humanely euthanized (day 9). The lower feed consumption due to decreased palatability was responsible for the decreased body weights.

Feed consumption at 300, 1000 or 2000 ppm was decreased by 5%, 29% or 54% in males and by 7%, 26% or 48% in females, respectively, after 1 day of administration, but increased for the remainder of the study. By the end of the study, feed consumption for males given 300 or 1000 ppm was comparable to that of controls and was decreased by 6% at 2000 ppm. Females given 300, 1000 or 2000 ppm consumed 8%, 6% or 11% less than controls at the end of the study, respectively.

Animals given 1000 or 2000 ppm had decreased body weight after 1 day of exposure, and this was considered secondary to the reduced feed consumption. These animals gained weight for the remainder of the study. By day 28, males and females given 2000 ppm weighed 8.5% and 10% less, respectively, than controls. Males and females given 300 or 1000 ppm had body weights comparable to those of controls on day 28.

Males (all treated doses) and females (1000 or 2000 ppm) had dose-related increases in serum total cholesterol levels (Table 14). Males and females given 1000 or 2000 ppm also had total serum protein levels that were higher than those of controls. Albumin and globulin levels were marginally increased in males given 1000 or 2000 ppm and in females given 2000 ppm.

Males and females given 1000 or 2000 ppm had increased absolute and relative liver weights that were dose and treatment related. There were a number of additional differences in organ weights of males and females given 2000 ppm that were statistically significant, including relative brain (males and females; increased), relative kidney (males; increased), relative testes (males; increased), relative thyroid (males and females; increased), absolute heart (males; decreased) and absolute spleen (males and females; decreased). These differences in organ weights were considered to be secondary to the lower body weights of this dose group and did not reflect a primary target organ effect of sulfoxaflor. This conclusion is supported by the absence of histopathological changes in these organs.

Treatment-related histological effects were observed in the livers of males and females given 1000 or 2000 ppm and consisted of a dose-related increase in the severity (very slight to moderate)

Table 14. Summary of selected findings in rats fed diets containing sulfoxaflor for 28 days

	Males				Females			
	Dietary concentration (ppm)							
	0	300	1000	2000	0	300	1000	2000
Dose (mg/kg bw per day), day 27	0	20.2	67.3	137	0	22.2	74.8	151
Plasma concentration (µg/g), day 27	0	9.0	29.4	59.3	0	7.3	25.5	51.5
Plasma $AUC_{24\,h}$ (µg·h/ml), day 27	0	210	693	1371	0	167	591	1183
Plasma $t_{½}$ (h), day 27	0	7.88	8.30	7.19	0	4.46	5.46	4.92
Cholesterol (mg/dl)	51	74*[a]	110*	157*	74	82	104*	142*
Total protein (g/dl)	6.9	6.9	7.4[#]	7.6[#]	6.2	6.1	6.3[#]	6.6[#]
Albumin (g/dl)	4.1	4.0	4.3	4.3[#]	3.8	3.7	3.8	3.9[#]
Globulin (g/dl)	2.8	2.8	3.1	3.3	2.4	2.4	2.5	2.7
Body weight, terminal (g)	199.7	201.3	195.6	181.7*	134.4	125.7	129.3	119.3*
Liver weight, absolute (g)	6.46	6.50	8.14*	9.36*	4.20	3.82	4.34	5.05*
Liver weight, relative (% of body weight)	3.23	3.23	4.16*	5.15*	3.12	3.04	3.36	4.23*
Hepatocytes, hypertrophy (no.)								
- very slight	0	0	0	0	0	0	3	2
- slight	0	0	2	0	0	0	0	3
- moderate	0	0	3	5	0	0	0	0
Hepatocytes, vacuolation/fatty change (no.)								
- very slight	0	0	2	3	0	0	0	1
- slight	0	0	3	0	0	0	0	0

From Yano et al. (2009b)
AUC, area under the curve; $t_{½}$, half-life; * $P < 0.05$ (Dunnett's test, males and females analysed separately); [#] $P < 0.05$ (Dunnett's test, males and females analysed together)
[a] Outside historical control range (36–51 mg/dl) from five studies.

of hepatocellular hypertrophy (with altered tinctorial properties) involving the centrilobular to mid-zonal regions of the hepatic lobule (Table 14). Effects were more prominent in males than in females, increasing to moderate severity in 1000 and 2000 ppm males. Vacuolation, consistent with fatty change, involving hepatocytes primarily in the right lateral lobe was also occasionally seen in a multifocal distribution in males given 1000 or 2000 ppm and in one female given 2000 ppm. The restriction of this alteration to only one liver lobe, the minor nature of the effect (very slight or slight) and the lack of a clear dose–response relationship in regards to severity suggest that this was not a toxicologically significant effect.

Toxicokinetic analysis of the plasma showed that levels of sulfoxaflor ($AUC_{24\,h}$) were effectively proportional to dose, with a 3.3- to 3.6-fold increase between the 300 and 1000 ppm groups and a 2.0-fold increase between the 1000 and 2000 ppm dose groups. Females were more efficient than males in eliminating the test material. The 24-hour systemic dose as measured by the $AUC_{24\,h}$ was 21%, 15% and 14% lower in females than in males in the 300, 1000 and 2000 ppm nominal dose groups, respectively (corresponding to 210, 693 and 1371 µg·h/ml versus 167, 591 and 1183 µg·h/ml at the low, middle and high doses, respectively). The plasma elimination half-life of sulfoxaflor in male rats was between 7 and 8 hours, whereas it was 32–43% shorter in females (between 4 and 5 hours). The chromatograms of the plasma samples taken from sulfoxaflor-dosed rats contained up to five peaks in addition to the parent; however, these impurities and/or metabolites made up less than 1% of the total test material in the plasma.

The NOAEL was 300 ppm (equal to 24.8 mg/kg bw per day), based on marginal liver toxicity (increased serum cholesterol and total protein levels) in males at 1000 ppm (equal to 79.4 mg/kg bw per day) and above (Yano et al., 2009b).

In a study of toxicity conducted according to OECD test guideline 408, groups of 10 male and 10 female F344/DuCrl rats were fed diets containing sulfoxaflor (purity 96.6%) at a concentration of 0, 100, 750 or 1500 ppm (equal to 0, 6.36, 47.6 and 94.9 mg/kg bw per day for males and 0, 6.96, 51.6 and 101 mg/kg bw per day for females) for 90 days. An additional 10 males and 10 females fed either 0 or 1500 ppm of test diet for 90 days were maintained for 28 days after withdrawing the test diets to examine the reversibility of any effects seen. Parameters evaluated were daily cage-side observations, weekly detailed clinical observations, ophthalmic examinations, functional observational battery (FOB) (pre-exposure and prior to necropsy, comprising cage-side, hand-held and open-field observations, rectal temperature, forelimb and hindlimb grip performance, landing foot splay and motor activity), body weights, feed consumption, prothrombin time, haematology, urinalysis, clinical chemistry, selected organ weights and gross pathological and histopathological examinations, which included a specifically detailed review of the nervous system. The study also included integrated toxicokinetics and an assessment of immunotoxicity, as measured by immune responsiveness in the sheep red blood cell antibody-forming cell (AFC) assay.

There were no mortalities, no treatment-related clinical observations and no ophthalmic findings at any dose level. Also, there were no statistically significant or treatment-related FOB observations and no treatment-related effects on grip performance, landing foot splay or motor activity.

Feed consumption was dose-relatedly decreased in males (all treated doses) and females (750 and 1500 ppm), due to decreased palatability of diets containing sulfoxaflor. During the first 4 days, males given 100, 750 or 1500 ppm consumed 5%, 12% or 24% less feed than controls, and females given 750 or 1500 ppm consumed 8% or 21% less feed than controls. However, feed consumption for males at all dose levels was comparable to that of controls by the end of the study. Female feed consumption in the 750 and 1500 ppm groups was 5% or 8% lower than that of controls, respectively, at 90 days and statistically significantly different.

After 4 days of treatment, body weight gains of males given 750 or 1500 ppm were 24% or 45% lower than those of controls, respectively, and body weight gains of females given 750 or 1500 ppm were 13% or 60% lower than those of controls, respectively. These animals gained weight for the remainder of the study. By day 90, males and females given 750 or 1500 ppm weighed 8% or 9% and 3% or 8% less than controls, respectively. By day 90, the body weight gain of males and females given 750 or 1500 ppm was 11% or 13% and 9% or 20% less than that of controls, respectively. All body weight effects were considered secondary to the lower feed consumption owing to decreased palatability of the test material in the feed. Males and females at 100 ppm had body weights comparable to those of the controls at the end of the 90-day study. During the 28-day recovery period, feed consumption was comparable to that of controls in both sexes, and nearly complete recovery was seen in body weights (~5% lower for both sexes).

Serum cholesterol levels in males and females at 750 or 1500 ppm were increased by 51% or 127% and 32% or 83%, respectively (Table 15). Total protein and albumin levels were increased in high-dose males and females, but these were not considered to be of toxicological significance. Other clinical pathology values were comparable to control values. Following the 28-day recovery period, all clinical chemistry parameters in males and females at 1500 ppm were comparable to those of controls.

The absolute liver weights of males and females given 750 or 1500 ppm were 6% or 5% and 25% or 17% higher than controls, respectively, and the relative liver weights of males and females given 750 or 1500 ppm were 14% or 8% and 41% or 27% higher than controls, respectively (Table 15). There were a number of additional differences in organ weights of males and females

Table 15. Summary of selected findings in rats fed diets containing sulfoxaflor for 90 days

	Males				Females			
	Dietary concentration (ppm)							
	0	100	750	1500	0	100	750	1500
Dose (mg/kg bw per day), day 89	0	4.7	36.4	74.0	0	4.8	38.3	75.2
Plasma concentration (µg/g), day 89	0	1.5	12.0	24.1	0	1.3	10.1	20.2
Plasma $AUC_{24\,h}$ (µg·h/ml), day 89	0	35	281	555	0	30	235	476
Plasma $t_{½}$ (h), day 89	0	8.73	9.16	9.09	0	7.93	8.47	7.73
Cholesterol (mg/dl)	59	60	89*	134*	77	86	102*	141*
Total protein (g/dl)	6.9	6.7	7.1	7.5*	7.1	7.4	7.3	7.6*
Albumin (g/dl)	4.1	3.9	4.1	4.3#	4.4	4.5	4.4	4.6#
Body weight, terminal (g)	313.1	308.2	289.7	277.9*	168.8	170	164.6	155.3
Liver weight (g)	8.14	8.09	8.60	10.2*	4.20	4.36	4.40*	4.92*
Liver weight, relative (% of body weight)	2.60	2.62	2.97*	3.67*	2.49	2.56	2.67*	3.17*
Incidence of finding (*n* = 10)								
Hepatocytes, hypertrophy								
- very slight	0	0	0	0	0	0	9	5
- slight	0	0	7	1	0	0	0	5
- moderate	0	0	3	9	0	0	0	0
Hepatocytes, necrosis								
- very slight	0	0	8	5	0	0	3	10
- slight	0	0	2	5	0	0	0	0
Hepatocytes, vacuolation/fatty change								
- very slight	0	0	6	2	0	0	0	0
- slight	0	0	4	3	0	0	0	0
- moderate	0	0	0	5	0	0	0	0
Liver/aggregates of macrophages								
- very slight	9	10	7	4	10	10	10	8
- slight	1	0	3	6	0	0	0	2
Incidence after recovery period (*n* = 10)								
Hepatocytes, hypertrophy								
- very slight	0	—	—	2	—	—	—	0
Hepatocytes, necrosis								
- very slight	0	—	—	1	—	—	—	0
Hepatocytes, vacuolation/fatty change								
- very slight	0	—	—	7	—	—	—	0
- slight	0	—	—	1	—	—	—	0

From Yano et al. (2009c)
* $P < 0.05$ (Dunnett's test, males and females analysed separately); # $P < 0.05$ (Dunnett's test, males and females analysed together)

given 750 or 1500 ppm that were statistically significant. These differences in organ weights were secondary to the lower body weights of these dose groups and did not reflect a primary target organ effect of sulfoxaflor. This conclusion was supported by the absence of histopathological changes in these organs.

Treatment-related histological liver effects occurred in males and females given 750 or 1500 ppm and consisted of a dose-related increase in the severity (slight to moderate) of hepatocellular hypertrophy (with altered tinctorial properties) involving the centrilobular to midzonal regions of the hepatic lobule. Individual hepatocyte necrosis was also observed in the centrilobular region with a multifocal distribution to a very slight or slight degree (Table 15). All effects were seen in both sexes but were more prominent in males than in females. Vacuolation of hepatocytes, consistent with fatty change, was also observed in all males in the 750 and 1500 ppm groups at very slight, slight or moderate degrees. In addition, in the rats with the greatest degree of hepatocellular hypertrophy, necrosis and vacuolation, there was an increase in the incidence of multifocal aggregates of macrophages/histiocytes. The microscopic changes were present in all three lobes of the liver examined in male and female rats; however, they were more readily apparent in the right lateral lobe. The microscopic changes in the liver were consistent with the increased liver weights and cholesterol levels noted for these rats.

Following the 28-day recovery period, a complete recovery was seen in the absolute and relative liver weights of males and females given 1500 ppm. There was partial recovery of the microscopic hepatic effects. Two male rats in the 1500 ppm group still had recognizable hepatocellular hypertrophy of a very slight degree in the centrilobular and midzonal regions. One of these two rats also had multifocal, very slight individual hepatocellular necrosis. Multifocal, very slight or slight hepatocellular vacuolation consistent with fatty change was present in most of the recovery males; however, the degree of involvement was substantially less severe in the recovery group. There were no microscopic treatment-related changes present in the liver of females given 1500 ppm.

There was no effect on immune responsiveness in female rats up to and including the high dose level of 1500 ppm. In males, there was no effect on immune responsiveness at 100 or 750 ppm, whereas the 1500 ppm group displayed a lower, non-statistically significant response (26% lower) when compared with the vehicle control group. The lower AFC response in the high-dose male group coincided with considerable general toxicity, including decreased body weights and increases in liver weights (absolute and relative), hepatocellular hypertrophy, necrosis, vacuolation consistent with fatty change, multifocal aggregates of macrophages and elevated serum cholesterol, for which overall the NOAEL was 100 ppm. Therefore, the lower AFC response in the high-dose males was considered secondary to systemic toxicity and thus does not reflect primary immunotoxic potential for sulfoxaflor.

Toxicokinetic analysis of the plasma showed that the systemic exposure of sulfoxaflor was dose proportional, with an approximate 8-fold increase in $AUC_{24\,h}$ between the 100 and 750 ppm groups and an approximate 2-fold increase between the 750 and 1500 ppm doses. Females were more efficient than males at eliminating the test material from their system. The 24-hour systemic dose as measured by the plasma $AUC_{24\,h}$ was 15%, 16% and 14% lower in females than in males in the 100, 750 and 1500 ppm nominal dose groups, respectively. The plasma elimination half-life of sulfoxaflor in male rats was approximately 9 hours, whereas it was approximately 8 hours in females (12% lower). The chromatograms of the plasma samples taken from sulfoxaflor-dosed rats (via diet) contained up to five minor peaks in addition to the parent compound. These peaks may represent metabolites of the test material or metabolites of test material impurities. Absolute quantification of the minor metabolites was not possible, because of the lack of reference standards. Elimination of sulfoxaflor in urine over 24 hours ranged between 51% and 61% of the ingested dose, with the exception of high-dose males, from which 37% of the ingested dose was excreted, 26 days after the initiation of the study. Elimination of test material in 0- to 24-hour urine on days 84 and 85 ranged between 52% and 69% for the lower two dose levels, but was somewhat lower at the high dose for both sexes (32–36% of ingested dose). In addition to parent sulfoxaflor, four urinary metabolites were detected. One peak was a known impurity in this lot of the test material. No definitive quantification of the other three metabolites was obtained.

The NOAEL was 100 ppm (equal to 6.36 mg/kg bw per day), based on liver toxicity (increased serum cholesterol level, vacuolation/fatty change of hepatocytes) in males at 750 ppm (equal to 47.6 mg/kg bw per day) and above. All effects had recovered during the 28-day recovery phase, with the exception of very slight histopathological changes in the liver (vacuolation/fatty change of hepatocytes) in males at 1500 ppm (equal to 94.9 mg/kg bw per day) (Yano et al., 2009c).

In a study of dermal toxicity conducted in compliance with OECD test guideline 410, groups of 10 male and 10 female F344/DuCrl rats were administered sulfoxaflor (purity 95.6%) dermally at a dose level of 0, 100, 500 or 1000 mg/kg bw per day for 28 days (6 hours/day, 7 days/week; semi-occlusive dressing). Parameters evaluated were daily cage-side and weekly detailed clinical observations, dermal observations, ophthalmic examinations, body weight, feed consumption, haematology, clinical chemistry, urinalysis, toxicokinetics of blood plasma, selected organ weights, and gross pathological and histopathological examinations.

There were no mortalities, no treatment-related clinical observations and no ophthalmic findings at any dose level. No effects on body weights or on feed consumption were observed.

Serum cholesterol levels were slightly but statistically significantly increased (17%) in males exposed to 1000 mg/kg bw per day. This effect was considered non-adverse because it was marginal and was well within the laboratory's historical control range.

Absolute and relative liver weights of males exposed to 1000 mg/kg bw per day were marginally higher (6.5% and 4.4%, respectively) than those of the controls. Also, treatment-related histopathological changes were observed in the livers of 6 of 10 males exposed to 1000 mg/kg bw per day and consisted of very slight hepatocyte hypertrophy, with altered tinctorial properties (increased cytoplasmic eosinophilia), involving the centrilobular/midzonal regions of the hepatic lobule.

There were no treatment-related gross pathological or histopathological changes in the skin at the dermal test site in males or females exposed to doses up to and including 1000 mg/kg bw per day.

The results of toxicokinetic analysis showed that following dermal application, the average plasma concentration of the test material (< 0.14, < 0.14, 0.36 and 4.37 µg/g in males and < 0.14, < 0.14, 0.44 and 2.35 µg/g in females at 0, 100, 500 or 1000 mg/kg bw per day, respectively) at the high dose was greater than dose proportional. Similar plasma concentrations were found prior to and 16 hours after test material removal, indicating some retention of test material at the application site.

The NOAEL for systemic toxicity was 500 mg/kg bw per day, based on slight histopathological changes in the liver of males at 1000 mg/kg bw per day. There were no substance-related signs of local irritation up to 1000 mg/kg bw per day, the highest dose tested (Thomas, Murray & Saghir, 2009).

Dogs

In a palatability and tolerability pilot study, six male and three female Beagle dogs were given sulfoxaflor (purity 95.6%) by dietary or capsule administration using six different exposure procedures (phases). Phase A (six males, two females) evaluated the palatability of the test material in ground diet offered for 3 days at a concentration of 0, 300, 750 or 1500 ppm. Phase B (six males) evaluated the palatability of the test material in ground diet offered for 3 days at a concentration of 0, 300 or 750 ppm with bacon flavouring added. Phase C (four males, two females) evaluated the palatability of the test material in pre-formulated pelletized diet offered for 3 days at a concentration of 0, 300 or 750 ppm with bacon flavouring added. Phase D (four males) evaluated the tolerability of the test material administered in a gelatine capsule twice per day for 3 days at a dose level of 10 or 25 mg/kg bw per day (5 or 12.5 mg/kg bw per dose). Phase E (two males, one female) evaluated the palatability of the test material in ground diet offered for 2 hours/day for 2, 4 or 5 days, as dry diet or with water added to form a slurry, at respective concentrations of 50, 100 or 250 ppm. Phase F (six males, three females) evaluated the tolerability of the test material administered in a gelatine capsule after offering canned diet once per day for 11 days at a dose level of 10 or 15 mg/kg bw per day.

Observations for morbidity, mortality, injury and the availability of feed and water were conducted twice daily. Clinical observations were conducted as needed during the study. Body weights and feed consumption were measured and recorded daily during the treatment period of each phase. Ophthalmoscopic and physical examinations were conducted pretest. Blood samples for clinical pathology evaluations were collected pretest, prior to initiation of dosing of phase F and at the termination of phase F. Additional blood samples for clinical chemistry were also collected on day 8 of phase F. Urine and blood samples for potential evaluation of test material exposure were collected at the termination of phase F. Necropsy examinations were performed, liver weights were measured and recorded, and selected tissues were collected at the termination of phase F.

All animals survived each exposure phase. Individual clinical observations were associated with lack of palatability or intolerance to the test material. These observations included emesis, loss of skin elasticity, thinness and altered faecal appearance.

Feed consumption values during each test material exposure period were consistently well below acceptable intake limits (at least 75% of basal intake). In most cases, feed consumption values were as low as 10–20% of basal intake levels. Body weights were consistently decreased during test material exposure periods, which was directly associated with decreased feed consumption.

Erythrocytes, haemoglobin and haematocrit tended to decrease slightly in the females at 10 mg/kg bw per day at termination. Individual values remained within expected ranges, but reticulocytes in females at 10 mg/kg bw per day and in males at 15 mg/kg bw per day were markedly reduced, suggestive of potential suppressed erythropoiesis.

Moderate to marked dose-related decreases in sodium levels were present in males at 15 mg/kg bw per day at the interim and terminal intervals and in females at 10 mg/kg bw per day at termination relative to predose values and expected ranges. Decreases in chloride levels of similar magnitude were present in males and females at 10 mg/kg bw per day at the interim and terminal intervals and in males at 15 mg/kg bw per day at both intervals. Potassium levels also tended to decrease gradually over the duration of the study, with values below expected ranges at termination in males at both dose levels and in females at 10 mg/kg bw per day. These changes were attributed to the emesis and loose faeces noted throughout the study, suggesting indirect test article effects.

A dose-related increase in ALT activity was present in males at 15 mg/kg bw per day at termination and in females at 10 mg/kg bw per day at termination relative to expected ranges, which may suggest a treatment-related effect.

There were no treatment-related organ weight changes or macroscopic observations in males or females. Histopathological evaluation was not conducted for any phase of this study.

In conclusion, none of the exposure procedures of this palatability and tolerability study produced feed consumption results that were considered acceptable for repeated exposure over 28 or 90 days (Stewart, 2009a).

In a second palatability and tolerability study, six female Beagle dogs were given sulfoxaflor (purity 95.6% for technical grade, 99.7% for analytical grade) by dietary, capsule or oral gavage administration for up to 28 days. Using the same animals, routes of test material administration and dosing regimens were altered during the course of the study as requested by the sponsor, with a 1- or 2-week interval between regimens. Two groups of three female Beagle dogs were administered the test material as follows: One group of three animals received the test material via the diet, ad libitum for 6 consecutive days, at a dose level of 500 ppm (group 1). The dosing route for these animals was changed to oral gavage using analytical-grade sulfoxaflor, once daily for 28 consecutive days, at a dose level of 15 mg/kg bw per day (group 3). Another group of three animals received the test material via capsule, twice daily for 6 consecutive days, at a dose level of 15 mg/kg bw per day (group 2). The dosing route for these animals was changed to dietary, ad libitum for 5 consecutive days, at a

dose level of 100 ppm (group 4). The dosing route for these animals was once again changed to oral gavage using technical-grade sulfoxaflor, once daily for 28 consecutive days, at a dose level of 15 mg/kg bw per day (group 5). For both oral gavage groups, the vehicle was 0.5% methylcellulose in deionized water, and the dose volume was 10 ml/kg bw.

Observations for morbidity, mortality, injury and the availability of feed and water were conducted twice daily for all animals. Clinical observations were conducted daily. Body weights and feed consumption were measured and recorded daily. Blood samples for clinical pathology evaluations were collected from all animals pretest and on days 6 and 29 (prior to necropsy), and urine samples were collected at necropsy from all animals on day 29. Blood samples for determination of the plasma concentrations of the test article were collected from animals in group 4 at designated time points on day 5 (the last day of dietary dosing) and from animals in groups 3 and 5 at designated time points on each respective day 28 (prior to necropsy). At study termination, necropsy examinations were performed and organ weights were recorded. A complete set of tissues from group 5 animals was sent to the sponsor for microscopic evaluation.

All animals survived until the scheduled termination intervals. However, there were clinical findings and body weight findings that were associated with reduced feed consumption. In some instances, the reduced feed consumption was accompanied by a lack of sufficient fluid intake, which resulted in a loss of skin elasticity. In addition, intermittent emesis and faecal alterations (discoloured, mucoidal and soft/watery faeces) were likely treatment related.

All exposures resulted in decreases in feed consumption and body weight, with the exception of dietary exposure at 100 ppm. However, feed consumption and body weights during oral gavage exposure tended to stabilize after an initial decrease.

There were no treatment-related effects on haematology, clinical chemistry or urinalysis parameters, with the possible exception of low urine volumes at 15 mg/kg bw per day (both groups).

The only organ weight alteration that was likely related to treatment was the lower thymus weights of all group 3 animals; however, no histopathological examination was conducted.

There were no treatment-related gross pathological observations in group 5 animals, and no treatment-related histopathological changes were observed.

Toxicokinetic analysis showed that the orally gavaged sulfoxaflor was rapidly absorbed from the gastrointestinal tract without any lag time and appeared in blood within 15 minutes of dosing. The peak plasma concentration (C_{max}) of sulfoxaflor was reached within 4 ± 2 hours after dosing. Elimination of sulfoxaflor from plasma was slow (elimination half-life = 26 ± 18 hours and clearance = 18 ± 13 ml/kg bw per hour). The daily systemic dose ($AUC_{24\,h}$) of sulfoxaflor to dogs after multiple gavage dosing at 15 mg/kg bw per day was 479 ± 111 µg·h/ml, and the dose-corrected $AUC_{24\,h}$ was 32 ± 7 µg·h/ml per milligram per kilogram body weight administered.

In conclusion, oral gavage administration at 15 mg/kg bw per day was a tolerable route of exposure and concentration for the technical-grade test material exposure, as determined by adequate feed consumption and tolerable in-life clinical signs. Also, exposure at 100 ppm via the dietary route was well tolerated, as determined by adequate feed consumption, but the amount of test material consumed was not sufficient to justify it as a potential high dose level for subsequent studies.

Exposure at 500 ppm via the dietary route or 15 mg/kg bw per day via the capsule route was not well tolerated, as determined by insufficient feed consumption. Therefore, these concentrations and routes could not be considered a viable method of test material exposure over a sustained duration. Based on this information, oral gavage exposure was determined to be the most appropriate route for a sustained duration of test material exposure in Beagle dogs (Stewart, 2009b).

In a study of toxicity conducted according to OECD test guideline 409, groups of four male and four female Beagle dogs were administered sulfoxaflor (purity 95.6%) by oral gavage in 0.5%

aqueous methylcellulose (Methocel A4C) at a dose level of 0, 1, 3 or 10 mg/kg bw per day for 90 days. However, on day 5, the highest dose level was reduced to 6 mg/kg bw per day because of intolerance (as determined by a lack of feed consumption). The dose volume was 10 ml/kg bw, except from day 5 to day 8, when the 10 mg/kg bw per day dose was administered at 6 ml/kg bw to achieve the 6 mg/kg bw per day dose.

Observations for morbidity, mortality, injury and the availability of feed and water were conducted twice daily for all animals. Clinical observations were conducted and body weights were measured and recorded weekly. Feed consumption was measured daily from days 1 to 7 and days 9 to 35, at least twice weekly from weeks 6 to 12 and at least once during week 13. Physical examinations were conducted by a veterinarian pretest to confirm the good health status of each animal placed on study. Blood and urine samples for clinical pathology evaluations were collected from all animals pretest and during weeks 6 and 13. Blood and urine samples were collected from all animals at designated intervals during week 13 for determination of the plasma and urine concentrations of the test material. At study termination, necropsy examinations were performed, organ weights were recorded and selected tissues were examined microscopically.

There were no mortalities and no treatment-related clinical observation findings at any dose level.

Treatment-related decreased mean feed consumption values were observed at the high dose level during the first 2 weeks of the study. Beginning in week 3, the mean feed consumption values at this exposure level stabilized and were similar to control values. Based on the data, the effect on feed consumption was considered to be related to exposure at 10 mg/kg bw per day, with residual effects prolonging the instability into week 2 of the study. There were no other notable feed consumption findings for the remainder of the study.

Treatment-related decreased mean body weights were observed at 10 mg/kg bw per day during the 1st week of exposure. However, mean body weights at this exposure level did not decrease significantly beyond week 1, effectively after the dose was reduced to 6 mg/kg bw per day on day 5, and by approximately week 9, the mean body weights at this exposure level had returned to pre-exposure values. The transient nature of the decreased mean body weights was a clear indication that the effect was related to exposure at 10 mg/kg bw per day and not related to exposure at 6 mg/kg bw per day.

There were no treatment-related effects on haematology, clinical chemistry or urinalysis parameters.

There were no treatment-related gross pathological observations, organ weight changes or histopathological changes at any exposure level.

The toxicokinetic analysis after 88 days of dosing at 1, 3 or 6 mg/kg bw per day showed that the daily systemic dose ($AUC_{24\,h}$) was 32 ± 6, 84 ± 23 and 147 ± 13 µg·h/ml in males and 22 ± 3, 71 ± 26 and 119 ± 19 µg·h/ml in females, respectively. The increase in systemic dose was clearly dose proportional in female dogs and was approximately dose proportional in male dogs across all three dose levels. The dose-corrected $AUC_{24\,h}$ was 32, 28 and 25 µg·h/ml per milligram per kilogram body weight administered in males and 22, 24 and 20 µg·h/ml per milligram per kilogram body weight administered in females, respectively. The mean plasma elimination half-life of sulfoxaflor at 1, 3 or 6 mg/kg bw per day was 20.3 ± 4.2, 28.2 ± 7.1 and 17.1 ± 6.1 hours in males and 27.5 ± 26.1, 20.9 ± 8.5 and 16.9 ± 1.9 hours in females, respectively.

Urinary elimination of sulfoxaflor was 70% ± 6%, 76% ± 12% and 59% ± 33% of the administered dose in male dogs and 69% ± 4%, 80% ± 6% and 74% ± 13% in female dogs at 1, 3 and 6 mg/kg bw per day, respectively.

The NOAEL was 6 mg/kg bw per day, based on decreased feed consumption and decreased body weights during the 1st week of exposure at 10 mg/kg bw per day. The reduction of this initial high dose to 6 mg/kg bw per day on day 5 allowed for recovery, and no treatment-related adverse effects occurred at any dose level thereafter (Stewart, 2010).

In a study of toxicity conducted according to OECD test guideline 452, groups of four male and four female Beagle dogs were administered sulfoxaflor (purity 95.6%) by oral gavage in 0.5% aqueous methylcellulose (Methocel A4C) at a dose level of 0, 1, 3 or 6 mg/kg bw per day for 1 year. The dose volume was 10 ml/kg bw. Observations for morbidity, mortality, injury and the availability of feed and water were conducted twice daily for all animals. Toxicity was assessed by weekly detailed clinical observations, feed consumption and body weight measurements, ophthalmoscopic examinations and clinical pathology evaluations. Blood and urine samples for the determination of the plasma concentrations of the test material were collected from all animals at designated time points during weeks 13, 26 and 52, and systemic exposure was determined. The toxicokinetic parameters were determined for the test article from concentration–time data. At study termination, necropsy examinations were performed, select organ weights were recorded and tissues were preserved for subsequent microscopic examination.

No treatment-related deaths occurred. An increase in the incidence of both soft and watery faeces occurring in two males (animal numbers 125 and 126) at 6 mg/kg bw per day was considered treatment related; the other two high dose level males were normal in this respect. A transient, treatment-related decrease in feed consumption and body weight was observed in two females (animal numbers 129 and 132) at 6 mg/kg bw per day during the first 2 weeks of dosing (Table 16).

One male at 6 mg/kg bw per day (animal number 125) exhibited increased ALP, total bilirubin, triglycerides, total cholesterol, high-density lipoprotein cholesterol and low-density lipoprotein cholesterol at 3 months. The triglyceride levels remained within expected ranges, but were increased relative to controls and other animals within the 6 mg/kg bw per day group. These changes were decreasing at 6 months and resolved at 12 months. Whether these were transient treatment-related effects to which there was adaptation or were incidental cannot be clearly ascertained. No other treatment-related effects on clinical pathology parameters were noted.

There were no treatment-related macroscopic findings, effects on organ weights or histopathological effects at any dose.

The systemic exposure to sulfoxaflor ($AUC_{24\ h}$) in plasma was proportional across all dose levels in both sexes. Except in female dogs at 52 weeks of exposure, toxicokinetic analysis of parent compound in urine showed that the systemic exposure to sulfoxaflor was proportional across all dose levels and time points. The deviation in females at 52 weeks was relatively modest.

The NOAEL was 6 mg/kg bw per day, the highest dose tested. The increased incidences of soft/watery faeces in two males at 6 mg/kg bw per day were not considered adverse, as these changes were not accompanied by any other toxicological effect. Also, the slight decreases in feed consumption and body weight in two females at 6 mg/kg bw per day during the first 2 weeks of dosing were not considered adverse, as there were no changes during the remainder of the study (Heward, 2010a).

2.3 Long-term studies of toxicity and carcinogenicity

Mice

In a study of carcinogenicity conducted according to OECD test guideline 451, groups of 50 male and 50 female Crl:CD1(ICR) mice were given diets containing sulfoxaflor (purity 95.6%) at a concentration of 0, 25, 100 or 750 ppm in males (equal to 0, 2.54, 10.4 and 79.6 mg/kg bw per day) and at a concentration of 0, 25, 250 or 1250 ppm in females (equal to 0, 3.43, 33.9 and 176 mg/kg bw per day) for up to 18 months. Animals were evaluated by daily cage-side, biweekly clinical observations, periodic hand-held detailed clinical examinations, body weights and feed consumption. Ophthalmic examinations were conducted pre-exposure and prior to necropsy. All mice had a complete necropsy examination with total and differential white blood cell counts and weights of selected organs at the scheduled necropsy. An extensive set of organs was examined histopathologically from all control and high dose group mice, as well as all mice that died or were euthanized in moribund

Table 16. Summary of selected findings in dogs fed diets containing sulfoxaflor for 1 year

	Males				Females			
	Dose (mg/kg bw per day)							
	0	1	3	6	0	1	3	6
Plasma $AUC_{24\,h}$ (µg·h/ml)								
- week 13	0	30	95	167	0	27	75	155
- week 26	0	31	83	146	0	27	76	165
- week 52	0	30	92	182	0	29	82	204
Faeces soft[a]	8/3	27/4	10/3	271/4	23/3	16/4	44/4	31/3
Faeces watery[a]	0/0	10/2	1/1	315/2	8/3	4/2	29/3	24/3
Body weight (kg)								
- week −1	9.36	9.40	9.63	9.33	7.60	7.71	7.77	7.70
- week 1	9.41	9.56	9.56	9.23	7.84	7.84	7.65	7.58
- week 2	9.62	9.86	9.62	9.23	7.86	7.87	7.77	7.60
- week 52	11.31	11.47	10.93	10.79	8.84	8.89	8.83	9.21
Feed consumption (g/animal per day)								
- week 1	231	275	199	188	253	242	209	155
- week 2	237	293	211	203	224	228	240	191

From Heward (2010a)
[a] Number of times observed/number of animals affected.

condition. The kidneys, liver, lungs, adrenal glands (females only) and all relevant gross lesions of mice from the low- and intermediate-dose groups from the terminal necropsy were also examined histopathologically.

There were no treatment-related changes in clinical observations, body weights and body weight gains, feed consumption, ophthalmological observations, or total and differential white blood cell counts in any of the sulfoxaflor-treated groups.

Toxicokinetic analysis indicated that the daily systemic dose (steady-state plasma concentration) of sulfoxaflor in male and female mice remained essentially proportional to dose during the course of the study (3 and 12 months after study start), and concentrations of sulfoxaflor in urine also increased proportionally with dose for both male and female mice and at both collection times (3 and 12 months).

The liver was the primary target of sulfoxaflor. The absolute and relative liver weights of males given 750 ppm were increased by 87% and 79%, respectively; in females given 1250 ppm, they were increased by 51% and 47%, respectively, compared with their respective controls, which also reflected a treatment-related increased incidence of liver tumours, as described below. At necropsy, there was a treatment-related increase in the incidence of mass nodules and multifocal pale foci in the liver of males given 750 ppm, compared with controls. Females given 1250 ppm had a treatment-related increased incidence of mass nodules in the liver, albeit at lower numbers compared with the high-dose males. Histopathological treatment-related changes consisted of a statistically significant increase in the incidences of both hepatocellular adenomas and carcinomas in male mice given 750 ppm, with 60% of such animals exhibiting hepatocellular carcinomas and/or adenomas. There was no effect at 100 ppm. The incidence in female mice given 1250 ppm was 10%, compared with 2% in controls, although this difference was not statistically significant. Although there were no statistically identified differences in the overall mortality rates between the controls and any of the sulfoxaflor-treated groups, hepatocellular carcinomas or adenomas were attributed as the cause of death or moribundity in a small proportion of

Table 17. Summary of selected findings in mice fed diets containing sulfoxaflor for up to 18 months

	Males				Females			
	Dietary concentration (ppm)							
	0	25	100	750	0	25	250	1250
Dose (mg/kg bw per day), 3 months	0	2.8	10.6	89.6	0	3.7	45.2	197
Plasma concentration (µg/g), 3 months	0	0.7	2.7	18.2	0	0.6	5.6	28.3
Dose (mg/kg bw per day), 12 months	0	2.2	8.3	64.8	0	3.0	30.7	144
Plasma concentration (µg/g), 12 months	0	0.8	2.9	21.4	0	0.7	5.0	29.1
Terminal body weight (g)	46.9	50.0	47.9	48.0	39.0	41.9	41.8	40.1
Liver weight, absolute (g)	2.54	2.48	3.72	4.75*	1.89	2.72	2.07	2.86*
Liver weight, relative (% of body weight)	5.44	4.99	7.40	9.75*	4.882	5.95	5.02	7.21*
Liver, histopathology								
No. examined	*50*	*50*	*50*	*50*	*50*	*50*	*50*	*50*
Foci, basophilic	3	1	4	2	0	0	1	0
Foci, eosinophilic	3	2	3	10	0	0	0	0
Foci, vacuolated	1	0	1	6	0	0	0	0
Hypertrophy, centrilobular	6	8	7	35*	2	0	14	29*
Hypertrophy, panlobular	0	0	0	12*	3	2	4	12*
Necrosis, multifocal	9	4	3	28*	1	3	1	6
Vacuolation, fatty change	2	1	1	16*	1	0	0	5
Mitotic alteration	5	1	1	10	1	3	2	0
Adenoma, hepatocyte	12	6	10	24*	1	1	0	2
Carcinoma, hepatocyte	2	0	4	17*	0	1	0	4
Adenoma and/or carcinoma	13	6	12	30*	1	2	0	5

From Thomas et al. (2010b)
* $P < 0.05$

males (6/50) given 750 ppm. Treatment-related non-neoplastic liver effects consisted of increases in the incidences of eosinophilic and vacuolated foci of cellular alteration in males given 750 ppm; slight to moderate centrilobular/midzonal or panlobular hepatocellular hypertrophy, with altered tinctorial properties (increased cytoplasmic eosinophilia), consistent with liver enzyme induction in males and females given 750 or 1250 ppm; multifocal individual cell necrosis of hepatocytes in males given 750 ppm (very slight or slight) and females given 1250 ppm (very slight); very slight fatty change in centrilobular/midzonal hepatocytes in males and females given 750 or 1250 ppm; and increased incidence of hepatocytes in mitosis in males given 750 ppm (Table 17).

The only treatment-related change in females given 250 ppm was an increased incidence of slight centrilobular/midzonal hepatocyte hypertrophy, with altered tinctorial properties (increased cytoplasmic eosinophilia), consistent with liver enzyme induction. This was considered to be an adaptive and non-adverse response due to a lack of any associated changes, including increase in liver weight or any other treatment-related histopathological findings.

Males given 750 ppm had an exacerbation in the cumulative incidence of spontaneous dermatitis, which is common in CD-1 mice. Histologically, this was characterized by subacute to chronic inflammation, variable epidermal ulceration and acanthosis. Associated with the ulcerative dermatitis was an increased incidence of reactive plasmacytosis of the local submandibular lymph nodes of males given 750 ppm. The exacerbation of spontaneous dermatitis in males given 750 ppm

was interpreted as secondary to the excessive stress induced by the malignant and/or benign hepatocellular neoplasms.

The NOAEL for carcinogenicity was 100 ppm (equal to 10.4 mg/kg bw per day), based on an increased incidence of hepatocellular adenomas and/or carcinomas in males at 750 ppm (equal to 79.6 mg/kg bw per day). The NOAEL for non-neoplastic changes was 100 ppm (equal to 10.4 mg/kg bw per day), based on liver toxicity (vacuolation/fatty change of hepatocytes) in males at 750 ppm (equal to 79.6 mg/kg bw per day) (Thomas et al., 2010b).

Rats

In a combined study of chronic toxicity and carcinogenicity conducted according to OECD test guideline 453, groups of 60 male and 60 female F344/DuCrl rats were given diets containing sulfoxaflor (purity 95.6%) at a concentration of 0, 25, 100 or 500 ppm in males (equal to 0, 1.04, 4.24 and 21.3 mg/kg bw per day) and at a concentration of 0, 25, 100 or 750 ppm in females (equal to 0, 1.28, 5.13 and 39.0 mg/kg bw per day) for up to 24 months. Ten rats of each sex per dose level were necropsied after 1 year (chronic toxicity group), and the remaining 50 rats of each sex per dose level were fed the respective diets for up to 2 years.

Animals were evaluated by daily cage-side examinations, biweekly clinical observations, periodic detailed clinical observations, body weights, feed consumption and toxicokinetics (plasma and urine). Clinical pathology examinations (haematology, clinical chemistry and urinalysis) were conducted at regular intervals throughout the study. Ophthalmic examinations were conducted on all rats pre-study and on all surviving rats prior to the scheduled necropsies. All rats had a complete necropsy, with weights of multiple organs collected from all rats at the scheduled necropsies. Histopathological examination of an extensive set of organs was performed on all control and high dose level rats and all rats that died spontaneously or were euthanized due to their moribund condition. Histopathological examination of rats from the low and intermediate dose levels of the 12-month chronic toxicity group was limited to the liver, adrenal glands, testes and relevant gross lesions. Histopathological examination of survivors from the low and intermediate dose levels of the 24-month oncogenicity group was limited to the liver, testes, epididymides, coagulating glands, prostate, seminal vesicles and relevant gross lesions.

During the study, there were no treatment-related clinical signs, and there were no statistical differences in mortality for either males or females at any dose level.

Males given 500 ppm had statistically significantly lower body weights at most measurement intervals starting on day 512 and continuing through day 729. On day 729, the mean body weight and body weight gain for males given 500 ppm were 5.0% and 5.7% lower than those of controls, respectively. Females given 750 ppm had statistically significantly lower body weights, first noted on day 57 and continuing through day 729. On day 729, the mean body weight and body weight gain for females given 750 ppm were 6.3% and 9.1% lower than those of controls, respectively. The body weights of males and females given 100 or 25 ppm were unaffected by treatment with sulfoxaflor (Table 18).

High dose level males and females had statistically significantly higher cholesterol concentrations at 3, 6 and 12 months and at 3, 6, 12 and 18 months, respectively, with increases ranging from 17.5% to 32.9%, which exceeded the respective historical control ranges. Values in both males and females at 24 months were similar to those in control animals.

The liver was the primary target organ for histopathological effects in males given 500 ppm and in females given 750 ppm at 12 and 24 months. The absolute and relative liver weights for high-dose males (500 ppm) and females (750 ppm) were increased at 12 months, in the range of 3.2–17% (Table 19), and relative weights were also statistically significantly increased at 24 months (Table 20). Non-neoplastic liver effects at 12 and 24 months consisted of hypertrophy of centrilobular and midzonal hepatocytes, necrosis of individual centrilobular hepatocytes, vacuolation (consistent with

Table 18. Summary of selected findings in rats fed diets containing sulfoxaflor for up to 2 years

	Males				Females			
	Dietary concentration (ppm)							
	0	25	100	500	0	25	100	750
Dose (mg/kg bw per day), 3 months	0	1.2	4.7	25.2	0	1.4	5.9	42.0
Plasma concentration (µg/g), 3 months	0	0.7	2.7	14.0	0	0.7	2.6	19.1
Dose (mg/kg bw per day), 12 months	0	0.9	3.8	19.1	0	1.3	5.2	35.8
Plasma concentration (µg/g), 12 months	0	0.4	1.8	9.8	0	0.6	2.2	19.1
Plasma $AUC_{24\,h}$ (µg·h/ml), 12 months	0	9.7	42.1	228	0	12.7	50.8	422
Mortality (no. of animals), 24 months	16	18	21	17	12	10	13	6
Body weight (g)								
- day 176	372.8	375.0	369.8	372.0	200.6	199.1	203.6	193.2*
- day 365	432.7	435.4	433.7	429.1	233.1	230.6	237.1	221.8*
- day 624	463.0	465.4	454.8	442.3*	291.4	289.1	299.9	274.3*
- day 729	439.3	443.2	418.6	417.4*	295.4	293.6	305.0	276.8*
Cholesterol (mg/dl)								
- 3 months	59	64	62	77*	93	101	103*	118*
- 6 months	72	78	74	85*	116	112	121	138*
- 12 months	79	95*	89	105*	143	137	140	168*
- 18 months	111	131	116	127	133	134	124	158*

From Stebbins et al. (2010a)
* $P < 0.05$

fatty change) of hepatocytes and an increase in the severity of aggregates of macrophages/histiocytes. An additional treatment-related liver effect in females given 750 ppm at 24 months consisted of a lower number of basophilic foci of altered hepatocytes (quantified as 21 or more basophilic foci in the three standard liver sections examined microscopically). A treatment-related neoplastic liver effect at 24 months consisted of a statistically significant increase in the incidence of benign hepatocellular adenomas in males given 500 ppm. Females given 750 ppm did not have any change in the incidence of liver tumours. There were no treatment-related liver effects in males or females given 25 or 100 ppm (Tables 19 and 20).

At 24 months, males given 100 or 500 ppm had treatment-related statistically significant higher absolute and relative testes weights. Absolute testes weights of males given 100 or 500 ppm were approximately 46% and 62% higher than those of controls, respectively. The higher testes weights were reflective of larger interstitial (Leydig) cell adenomas in the testes of males at these dose levels (Table 20).

The incidence of interstitial cell adenomas in at least one testis of males from all dose groups was comparable to that of controls at 24 months. However, males given 500 ppm had a statistically significant increase in the incidence of bilateral interstitial cell adenomas of the testes and a corresponding decrease in the incidence of unilateral interstitial cell tumours, relative to controls. An additional treatment-related testicular effect consisted of a statistically significant increase in the incidence of severe bilateral atrophy of seminiferous tubules in males given 100 or 500 ppm (Table 20).

At 24 months, males given 100 or 500 ppm had treatment-related statistically significantly lower absolute and relative epididymal weights. The lower epididymal weights were associated with a higher incidence of decreased spermatic elements (bilateral, severe) in the lumen of the epididymides of these males.

Table 19. Summary of selected morphological findings in rats at 12 months

	Males				Females			
	Dietary concentration (ppm)							
	0	25	100	500	0	25	100	750
Body weight (g)	391.5	416.5	410.3	404.0	214.2	215.8	216.3	207.9
Liver weight, absolute (g)	9.43	10.19	10.01	11.04*	5.59	5.50	5.69	5.77
Liver weight, relative (% of body weight)	2.41	2.44	2.44	2.73*	2.62	2.55	2.63	2.78*
Hepatocytes, hypertrophy (no.)								
- very slight	0	0	0	2	0	0	0	8
- slight	0	0	0	6	0	0	0	0
- moderate	0	0	0	2	0	0	0	0
Hepatocytes, necrosis (no.)								
- very slight	1	0	0	7	0	0	0	3
- slight	0	0	0	1	0	0	0	0
Hepatocytes, vacuolization (no.)								
- very slight	6	10	9	2	4	2	6	5
- slight	0	0	0	7	0	0	0	4
Testes; interstitial cell, hyperplasia, bilateral (no.)	9	8	10	10	—	—	—	—
Testes, interstitial cell adenoma; unilateral (no.)[a]	0	1	3	3	—	—	—	—

From Stebbins et al. (2010a)
* $P < 0.05$
[a] Historical control incidences (12-month sacrifice) from six studies: testicular interstitial cell adenomas: 0/10 to 3/10 (0–30%).

Table 20. Summary of selected morphological findings in rats at 24 months

	Males				Females			
	Dietary concentration (ppm)							
	0	25	100	500	0	25	100	750
Terminal body weight (g)	415.2	418.4	396.0	394.2	278.2	275.4	283.4	257.2*
Liver weight, absolute (g)	12.15	11.87	12.06	12.02	7.59	7.33	7.79	7.67
Liver weight, relative (% of body weight)	2.99	2.86	3.08	3.08*	2.75	2.66	2.75	2.99*
Testes weight, absolute (g)	3.72	3.93	5.42*	6.03*	—	—	—	—
Testes weight, relative (% of body weight)	0.91	0.94	1.36*	1.52*	—	—	—	—
Epididymides weight, absolute (g)	0.56	0.49	0.43*	0.41*	—	—	—	—
Epididymides weight, relative (% of body weight)	0.14	0.12	0.11*	0.11*	—	—	—	—
Liver								
No. examined	*50*	*50*	*50*	*50*	*50*	*50*	*50*	*50*
Aggregates of macrophages								
- very slight	32	40	26	27	38	38	34	26*
- slight	4	1	4	16*	4	0	8	21*
Foci of cellular alteration, basophilic								
- 1–5	17	15	13	18	2	4	3	9
- 6–10	22	14	14	23	7	3	7	17*
- 11–20	5	12	11	1	19	21	18	18
- > 20	0	1	1	0	19	18	18	2*

Table 20 (continued)

	Males				Females			
	Dietary concentration (ppm)							
	0	25	100	500	0	25	100	750
Foci of cellular alteration, eosinophilic								
- 1–5	26	33	26	20	26	32	30	36
- 6–10	7	2	7	11	0	0	1	2
- 11–20	2	0	3	7	0	1	0	0
- > 20	0	0	0	1	0	0	0	0
Hepatocellular hypertrophy								
- very slight	2	0	0	0	0	1	0	33*
- slight	0	0	0	34*	0	0	0	5
Hepatocellular necrosis								
- very slight	2	0	0	24*	0	0	1	22*
- slight	0	0	0	1	0	0	0	1
Hepatocellular vacuolation/fatty change								
- very slight	21	25	21	23	27	35	36	9*
- slight	17	8	12	20	9	7	5	28*
- moderate	1	1	2	0	0	0	1	7*
Hepatocellular adenoma	4	2	5	16*	3	2	2	2
Hepatocellular carcinoma	3	1	1	0	0	0	0	0
Hepatocellular adenoma/carcinoma	7	3	6	16	3	2	2	2
Testes								
No. examined	*50*	*50*	*50*	*50*	—	—	—	—
Seminiferous tubule, atrophy, bilateral	13	15	25*	34*	—	—	—	—
Interstitial cell hyperplasia								
- unilateral	1	2	0	2	—	—	—	—
- bilateral	4	0	3	2	—	—	—	—
Interstitial cell adenoma								
- unilateral[a]	12	8	5	2*	—	—	—	—
- bilateral[a]	32	38	40	44*	—	—	—	—
- unilateral/bilateral[a]	44	46	45	46	—	—	—	—
Epididymides								
No. examined	*50*	*50*	*50*	*50*	—	—	—	—
Decreased spermatic elements, bilateral	21	23	29	37*	—	—	—	—
Coagulating gland								
No. examined	*50*	*50*	*50*	*50*	—	—	—	—
Decreased secretory material	10	11	16	21*	—	—	—	—
Prostate								
No. examined	*50*	*50*	*50*	*50*	—	—	—	—
Decreased secretory material	13	15	17	25*	—	—	—	—
Seminal vesicle								
No. examined	*50*	*50*	*50*	*50*	—	—	—	—
Decreased secretory material	10	11	16	21*	—	—	—	—
Preputial gland								
No. examined	*8*	*8*	*7*	*10*	—	—	—	—
Carcinoma	5	7	7	10	—	—	—	—

From Stebbins et al. (2010a)

* $P < 0.05$

[a] Historical control incidences (24-month sacrifice) from five studies conducted in 2004–2009: interstitial cell adenomas, unilateral: 4–8 (8–16%); interstitial cell adenomas, bilateral: 32–39 (64–78%); interstitial cell adenomas, unilateral or bilateral: 38–46 (76–92%).

Males given 500 ppm had statistically significant increases in the incidence of decreased secretory material in the coagulating glands (severe), prostate (moderate) and seminal vesicles (severe) at 24 months. Males given 500 ppm also had an increased incidence of carcinoma of the preputial gland that was interpreted to be treatment related.

Toxicokinetic analyses of plasma samples at 3 and 12 months indicated dose proportionality in systemic dose levels that were comparable between the two time points. There were no sex differences in plasma concentrations of sulfoxaflor across the dose levels and times analysed. Urinary elimination of sulfoxaflor was also dose proportional for both male and female rats at 3, 6 and 12 months, representing 58–127% of the average test material consumed in a 24-hour period.

Although the mode of action (MOA) for the male reproductive organ effects was not investigated as part of this study, the primary effect was interpreted to be larger interstitial cell adenomas. The effects on seminiferous tubules, epididymides, accessory sex glands and preputial gland were thought to be secondary to loss of normal testicular function due to the large size of the interstitial cell adenomas and possible alterations in the endocrine balance of these rats, as there was a high correlation between the presence of large interstitial cell adenomas and effects noted in the testicular seminiferous tubules, epididymides and accessory sex glands. In regards to severe bilateral atrophy of testicular seminiferous tubules, there was 100% correlation of this finding with the presence of large interstitial cell adenomas (adenomas that occupied approximately 75% or more of the cross-sectional area of the testes). The majority of control and treated males that had severe bilateral decreased spermatic elements of the epididymides, severe bilateral decreased secretory material of the coagulating glands and seminal vesicles and moderate bilateral decreased secretory material of the prostate had large interstitial cell adenomas. These correlations, noted in control and treated males, suggest that the enlargement of the interstitial cell adenomas is the primary effect, and the other effects are secondary to loss of normal testicular function due to the overwhelming size of the interstitial cell adenomas, and not primary responses to administration of sulfoxaflor.

The NOAEL for carcinogenicity was 100 ppm (equal to 4.24 mg/kg bw per day), based on an increased incidence of hepatocellular adenomas in males at 500 ppm (equal to 21.3 mg/kg bw per day). Also at 500 ppm, there was an increased incidence of bilateral Leydig (interstitial) cell adenomas of the testes, whereas there was no effect on the incidence of combined unilateral/bilateral Leydig cell adenomas. The size and weight of the testes and the size of Leydig cell adenomas were increased at 100 and 500 ppm and were associated with the secondary changes in the testes and epididymides listed below.

The NOAEL for non-neoplastic effects was 25 ppm (equal to 1.04 mg/kg bw per day), based on changes in the testes (increased testes weights, increased incidence of severe bilateral atrophy of seminiferous tubules) and epididymides (decreased epididymal weights, increased incidence of severe bilateral decreased spermatic elements of the epididymides) in males at 100 ppm (equal to 4.24 mg/kg bw per day) and above.

In females, the NOAEL for non-neoplastic effects was 100 ppm (equal to 5.13 mg/kg bw per day), based on degenerative changes in the liver (vacuolation/fatty change of hepatocytes) at 750 ppm (equal to 39.0 mg/kg bw per day) (Stebbins et al., 2010a).

2.4 Genotoxicity

Genotoxicity studies with sulfoxaflor are summarized in Table 21.

In a reverse gene mutation assay in bacteria conducted according to OECD test guideline 471 (1997), *Salmonella typhimurium* (strains TA98, TA100, TA1535, TA1537) and *Escherichia coli* (strain WP2*uvr*A) were exposed to sulfoxaflor (purity 96.6%), using DMSO as solvent, in the

Table 21. Summary of genotoxicity studies with sulfoxaflor

End-point	Test object	Concentration or dose	Purity (%)	Result	Reference
In vitro					
Reverse mutation	*Salmonella typhimurium* TA98, TA100, TA1535, TA1537	± S9 mix: 0–5000 µg/plate	96.6	Negative	Mecchi (2007)
	Escherichia coli WP2*uvr*A				
Gene mutation, *HPRT* locus	Chinese hamster ovary cells	± S9 mix: 0, 173.3–2773 µg/ml	96.6	Negative	Schisler, Geter & Trombley (2007)
Chromosomal aberration	Rat lymphocytes (whole blood)	± S9 mix: 0, 173.3–2773 µg/ml	96.6	Negative	Schisler, Geter & Kleinert (2007)
In vivo					
Micronucleus induction	Male and female Crl:CD1(ICR) mice, bone marrow erythroblasts	0, 100, 200 and 400 mg/kg bw; twice (24 h apart); oral administration	95.6	Negative	LeBaron & Schisler (2009)

S9, 9000 × *g* rat liver supernatant

presence and absence of S9 metabolic activation in two independent sets of experiments. Doses were selected based on the results of a range-finding study. For the initial preincubation test using doses of up to and including 5000 µg/plate, three plates were used for each strain, condition and dose. Vehicle and positive controls were included in each experiment. The independent repeat utilized the same conditions as the initial test. Doses up to and including 5000 µg/plate did not cause any bacteriotoxic effects. Total bacteria counts remained unchanged, and no inhibition of growth was observed.

No evidence for mutagenic activity of sulfoxaflor was seen. No biologically relevant increase in the mutant count, in comparison with the negative controls, was observed. The positive controls induced the appropriate responses in the corresponding strains. Therefore, sulfoxaflor was considered to be not mutagenic in the bacterial strains tested, in either the presence or absence of metabolic activation (Mecchi, 2007).

In an in vitro mammalian cell gene mutation test conducted according to OECD test guideline 476 (1997), sulfoxaflor (purity 96.6%) dissolved in DMSO was tested for its ability to induce forward mutations at the *HPRT* locus in Chinese hamster ovary (CHO) cells. Two independent sets of experiments were conducted in the presence and absence of S9 metabolic activation. Based on the guideline limit dose (10 mmol/l), concentrations of 173.3–2773 µg/ml were used in the main study both with and without metabolic activation, and the same concentrations were used for the independent repeats. Ethyl methanesulfonate (EMS) and 20-methylcholanthrene (20-MCA) served as positive controls in the experiments without and with metabolic activation, respectively. The cells were treated for 4 hours in both experiments, without and with metabolic activation. After this, the incubation media were replaced by culture medium, and the cells were incubated (and subcultured every 2–3 days) for about 7–9 days for expression of mutant cells. This was followed by incubation of cells for 6–10 days in selection medium containing 6-thioguanine.

Neither in the initial nor in the confirmatory study was any increase in the mutant frequency observed. In contrast to this, the positive control substances EMS and 20-MCA resulted in a marked increase in mutant frequency. Based on the results of the study, sulfoxaflor was considered to be not mutagenic in the CHO/HPRT forward mutation assay, in either the presence or absence of metabolic activation (Schisler, Geter & Trombley, 2007).

In an in vitro mammalian chromosomal aberration test conducted according to OECD test guideline 473 (1997), the clastogenic potential of sulfoxaflor (purity 96.6%) dissolved in DMSO was

tested in rat lymphocytes (whole blood). Based on the results of the mitotic index, concentrations of 693.3, 1386.5 and 2773 µg/ml were used with and without metabolic activation in an experiment with 4 hours of treatment and harvest at 24 hours (i.e. 20 hours post-exposure). In addition, with 24 hours of treatment without S9 mix, concentrations of 173.3, 346.6 and 693.3 µg/ml were chosen. Vehicle (DMSO) and positive controls (cyclophosphamide and mitomycin C for the test with and without metabolic activation, respectively) were included to demonstrate the sensitivity of the test system. Cultures were set up in duplicate. After slide preparation and staining of the cells, 200 metaphases per dose and treatment condition were analysed for chromosomal aberrations.

None of the cultures treated with sulfoxaflor in the absence or in the presence of S9 mix showed any biologically relevant or statistically significant increase in the numbers of aberrant metaphases. No change in the frequency of polyploid metaphases was observed. The positive controls mitomycin C and cyclophosphamide induced clastogenic effects and demonstrated the sensitivity of the test system and the activity of the S9 mix used. Based on the results of this test, sulfoxaflor was considered not to be clastogenic for mammalian cells in vitro, in either the presence or absence of metabolic activation (Schisler, Geter & Kleinert, 2007).

In a mammalian erythrocyte micronucleus test conducted according to OECD test guideline 474 (1997), groups of six male and six female Crl:CD1(ICR) mice received two oral (gavage) doses (24 hours apart) of sulfoxaflor (purity 95.6%) at 0 (0.5% aqueous hydroxypropyl methylcellulose/ methylcellulose polymers), 100, 200 or 400 mg/kg bw in a volume of 10 ml/kg bw. The doses were selected based on the results of a range-finding experiment (250, 500, 1000, 1500, 2000 mg/kg bw per day, 3–4 animals of each sex). The vehicle served as negative control, and cyclophosphamide (one dose at 120 mg/kg bw) served as positive control. The animals were terminated 24 hours after the last administration, the bone marrow of the two femora was prepared and 2000 polychromatic erythrocytes were evaluated per animal (five animals) and investigated for micronuclei. The ratio of polychromatic erythrocytes to normochromatic erythrocytes was determined in 200 erythrocytes.

In the range-finding experiment, most of the animals (both sexes) died within 2 hours after administration of doses of 1000 mg/kg bw per day and above. Animals dosed with 250 or 500 mg/kg bw per day showed little or no clinical signs of toxicity.

In the main study, decreased activity was observed in sulfoxaflor-treated mice at 400 mg/kg bw. There was no substance-induced mortality. No signs were recorded for the control groups or other dose groups, and no animals died in these groups. Body temperature was decreased in animals dosed with 400 mg/kg bw per day.

Two oral doses of sulfoxaflor up to and including 400 mg/kg bw did not lead to any increase in the number of polychromatic erythrocytes with micronuclei. No inhibition of erythropoiesis, determined from the ratio of polychromatic to normochromatic erythrocytes, was observed. The positive control caused a clear increase in the number of polychromatic erythrocytes with micronuclei, thus demonstrating the sensitivity of the test system. Based on the results of this test, sulfoxaflor was considered not to be clastogenic in vivo in mice (LeBaron & Schisler, 2009).

2.5 Reproductive toxicity

(a) Multigeneration studies

In a reproduction/developmental toxicity screening study conducted according to OECD test guideline 421, groups of 12 male and 12 female Crl:CD(SD) rats were given diets containing sulfoxaflor (purity 95.6%) at a concentration of 0, 100, 500 or 1000 ppm (equal to 0, 8.26, 40.7 and 79.1 mg/kg bw per day in males and 0, 8.30, 42.2 and 81.6 mg/kg bw per day in females, respectively). Males were fed the test diets for 2 weeks prior to breeding and continuing throughout breeding until

termination. The females were fed the test diets for 2 weeks prior to breeding, continuing through breeding (up to 2 weeks), gestation, lactation and weaning; pups were weaned on postnatal day (PND) 21. Effects on gonadal function, mating behaviour, conception, development of the conceptus, parturition and postnatal growth and survival were evaluated. In addition, a gross necropsy and histopathological examination of the adults were conducted with an emphasis on organs of the reproductive system. In the offspring, litter size, pup survival, sex, body weight and the presence of gross external morphological alterations were assessed.

All parental animals survived until termination, and there were no treatment-related clinical observations at any dose level throughout the study. Males in the 1000 and 500 ppm dose groups had a treatment-related decrease in feed consumption and body weight (1000 ppm only) during the 1st week of treatment. Females in the 1000 and 500 ppm dose groups had treatment-related decreases in body weight gain during the 1st week of gestation, and females in the 1000 ppm dose group had slightly lower feed consumption during the pre-breeding and gestation phases.

Males of the 1000 and 500 ppm dose groups had increased absolute and relative liver weights that were dose and treatment related (liver weights for females at 1000 ppm were not recorded due to an effect on litter survival; there was no effect at 500 ppm). Treatment-related histological effects were observed in the livers of males given 1000 and 500 ppm and females given 1000 ppm and consisted of a dose-related increase in the severity of hepatocellular hypertrophy, with altered tinctorial properties, involving the centrilobular to midzonal regions of the hepatic lobule. Histological effects were of very slight or slight severity in males and very slight severity in females. In addition, there was treatment-related multifocal hepatocyte vacuolation (slight severity) in 1000 ppm females (Table 22).

There were no reproductive or developmental toxicity effects observed in any group up to PND 0 (birth). Shortly after birth, there was a significant decrease in pup survival in the 1000 ppm dose group, such that PND 1 survival was 46.3%, compared with 98.3% in controls. In addition, PND 1 pup body weights were significantly decreased (22–25%) relative to controls. By PND 4, 11 of 12 dams had total litter loss, resulting in 7.3% pup survival, compared with 95.4% in controls. Because only 1 of 12 litters remained, this dose group was terminated on PND 6. Pup survival was also affected in the 500 ppm group, with 4 of 12 dams losing approximately half of their litters by PND 4, which resulted in a pup survival rate of 81.2% compared with 95.4% in controls. There were no effects on pup body weight in the 500 ppm group. There were no treatment-related effects on any other reproductive parameters at 1000 or 500 ppm, and no reproductive effects whatsoever at 100 ppm (Table 23).

Analyses of plasma samples from PND 4 culled pups indicated a dose-proportionate increase in the systemic concentration of sulfoxaflor. In plasma and milk samples taken from three dams of the 1000 ppm group on PND 4 or PND 6, the concentrations of sulfoxaflor were equivalent (26.8 µg/g in plasma, 28.3 µg/g in milk).

The NOAEL for parental toxicity was 100 ppm (equal to 8.26 mg/kg bw per day), based on decreased body weight gains in females during the 1st week of gestation at 500 ppm (equal to 40.7 mg/kg bw per day) and above.

The NOAEL for effects on offspring was 100 ppm (equal to 8.26 mg/kg bw per day), based on reduced pup survival at 500 ppm (equal to 40.7 mg/kg bw per day) and above (Rasoulpour et al., 2010b).

In a two-generation reproductive toxicity study conducted according to OECD test guideline 416, groups of 27 male and 27 female Crl:CD(SD) rats were given diets containing sulfoxaflor (purity 95.6%) at a concentration of 0, 25, 100 or 400 ppm, equal to 0, 1.52–1.74, 6.07–6.86 and 24.6–28.1 mg/kg bw per day in males (F_0 and F_1 generation, respectively, for the entire dosing period) and 0, 1.91–2.11, 7.82–8.39 and 30.5–34.3 mg/kg bw per day in females (F_0 and F_1 generation, respectively, for the pre-mating period), for approximately 10 weeks prior to breeding and continuing through

Table 22. Summary of selected parental findings in a reproduction/developmental toxicity screening study in rats

	Males				Females			
	Dietary concentration (ppm)							
	0	100	500	1000	0	100	500	1000
Body weight (g), pre-mating								
- day 7	312.4	309.1	304.0	293.9*	212.5	206.3	205.8	202.1
- day 14	366.6	360.1	354.4	343.4*	239.1	225.9	225.9	226.0
Body weight gain (g), gestation								
- days 0–7	—	—	—	—	49.2	48.9	40.6*	37.0*
- days 0–20	—	—	—	—	162.1	171.0	151.5	150.7
Feed consumption (g/day), pre-mating								
- days 1–7	26.4	25.2	24.0*	22.2*	18.1	17.3	17.0	16.0
- days 7–14	27.5	25.9	25.7	24.8	19.3	17.0*	17.8	17.4*
Terminal body weight (g)	405.3	402.2	397.0	386.9	298.3	291.7	287.1	284.7
Liver weight, absolute (g)	12.80	12.66	14.08	14.55	12.34	12.18	12.43	12.74
Liver weight, relative (% of body weight)	3.14	3.14	3.54*	3.75*	4.13	4.18	4.33	4.47
Hepatocellular hypertrophy (no.)								
- very slight	0	0	10	0	1	0	0	12
- slight	0	0	1	12	0	0	0	0
Hepatocellular vacuolation (no.)								
- very slight	7	8	8	7	11	10	6	8
- slight	0	0	0	0	0	0	0	2

From Rasoulpour et al. (2010b)
* $P < 0.05$

Table 23. Summary of selected findings for reproductive performance and litter parameters in rats

	0 ppm	100 ppm	500 ppm	1000 ppm
Mating index (%)	100	100	100	100
Fertility index (%)	100	100	100	100
Gestation index (%)	100	100	100	100
Gestation length (days)	21.4	21.3	21.8	21.7
Time to mating (days)	2.5	2.8	3.4	3.3
Mean litter size (born live)				
- PND 0	14.4	14.9	13.8	14.8
- PND 1	14.2	14.9	13.0	6.8*
- PND 4 (before/after culling)	13.8/8.0	14.6/8.0	11.2/7.8	1.6*/1.0*
- PND 7	8.0	7.8	7.8	—
- PND 21	8.0	7.8	7.8	—
Survival index (%)				
- PND 1	98.3	100.0	94.5[a]	46.3*
- PND 4, before culling	95.4	97.8	81.2[a]	7.3*
- PND 21, after culling	100.0	97.9	100.0	—
Mean pup body weight (g), males/females				
- PND 1	6.8/6.3	6.6/6.4	6.7/6.3	5.1*/4.9*
- PND 4, before culling	9.5/9.0	9.0/8.5	9.3/8.8	7.1/7.3
Plasma concentration of sulfoxaflor (µg/g), PND 4	0	1.3	7.4	15.8

From Rasoulpour et al. (2010b)
* $P < 0.05$
[a] Outside historical control range (PND 1: 96.2–100%; PND 4: 94.0–100%).

breeding, gestation and lactation for two generations. In-life parameters included clinical observations, feed consumption, body weights, estrous cyclicity, reproductive performance, pup survival, pup body weights, puberty onset and anogenital distance. In addition, postmortem evaluations included gross pathology and organ weights in weanlings and toxicokinetic analyses, gross pathology, organ weights, oocyte quantification and sperm count, motility and morphology, and histopathology in adults.

In the parental animals, there were no treatment-related mortalities or clinical observations and no treatment-related effects on body weight or feed consumption in either generation at any dose level. Also, there were no treatment-related effects on estrous cyclicity, sperm analysis parameters, mating, conception, fertility or gestation indices, time to mating or gestation length in either generation at any dose level.

Parental postmortem observations consisted of increased absolute and relative liver weights in the F_0 (12.8% and 10.9%, respectively) and F_1 (6.5% and 7.8%, respectively) males at 400 ppm. This effect on liver weight correlated with histopathological findings of very slight to slight centrilobular hepatocyte hypertrophy, often with a very slight increase in individual cell necrosis of centrilobular hepatocytes (Table 24). No other systemic effects were noted at 400 ppm, and there were no treatment-related effects on F_0 or F_1 parameters in male or female rats at 25 or 100 ppm.

Reproductive effects were limited to the 400 ppm dose group and comprised slightly decreased neonatal (PNDs 1 and 4) survival in both generations. As a consequence of this effect on neonatal pup survival, there was a statistically significant decrease in gestation survival index (percentage of live born pups/total pups delivered) in the high-dose F_2 litters. As gestation survival index is a ratio of live pups over total pups delivered, this slight decrease in the high-dose group index is attributed to treatment-related neonatal deaths known to occur at or shortly after birth with high exposures to sulfoxaflor (Table 25).

Evidence indicating that survival is not affected before birth comes from a cross-fostering study, developmental toxicity study and two critical windows of exposure studies, all demonstrating that in utero exposure to sulfoxaflor caused postnatal, and not gestational, death. Given the clear profile of neonatal deaths, effectively coincident with birth and during the very early postnatal period, it is most appropriate to combine the gestation survival index data with PND 1–4 survival to create a combined "PND 0–4 survival" category. Here, there is a clear treatment-related, statistically significant decrease in total pup survival in the 400 ppm F_2 litters (Table 25).

In addition, there was an apparent treatment-related, statistically significant delay (2.4 days) in puberty onset (preputial separation) for F_2 males at 400 ppm without a corresponding decrement in body weight at the time of attainment (Table 25). This external marker of male puberty onset is androgen dependent, but the underlying mechanism to explain how sulfoxaflor induced this effect is not known; however, there were no other indications of androgenic or anti-androgenic effects. This included no treatment-related effects on anogenital distance, no effects on testis or accessory sex gland (i.e. prostate, seminal vesicle and epididymis) weight or histopathology, no evidence of malformations, such as hypospadias or ectopic testes, no effects on mating, fertility, time to mating or gestation length, and no treatment-related effects on preputial separation at the same dose level in a developmental neurotoxicity study with sulfoxaflor. Taken together, the weight of evidence across androgen-sensitive end-points does not support any other sulfoxaflor-mediated anti-androgenic effects. There were no effects on puberty onset or any other parameter of reproductive performance or offspring growth and survival at 25 or 100 ppm.

Owing to the statistically significant delay in male puberty onset at 400 ppm in the F_1 offspring, anogenital distance measurements on F_2 litters were triggered as specified in the relevant test guidelines. There were no treatment-related effects on absolute or relative anogenital distance in male or female pups at any dose level. Although female anogenital distance and relative anogenital distance were statistically significantly reduced at 25 and 400 ppm, these differences were not considered treatment related, as they did not exhibit a dose–response relationship (Table 25). Furthermore, anogenital

Table 24. Summary of selected parental findings in a two-generation reproductive toxicity study in rats

	Males				Females			
	Dietary concentration (ppm)							
	0	25	100	400	0	25	100	400
F_0 generation								
Body weight (g), pre-mating, day 71	523.8	543.7	529.5	540.1	303.4	295.1	298.4	290.2
Terminal body weight (g)	568.0	584.6	578.3	584.4	313.1	309.1	306.6	308.3
Liver weight, absolute (g)	15.09	15.98	15.49	17.02*	9.61	9.91	9.57	10.02
Liver weight, relative (% of body weight)	2.65	2.73	2.68	2.91*	3.06	3.20	3.12	3.26
Liver, no. examined histopathologically	*27*	*27*	*27*	*27*	*27*	*6*	*6*	*27*
Hepatocellular hypertrophy								
- very slight	0	0	0	2	0	0	0	0
- slight	0	0	0	24	0	0	0	0
Hepatocellular necrosis, single cell, centrilobular								
- very slight	9	7	9	25	3	0	0	5
Hepatocellular vacuolation/fatty change, centrilobular								
- very slight	0	0	2	4	0	0	0	0
- slight	1	0	0	2	0	0	0	0
F_1 generation								
Body weight (g), pre-mating, day 71	559.7	573.1	562.8	556.0	302.7	301.5	301.5	292.5
Terminal body weight (g)	630.3	640.0	633.4	624.5	322.0	323.1	320.6	309.9
Liver weight, absolute (g)	17.45	17.47	17.32	18.59	10.10	10.44	10.14	10.15
Liver weight, relative (% of body weight)	2.76	2.72	2.74	2.98*	3.14	3.23	3.17	3.27
Liver, no. examined histopathologically	*27*	*27*	*27*	*27*	*27*	*6*	*6*	*27*
Hepatocellular hypertrophy								
- very slight	0	0	1	19	0	0	0	0
- slight	0	0	0	7	0	0	0	0
Hepatocellular necrosis, single cell, centrilobular								
- very slight	6	6	4	12	1	0	0	0
Hepatocellular vacuolation/fatty change, centrilobular								
- very slight	1	0	0	0	0	0	0	0

From Rasoulpour et al. (2010c)
* $P < 0.05$

distance was decreased in treated females, relative to controls, whereas alterations in endocrine function would typically result in an increase in anogenital distance in females (i.e. masculinization).

Toxicokinetic data from lactation day (LD) 4 dams and culled PND 4 pups in the second generation show dose-proportional systemic exposure to sulfoxaflor in dams and their offspring. Plasma concentrations of sulfoxaflor in rat pups were, on average, 32% of the levels measured in the dams (Table 25).

The NOAEL for parental toxicity was 100 ppm (equal to 6.07 mg/kg bw per day), based on liver toxicity (increased incidence of vacuolation/fatty change of centrilobular hepatocytes) in F_0 males at 400 ppm (equal to 24.6 mg/kg bw per day).

Table 25. Summary of selected findings for reproductive performance and litter parameters in rats

	0 ppm	25 ppm	100 ppm	400 ppm
F_1 generation				
Mating index (%), males/females	92.6/92.6	100/100	100/100	100/100
Fertility index (%), males/females	92.6/92.6	100/96.3	88.9/88.9	100/100
Gestation index (%)	100	100	100	100
Gestation length (days)	21.7	21.5	21.6	21.6
Time to mating (days)	2.7	3.3	2.3	3.3
Gestation survival (%)	99.2	99.5	99.7	100
Postimplantation loss (%)	5.92	5.85	8.23	7.24
Mean litter size (born live)				
- PND 0	14.8	14.7	14.5	13.8
- PND 1	14.7	14.6	14.3	13.5
- PND 4	14.5	14.4	14.2	13.1
- PNDs 7, 14 and 21	8.0	8.0	7.8	7.7
PND 1 survival (%)	99.4	99.2	99.1	98.1
PND 4 survival (%)	97.2	97.9	97.1	95.4
F_2 generation				
Mating index (%), males/females	96.3/96.3	92.0/92.3	100/100	96.2/96.3
Fertility index (%), males/females	92.6/92.6	92.0/92.3	92.3/92.3	92.3/92.6
Gestation index (%)	100	100	100	100
Gestation length (days)	21.6	21.7	21.7	21.5
Time to mating (days)	3.0	2.7	2.3	2.6
Gestation survival (%)	99.7	99.1	98.8	97.4*
Postimplantation loss (%)	7.35	8.11	6.87	14.03[a]
Mean litter size (born live)				
- PND 0	13.2	14.3	14.3	13.4
- PND 1	13.1	14.2	14.1	12.9
- PND 4	13.0	14.0	13.9	12.8
- PNDs 14 and 21	7.9	7.9	8.0	7.8
PND 1 survival (%)	99.7	99.1	98.5	96.7
PND 4 survival (%)	98.8	98.0	97.1	95.5
PND 0–4 survival (%)	98.5	97.1	96.0	93.0*[b]
Anogenital distance (mm)/body weight (g)				
- males	3.76/7.3	3.68/7.4	3.64/7.2	3.55/6.9
- females	2.13/6.9	1.97*/7.0	2.01/6.8	1.97*/6.6
Days to vaginal opening/body weight (g)	31.6/117.2	31.7/116.4	31.4/114.0	32.1/112.2
Days to preputial separation/body weight (g)	44.6/253.6	46.4/265.8	44.5/250.3	47.0*[c]/272.8
Test material intake by dams (mg/kg bw per day), LD 4	0	2.1	8.5	29.2
Plasma concentration in dams (µg/g), LD 4	0	1.1	4.5	15.9
Plasma concentration in male pups (µg/g), LD 4	0	< 0.6 (LLQ)	1.4	5.3
Plasma concentration in female pups (µg/g), LD 4	0	< 0.6 (LLQ)	1.5	5.8

From Rasoulpour et al. (2010c)

LD, lactation day; LLQ, lowest level quantified; PND, postnatal day; * $P < 0.05$

[a] Outside historical control range for postimplantation loss (5.06–10.7%).

[b] Outside historical control range for PND 0–4 survival (93.4–98.4%).

[c] Outside historical control range for preputial separation (44.3–45.7 days).

The NOAEL for effects on fertility was 400 ppm (equal to 24.6 mg/kg bw per day), the highest dose tested.

The NOAEL for effects on offspring was 100 ppm (equal to 6.07 mg/kg bw per day), based on reduced pup survival and delayed preputial separation (puberty onset) in F_2 males at 400 ppm (equal to 24.6 mg/kg bw per day) (Rasoulpour et al., 2010c).

In a cross-fostering study conducted to assess whether the observed effects of sulfoxaflor on neonatal survival in rats resulted from in utero and/or lactational exposure, groups of 32 time-mated female Crl:CD(SD) rats were given diets containing sulfoxaflor (purity 95.6%) at a concentration of 0 or 1000 ppm for 2 weeks prior to mating through weaning on LD 21. As the control and treated females mated, they were subdivided into foster dams and donor dams. A caesarean section was performed on gestation day (GD) 21. At this time, one or more batches of two of the offspring of each sex from donor dams were immediately cross-fostered to a foster dam that had its own litter removed that day (i.e. on LD 0). After cross-fostering was complete, each control and sulfoxaflor-treated foster dam had mixed litters composed of two pups of each sex that originated from control donor dams (five litters) and two pups of each sex that originated from sulfoxaflor-treated donor dams (eight litters). This design controlled for litter of origin effects and enabled comparison of the survival of pups exposed to sulfoxaflor during gestation alone or during lactation alone with unexposed control pups and pups exposed during both gestation and lactation.

Dams given 1000 ppm sulfoxaflor had treatment-related effects on body weight, body weight gain and feed consumption consistent with effects seen at this dose level in the previous reproduction/developmental toxicity screening study. Time-weighted average doses for treated animals were 81.2, 74.5 and 59.5 mg/kg bw per day in the pre-mating, gestation and lactation periods, respectively. These corresponded to maternal sulfoxaflor blood concentrations of 23.0–29.3 (mean 27.0) µg/g plasma on GD 21 and 19.6–25.0 (mean 22.4) µg/g plasma on LD 0. The average measured concentration of sulfoxaflor in plasma from male/female pups on GD 21 and LD 0 from these dams was 24.8/24.8 and 25.3/25.9 µg/g plasma, respectively. Thus, fetal and pup plasma levels of sulfoxaflor were very similar to one another and very similar to dam plasma levels. The measured sulfoxaflor concentrations in milk from the same dams on LD 0 were approximately half the corresponding plasma levels and ranged from 12.3 to 14.0 (mean 13.3) µg/g milk.

All offspring from dams exposed to 1000 ppm sulfoxaflor prior to birth died by PND 4, irrespective of whether they were cross-fostered to control or treated foster dams (Table 26). Consistent with reduced viability, some offspring were cold to the touch, had bluish skin, were autolysed and cannibalized and had stomachs void of milk.

Conversely, there was no effect on neonatal survival for pups exposed to sulfoxaflor only after birth. Furthermore, PND 1 pup body weights were significantly decreased in prenatally exposed offspring.

In conclusion, these data demonstrate that the effect of sulfoxaflor on pup survival was due to in utero, not lactational, exposure (Rasoulpour & Zablotny, 2010a).

(b) Developmental toxicity

Rats

In a prenatal developmental toxicity range-finding study, groups of seven time-mated female Crl:CD(SD) rats were given diets containing sulfoxaflor (purity 95.6%) at a concentration of 0, 500, 1000, 1500 or 2000 ppm (equal to 0, 35.4, 68.0, 86.7 and 94.2 mg/kg bw per day) on GD 6 through GD 21. In-life parameters evaluated for all groups included clinical observations, body weight, body weight gain and feed consumption. On GD 21, all surviving rats were euthanized and examined for gross pathological alterations. Liver and kidney weights were recorded, along with the number of corpora lutea, implantations, resorptions and live/dead fetuses.

Table 26. Summary of selected findings in a cross-fostering study in rats.

	Dietary concentration (ppm), in gestation/in lactation			
	0/0	1000/0	0/1000	1000/1000
Litter size				
- PND 0	4.0	4.0	4.0	4.0
- PND 1	4.0	0.6	3.9	1.6
- PND 4	4.0	0.0	3.9	0.0
Survival (%)				
- PND 1	100.0	15.0	96.9	40.6
- PND 4	100.0	0.0	96.9	0.0
Pup body weights (g), males/females				
- PND 0	5.5/5.2	5.2/5.1	5.7/5.3	5.5/5.1
- PND 1	6.0/5.5	4.5[a]/4.8	6.3/5.8	4.8/4.6

From Rasoulpour & Zablotny (2010a)
PND, postnatal day
[a] Only one litter with pups remaining on PND 1.

Administration of sulfoxaflor to time-mated rats resulted in excessive systemic toxicity at 1500 and 2000 ppm, as evidenced by body weight loss, decreased body weight gain and decreased feed consumption. Therefore, all animals in these groups were euthanized for humane reasons on GD 13, with no further collection of data. Animals in the 1000 ppm dose group had transient and less severe treatment-related decreases in body weight/body weight gain, decreased feed consumption and increased relative liver weights. The 500 ppm dose group had treatment-related, transient decreases in feed consumption during the first 3 days of treatment; however, the body weights and body weight gains remained comparable to those of controls. There were no treatment-related clinical observations in any group tested and no treatment-related gross pathology observations, effects on pregnancy rates, effects on numbers of corpora lutea and implantations, increase in resorption rate or litter size or indication of embryo/fetal lethality in animals given diets containing 500 or 1000 ppm sulfoxaflor (Rasoulpour, Marshall & Yano, 2008).

Based on the results of this range-finding study, dietary concentrations of 25, 150 and 1000 ppm sulfoxaflor were selected for the definitive developmental toxicity study.

In a prenatal developmental toxicity study conducted according to OECD test guideline 414, groups of 26 time-mated female Crl:CD(SD) rats were given diets containing sulfoxaflor (purity 95.6%) at a concentration of 0, 25, 150 or 1000 ppm (equal to 0, 1.95, 11.5 and 70.2 mg/kg bw per day) on GD 6 through GD 21. In-life maternal study parameters included clinical observations, body weight, body weight gain and feed consumption. On GD 21, all rats were euthanized, and all dams and fetuses were examined for gross pathological alterations. In addition, blood was collected from dams and fetuses to determine blood levels of the test material. Liver, kidneys and gravid uterine weights were recorded, along with the numbers of corpora lutea, uterine implantations, resorptions and live/dead fetuses. All fetuses were weighed, sexed and examined for external alterations. Approximately one half of the fetuses were examined for visceral and craniofacial alterations, whereas skeletal examinations were conducted on the remaining fetuses.

Maternal toxicity was evidenced at 1000 ppm by decreases in body weight and body weight gains, relative to controls, with concomitant decreased feed consumption, throughout the treatment period and increased relative liver weights (Table 27).

Table 27. Summary of selected findings in a prenatal developmental toxicity study in rats

	0 ppm	25 ppm	150 ppm	1000 ppm
Dose in dams (mg/kg bw per day), last week	0	1.6	9.3	64.2
Plasma concentration in dams (µg/g), GD 21	0	0.84	4.94	35.2
Plasma concentration in fetuses (µg/g), GD 21	0	0.64	4.07	30.0
No. of females pregnant	24	23	25	25
Body weight gain (g), GDs 6–9	15.5	15.7	17.3	0.8*
Body weight gain (g), GDs 6–21	152.9	150.1	154.0	118.6*
Body weight gain (g), GDs 0–21	189.4	187.8	192.5	152.4*
Terminal body weight (g), corrected	319.0	317.9	319.9	294.9*
Liver weight, absolute (g)	14.7	14.4	14.6	14.2
Liver weight, relative (% of body weight)	3.45	3.40	3.43	3.66*
Feed consumption (g/day), GDs 6–7	22.5	22.8	21.7	15.7*
Feed consumption (g/day), GDs 7–8	22.5	22.8	22.8	13.7*
Feed consumption (g/day), GDs 8–9	23.5	22.8	23.6	17.7*
No. of litters with viable fetuses	24	23	25	25
Number of corpora lutea per dam	14.1	14.1	14.3	13.5
Number of implantations per dam	13.5	13.3	13.9	13.0
Number of resorptions per litter	0.2	0.7	0.6	0.7
Mean postimplantation loss (%)	1.4	4.9	5.1	5.2
No. of live fetuses per litter	13.3	12.7	13.3	12.3*
Fetal body weights (g), males	5.94	6.02	6.02	5.29*
Fetal body weights (g), females	5.67	5.71	5.63	4.99*
Gravid uterine weight (g)	106.4	102.8	106.6	92.3*
External examination				
No. of fetuses/litters	320/24	278/22	332/25	295/24
Forelimb flexure, slight (no. of fetuses/litters)	0/0	0/0	0/0	154*/20[a]
Forelimb flexure (no. of fetuses/litters)	0/0	0/0	0/0	122*/23
Hindlimb rotation (no. of fetuses/litters)	0/0	0/0	0/0	12*/7
Visceral examination				
No. of fetuses/litters	168/24	139/22	173/26	149/24
Convoluted ureter (no. of fetuses/litters)	0/0	1/1	0/0	19*/7
Skeletal examination				
No. of fetuses/litters	152/24	126/22	159/25	133/24
Delayed ossification, parietal (no. of fetuses/litters)	0/0	0/0	1/1	5*/4
Bent clavicle (no. of fetuses/litters)	0/0	0/0	0/0	40*/17
Fused sternebrae (no. of fetuses/litters)	0/0	0/0	1/1	6*/5

From Rasoulpour, Marshall & Saghir (2010)
* $P < 0.05$
[a] Four litters were excluded.

Developmental toxicity was evidenced at 1000 ppm by decreases in fetal body weight and gravid uterine weight and clear increases in several fetal abnormalities (see below). The external examination at 1000 ppm revealed that 40% of the fetuses (122/295) had unilateral or bilateral forelimb flexure and 12 fetuses had hindlimb rotation abnormalities, whereas approximately 60%

of the fetuses (154/248) had unilateral or bilateral slight forelimb flexure (variation). The incidences of severe forelimb flexure, hindlimb rotation and slight forelimb flexure were statistically significant and considered treatment related. Fetuses in this group exhibited a contracted or hunched posture of the body, limbs and neck. This did not appear to be a structural defect, but instead was noted during visceral examination as a difficulty in laying the fetuses flat due to skeletal muscle contracture.

At the visceral examination at 1000 ppm, 19 of 149 fetuses had unilateral or bilateral convoluted ureter, the incidence of which reached statistical significance and was deemed treatment related. Two of these fetuses also had hydroureter.

The skeletal examination at 1000 ppm showed that there were 40 of 133 fetuses with unilateral or bilateral bent clavicle, which co-occurred with limb abnormalities in 35 of 40 fetuses. This finding was statistically significant and considered treatment related. Also at 1000 ppm, there was a treatment-related increase in the incidence of a skeletal variation, fused sternebrae (6/133 fetuses).

Administration of 150 or 25 ppm sulfoxaflor produced no treatment-related maternal toxicity and no indications of embryo/fetal toxicity or teratogenicity.

The terminal plasma concentrations of sulfoxaflor in both dam and fetal blood were dose proportional throughout the entire range of dietary exposure concentrations, with similar levels in the maternal and fetal blood compartments.

The NOAEL for maternal toxicity was 150 ppm (equal to 11.5 mg/kg bw per day), based on decreases in body weight and body weight gain and decreased feed consumption at 1000 ppm (equal to 70.2 mg/kg bw per day).

The NOAEL for prenatal developmental toxicity was 150 ppm (equal to 11.5 mg/kg bw per day), based on increases in several fetal abnormalities (forelimb flexure, bent clavicle, hindlimb rotation, fused sternebrae, convoluted ureter and hydroureter) at 1000 ppm (equal to 70.2 mg/kg bw per day) (Rasoulpour, Marshall & Saghir, 2010).

A special developmental toxicity study was conducted to determine the critical window of susceptibility of rat fetuses and to test the hypothesis that late gestational exposure to sulfoxaflor induces fetal abnormalities and reduced neonatal survival via its pharmacological action on the fetal muscle nAChR. This receptor develops functional expression between GD 16 and GD 17 in the rat, resulting in synchronized fetal limb movements and diaphragmatic responsiveness important for the transition to extrauterine respiration.

Groups of 12 time-mated female Crl:CD(SD) rats were given diets containing sulfoxaflor (purity 95.6%) at a concentration of 0 or 1000 ppm beginning on GD 6 or 16. Group 1 received control feed (0 ppm) from GD 6 until termination on LD 4. Group 2 was administered feed containing 1000 ppm sulfoxaflor from GD 6 until the morning of GD 16, to cover all of embryogenesis up to, but not including, the start of early fetal movements, and was then switched to control feed (0 ppm) until termination on LD 4. Group 3 was administered control feed (0 ppm) from GD 6 until the morning of GD 16, offered feed containing 1000 ppm sulfoxaflor from GD 16 until parturition in order to cover development of the muscle nAChR and its role in development of synchronized fetal limb movements up to onset of parturition and switched back to control feed (0 ppm) until termination on LD 4. For toxicokinetic analysis, a blood sample was taken from four females per group on GD 16 (control and group 2) and GD 21 (control and group 3) to measure plasma sulfoxaflor concentration.

In the offspring, effects on litter size, survival, body weight and the presence of gross external morphological alterations, with particular focus on limb abnormalities (e.g. forelimb flexure and hindlimb rotation), were carefully assessed. All pups surviving to PND 4 were examined for gross external alterations and euthanized by oral administration of sodium pentobarbital solution. Following external examination, one randomly selected pup of each sex from groups 1 and 2 was examined internally for convoluted ureters. In addition, one randomly selected pup or all group 3 pups of each

sex per litter were viscerally examined for convoluted ureters. Following visceral examination, the pups were preserved in alcohol, macerated, stained with Alizarin Red-S in order to visualize ossified bone, cleared and examined for bent clavicles. Any pups found dead or that were euthanized in moribund condition were examined to the extent possible and discarded.

Offspring from animals given 1000 ppm sulfoxaflor from GD 6 to GD 16 (group 2) were completely normal and did not display previously described fetal abnormalities or reduced neonatal survival. In contrast, offspring given 1000 ppm sulfoxaflor from GD 16 to birth (group 3) had the same gross effects of limb contractures and reduced neonatal survival seen in the previous studies that had treatment with 1000 ppm sulfoxaflor throughout gestation (Table 28). This demonstrates that the critical window of susceptibility for both of these effects falls within GD 16 to birth.

In addition, daily examination of group 3 offspring born with limb abnormalities confirmed that these were reversible in a number of pups from five litters shortly after withdrawal of maternal dietary exposure to sulfoxaflor (Table 29). In some cases, full reversal of the limb abnormalities was evident the day after birth and occurred for all affected animals that survived to PND 4; reversal also occurred in some animals that subsequently died before PND 4. Likewise, the visceral and skeletal findings of abnormal ureter and bent clavicle, the latter of which had a high incidence (30.1% of fetuses), in the definitive developmental toxicity study were not present in this study at necropsy on PND 4, despite similar blood concentrations and limb abnormality indices between these two studies.

The study demonstrated that the critical period of developmental susceptibility to sulfoxaflor-induced fetal abnormalities and reduced neonatal survival is between GD 16 and birth and that the fetal limb abnormalities were rapidly reversible after birth in pups surviving to PND 4. These results support the hypothesis that late gestational exposure to sulfoxaflor induces fetal abnormalities and neonatal death via its pharmacological action on the fetal muscle nAChR, which develops functional expression during this stage of gestation (Rasoulpour & Zablotny, 2010b).

In a subsequent special developmental toxicity study conducted to determine the critical window of susceptibility of rat fetuses, the GD 16 to birth exposure period was divided into three 48-hour exposure windows starting on the morning of GD 16, 18 or 20. Groups of 10 female Crl:CD(SD) rats were given control diet (group 1) or diets containing 1000 ppm sulfoxaflor (purity 95.6%) from GDs 16 to 18 (group 2), GDs 18 to 20 (group 3) or GDs 20 to 22 (group 4). At the end of their respective 48-hour treatment periods, dams were switched to control feed (0 ppm) until termination on LD 4. For toxicokinetic analysis, blood samples were taken from dams in the treated groups at the end of their 48-hour treatment interval.

In the offspring, effects on litter size, survival, body weight and the presence of gross external morphological alterations, with particular focus on limb abnormalities (e.g. forelimb flexure and hindlimb rotation), were carefully assessed. All pups surviving to PND 4 were examined for gross external alterations and euthanized by oral administration of sodium pentobarbital solution. Following external examination, all pups of each sex from groups 1 and 4 were viscerally examined for convoluted ureters. Following visceral examination, the pups were preserved in alcohol, macerated, stained with Alizarin Red-S in order to visualize ossified bone, cleared and examined for bent clavicles. All pups from groups 2 and 3 were discarded following examination for gross external alterations. Any pups found dead or that were euthanized in moribund condition were examined to the extent possible and discarded.

Offspring from animals given 1000 ppm sulfoxaflor for 48 hours starting on the morning of GD 16 or 18 (groups 2 and 3) were similar to controls and did not display previously described fetal abnormalities or reduced neonatal survival. In contrast, offspring from animals given 1000 ppm sulfoxaflor for 48 hours starting on the morning of GD 20 (group 4) had fetal limb abnormalities (forelimb flexure and hindlimb rotation) as well as reduced neonatal survival, demonstrating that exposure shortly before birth (GD 21 or 22) is sufficient to induce developmental toxicity (Table 30).

Table 28. Summary of selected findings in a special developmental toxicity study in rats

	0 ppm	1000 ppm	1000 ppm
	Group 1	Group 2	Group 3
Treatment period	Control	GDs 6–16	GD 16–birth
Dose (mg/kg bw per day)			
- GDs 6–16	0	76.5 (65.7–86.1)	0
- GDs 16–21	0	0	38.6 (20.8–53.8)
Plasma concentration in dams (µg/g)			
- GD 16	0	35.4–40.9	—
- GD 21	0	—	32.1–43.2
Body weight gain (g)			
- GDs 6–9	14.1	−2.0*	12.4
- GDs 6–16	75.1	50.2*	67.6
- GDs 16–17	10.6	17.1*	5.6
- GDs 19–21	29.4	23.5	17.2*
- GDs 16–21	70.6	65.6	43.4*
- GDs 0–21	181.4	143.9*	142.9*
Feed consumption (g/animal per day)			
- GDs 6–7	22.6	16.3*	22.7
- GDs 7–8	24.4	13.4*	23.7
- GDs 19–20	23.8	20.7*	13.7*
- GDs 20–21	23.8	19.8	15.5*
- LDs 1–4	36.0	31.1	25.5*
Litter size			
- born live	13.6	12.0	11.9
- born dead	0.4	0.2	0.3
- PND 1	13.6	12.0	9.9
- PND 2	13.6	12.0	5.9*
- PND 3	13.4	12.0	5.7*
- PND 4	13.3	11.9	5.6*
Pup survival (%)			
- PND 1	100.0	100.0	83.2*
- PND 2	100.0	100.0	49.7*
- PND 3	99.20	100.0	47.6*
- PND 4	98.4	100.0	46.9*
Pup body weight (g), males/females			
- PND 1	7.2/6.8	7.3/7.0	5.7*/5.5*
- PND 4	10.6/10.1	10.5/10.3	8.6*/8.1*
External examination of pups			
PND 0			
No. of fetuses/litters examined	122/9	120/10	143/12
Forelimb flexure (no. of fetuses/litters)	0/0	0/0	50/11*
Hindlimb rotation (no. of fetuses/litters)	0/0	0/0	19/8*

Table 28 (continued)

	0 ppm	1000 ppm	1000 ppm
	Group 1	Group 2	Group 3
PND 1			
No. of fetuses/litters examined	122/9	120/10	119/12
Forelimb flexure (no. of fetuses/litters)	0/0	0/0	38/9*
Hindlimb rotation (no. of fetuses/litters)	0/0	0/0	8/6*
PND 2			
No. of fetuses/litters examined	122/9	120/10	71/8
Forelimb flexure (no. of fetuses/litters)	0/0	0/0	6/4*
Hindlimb rotation (no. of fetuses/litters)	0/0	0/0	2/2
PND 4			
No. of fetuses/litters examined	122/9	120/10	67/7
Forelimb flexure (no. of fetuses/litters)	0/0	0/0	0/0
Hindlimb rotation (no. of fetuses/litters)	0/0	0/0	0/0

From Rasoulpour & Zablotny (2010b)
GD, gestation day; LD, lactation day; PND, postnatal day; * $P < 0.05$

Table 29. Summary of limb alteration reversals in rat pups from group 3 (treatment period GD 16 to birth)

Day		Litter No.											
		708	709	710	711	712	713	714	715	716	717	718	719
LD 0	No. of pups alive	11	12	1	15	11	12	14	12	14	15	12	14
	No. w/LABN	8	4	0	6	2	6	6	9	5	3	3	3
	No. born dead	1	0	0	0	0	0	0	1	0	1	0	0
LD 1	No. of pups alive	7	11	1	13	3	5	14	12	14	15	12	12
	No. w/LABN	7	3	0	4	0	5	2	5	7	1	2	6
	No. dead	4	1	0	2	8	7	0	0	0	0	0	2
	No. of reversals	—	—	—	—	—	—	4	4	—	2	1	—
LD 2	No. of pups alive	0	0	0	10	3	1	14	0	12	15	12	4
	No. w/LABN	LL	LL	LL	2	0	1	1	LL	2	1	0	1
	No. dead	11	12	1	5	8	11	0	12	2	0	0	10
	No. of reversals	—	—	—	—	—	—	1	—	3	—	2	—
LD 3	No. of pups alive	0	0	0	9	3	0	14	0	12	14	12	4
	No. w/LABN	LL	LL	LL	0	0	LL	0	LL	0	1	0	0
	No. dead	11	12	1	6	8	12	0	12	2	1	0	10
	No. of reversals	—	—	—	—	—	—	1	—	2	—	—	—
LD 4	No. of pups alive	0	0	0	9	3	0	14	0	12	14	12	3
	No. w/LABN	LL	LL	LL	0	0	LL	0	LL	0	0	0	0
	No. dead	11	12	1	6	8	12	0	12	2	1	0	11
	No. of reversals	—	—	—	—	—	—	—	—	—	1	—	—
No. w/LABN per litter		8	4	0	6	2	6	6	9	7	3	3	6
No. dead per litter		11	12	1	6	8	12	0	12	2	1	0	11
No. of confirmed reversals		—	—	—	—	—	—	6	4	5	3	3	—

From Rasoulpour & Zablotny (2010b)
LD, lactation day; LL, lost litter; w/LABN, with limb abnormalities (forelimb flexure and hindlimb rotation)

Table 30. Summary of selected findings in a special developmental toxicity study in rats

	0 ppm	1000 ppm	1000 ppm	1000 ppm
	Group 1	Group 2	Group 3	Group 4
Treatment period	Control	GDs 16–18	GDs 18–20	GDs 20–22
Dose (mg/kg bw per day)	0	63.9 ± 4.5	42.5 ± 1.3	35.7 ± 9.4
Plasma concentration in dams (µg/g)				
- GD 18, 20 or 22	0	16.4–33.3	23.0–30.2	16.1
- LD 0	0	—	—	5.4–6.7
Body weight gain (g)				
- GDs 16–17	3.6	−4.1*	4.1	3.8
- GDs 18–19	9.3	14.7	0.1*	8.4
- GDs 19–20	12.6	14.1	5.4*	14.3
- GDs 20–21	8.4	12.1	15.5	3.1
Feed consumption (g/animal per day)				
- GDs 16–17	25.1	21.1*	26.9	26.5
- GDs 18–19	18.9	18.6	14.3*	19.0
- GDs 19–20	21.1	22.6	14.2*	21.9
- GDs 20–21	19.6	21.5	20.1	14.4*
- GDs 21–22	11.7	13.1	13.3	2.9*
Litter size				
- born live	11.4	11.0	11.7	12.0
- born dead	0	0.1	0	0
- PND 1	11.4	10.9	11.6	10.8
- PND 2	11.4	10.9	11.6	10.8
- PND 3	11.4	10.9	11.6	10.8
- PND 4	11.3	10.9	11.6	10.8
Pup survival (%)				
- PND 1	100	99.0	99.0	99.0
- PND 2	100	99.0	99.0	89.6
- PND 3	100	99.0	99.0	89.6
- PND 4	99.1	99.0	99.0	89.6
External examination of pups				
PND 0				
No. of fetuses/litters examined	114/10	99/9	105/9	96/8
Forelimb flexure (no. of fetuses/litters)	0/0	0/0	0/0	7/4*
Hindlimb rotation (no. of fetuses/litters)	0/0	0/0	0/0	11/6*
PND 1				
No. of fetuses/litters examined	114/10	98/9	104/9	95/8
Forelimb flexure (no. of fetuses/litters)	0/0	0/0	0/0	4/3
Hindlimb rotation (no. of fetuses/litters)	0/0	0/0	0/0	5/2
PND 2				
No. of fetuses/litters examined	114/10	98/9	104/9	86/8
Forelimb flexure (no. of fetuses/litters)	0/0	0/0	0/0	0/0
Hindlimb rotation (no. of fetuses/litters)	0/0	0/0	0/0	0/0
PND 4				
No. of fetuses/litters examined	113/10	98/9	104/9	86/8
Forelimb flexure (no. of fetuses/litters)	0/0	0/0	0/0	0/0
Hindlimb rotation (no. of fetuses/litters)	0/0	0/0	0/0	0/0

From Rasoulpour & Zablotny (2010c)
* $P < 0.05$

The sulfoxaflor plasma concentration of groups 2, 3 and 4 ranged from 16.4 to 33.3, 23.0 to 30.2 and 5.4 to 16.1 µg/g plasma, respectively (Table 30). The plasma concentration of sulfoxaflor in group 4 was lower than in the other groups because three of the four sampled rats had undergone parturition; therefore, feed consumption, and corresponding test material intake, in these animals was minimal. The sulfoxaflor plasma concentration from the one group 4 animal that had not given birth was 16.1 µg/g.

The daily examination of surviving group 4 offspring born with limb abnormalities indicated that these limb abnormalities were reversible in a number of surviving pups from six litters shortly after withdrawal of maternal dietary exposure to sulfoxaflor (Table 31). In some cases, full reversal of the limb abnormalities was evident the day after birth and occurred for all affected animals that survived to PND 4; reversal also occurred in some animals that subsequently died before PND 4. Likewise, the visceral and skeletal findings of abnormal ureter and bent clavicle, the latter of which had a high incidence (30.1% of fetuses), in the definitive developmental toxicity study were not present in this study at necropsy on PND 4.

This study demonstrated that the critical period of developmental susceptibility to sulfoxaflor-induced fetal abnormalities and reduced neonatal survival effects occurs shortly before birth and that the fetal limb abnormalities are rapidly reversible after birth. These results support the hypothesis that late gestational exposure to sulfoxaflor induces fetal abnormalities and neonatal death via its pharmacological action on the fetal muscle nAChR, which develops functional expression during this stage of gestation (Rasoulpour & Zablotny, 2010c).

Rabbits

In a prenatal developmental toxicity range-finding study, groups of seven time-mated female New Zealand White rabbits received sulfoxaflor (purity 95.6%) by oral gavage at a dose level of 0, 10, 15, 20 or 25 mg/kg bw per day from GD 7 to GD 27. The test substance was administered as an aqueous suspension in 0.5% methylcellulose at a dose volume of 2 ml/kg bw. In-life parameters evaluated for all groups included clinical observations, body weight, body weight gain and feed consumption. On GD 28, all surviving rabbits were euthanized and examined for gross pathological alterations. Liver and kidney weights were recorded, along with the number of corpora lutea, implantations, resorptions and live/dead fetuses. Blood samples from five rabbits from each surviving dose group were taken at 1, 2, 4, 8 and 24 hours after the final dose on GD 27 for analysis of sulfoxaflor levels.

Oral administration of sulfoxaflor by gavage to time-mated New Zealand White rabbits at 25 or 20 mg/kg bw per day caused severe inanition, and the animals were removed from study on GD 13 or 16, respectively. Animals in the 15 mg/kg bw per day group had treatment-related body weight loss (14–78 g) upon initiation of dosing (GDs 7–10) and an overall decreased mean body weight gain (approximately 39% lower than controls) throughout the dosing period (GDs 7–28). There was no maternal toxicity observed at 10 mg/kg bw per day. There was no indication of embryo/fetal lethality at any dose level.

Toxicokinetic analyses on GDs 27–28 indicated slow elimination of sulfoxaflor from plasma, with a half-life of 14 hours. A 1.5-fold increase in the dose (from 10 to 15 mg/kg bw per day) resulted in a 1.4-fold increase in the daily systemic dose ($AUC_{24\,h}$: 236 and 332 µg·h/ml, respectively). The maximum concentrations (C_{max}) in plasma were observed at 2 hours after administration, with levels of 14.9 or 21.8 µg/g at 10 or 15 mg/kg bw per day, respectively (Rasoulpour & Brooks, 2008).

In a prenatal developmental toxicity range-finding study, groups of five time-mated female New Zealand White rabbits were given diets containing sulfoxaflor (purity 95.6%) at a concentration of 0, 500 or 1000 ppm (equal to 0, 21.7 and 36.6 mg/kg bw per day) on GD 7 through GD 28. In-life parameters evaluated for all groups included clinical observations, body weight, body weight gain and feed consumption. In addition, blood was collected from all surviving rabbits at 1, 2, 4 and

Table 31. Summary of limb alteration reversals in rat pups from group 4 (treatment period GDs 20–22)

Day		Litter No.							
		1636	1637	1639	1640	1642	1643	1644	1645
LD 0	No. of pups alive	12	11	14	14	12	12	9	12
	No. w/LABN	2	1	0	1	1	3	0	5
	No. born dead	0	0	0	0	0	0	0	0
LD 1	No. of pups alive	12	11	14	14	12	11	9	12
	No. w/LABN	2	0	0	0	0	1	0	4
	No. dead	0	0	0	0	0	1	0	0
	No. of reversals	—	1	—	1	1	1	—	1
LD 2	No. of pups alive	12	11	14	14	12	10	9	4
	No. w/LABN	0	0	0	0	0	0	0	0
	No. dead	0	0	0	0	0	2	0	8
	No. of reversals	2	—	—	—	—	—	—	—
LD 3	No. of pups alive	12	11	14	14	12	10	9	4
	No. w/LABN	0	0	0	0	0	0	0	0
	No. dead	0	0	0	0	0	2	0	8
	No. of reversals	—	—	—	—	—	—	—	—
LD 4	No. of pups alive	12	11	14	14	12	10	9	4
	No. w/LABN	0	0	0	0	0	0	0	0
	No. dead	0	0	0	0	0	2	0	8
	No. of reversals	—	—	—	—	—	—	—	—
No. w/LABN per litter		2	1	0	1	1	3	0	5
No. dead per litter		0	0	0	0	0	2	0	8
No. of confirmed reversals		2	1	—	1	1	1	—	1

From Rasoulpour & Zablotny (2010c)
LD, lactation day; w/LABN, with limb abnormalities (forelimb flexure and hindlimb rotation)

8 hours (GD 27) and 24 hours (GD 28) after the offering of feed on GD 27 to determine blood levels of test material. On GD 28, all surviving rabbits were euthanized and examined for gross abnormalities. Liver and kidney weights were recorded, along with the numbers of corpora lutea, implantations, resorptions and live/dead fetuses.

Treatment-related effects at 1000 ppm consisted of a statistically significant mean body weight loss of 60 g (range +19 to −173 g) after initiation of treatment (GDs 7–10) and a 33% decrease in mean body weight gain, relative to controls, throughout the treatment period (GDs 7–28). One rabbit in this group had 6 consecutive days of inanition (GDs 9–14) and was euthanized for humane reasons on GD 14. There was no maternal toxicity observed in the 500 ppm rabbits, and there was no indication of embryo/fetal lethality at any dose level.

Toxicokinetic analysis of the time-course plasma concentration of sulfoxaflor from the rabbits exposed through the diet showed that the daily systemic dose ($AUC_{24\ h}$: 439 and 776 µg·h/ml at 500 and 1000 ppm, respectively) was dose proportional, with constant steady-state plasma concentrations (18.3–19.4 and 32.8–35.6 µg/g at 500 and 1000 ppm, respectively) with minimal diurnal fluctuation, compared with the 3-fold difference between C_{min} and C_{max} observed after oral gavage. Dietary administration resulted in a dose-corrected $AUC_{24\ h}$ of about 22 µg·h/ml per milligram per kilogram body weight administered, which was consistent with previously reported dose-corrected values of

20–22 µg·h/ml per milligram per kilogram body weight administered following gavage administration. The dietary route afforded a greater applied maximally tolerated dose (1000 ppm, equal to 36.6 mg/kg bw per day) relative to gavage (15 mg/kg bw per day caused excessive maternal toxicity). Therefore, the dietary route of administration was chosen for the definitive rabbit developmental toxicity study, as it allows for more than twice the applied dose and a correspondingly higher $AUC_{24\,h}$ compared with gavage administration (Rasoulpour & Brooks, 2009a).

In a prenatal developmental toxicity study conducted according to OECD test guideline 414, groups of 26 time-mated female New Zealand White rabbits were given diets containing sulfoxaflor (purity 95.6%) at a concentration of 0, 30, 150 or 750 ppm (equal to 0, 1.3, 6.6 and 31.9 mg/kg bw per day) on GDs 7 through 28. In-life parameters evaluated for all rabbits included clinical observations, body weight, body weight gain and feed consumption. Maternal blood was collected for sulfoxaflor analysis from four rabbits per group over a 24-hour period starting on the morning of GD 27 and also at termination on GD 28. Fetal umbilical cord blood was also taken at termination. All rabbits surviving to GD 28 were euthanized and examined for gross pathological alterations and changes in liver, kidney and gravid uterine weight. The numbers of corpora lutea, uterine implantations, resorptions and live/dead fetuses were determined. All fetuses were weighed, sexed and examined for external and visceral alterations. Also, the heads were examined for craniofacial alterations by serial sectioning in approximately one half of the fetuses in each litter, whereas skeletal examinations were performed on all fetuses.

Animals in the 750 ppm dose group exhibited treatment-related maternal toxicity in the form of decreased faeces in 7 of 26 animals, decreased mean body weight gain (55%) from GD 7 to GD 13, decreased mean body weight gain (12%) throughout treatment (GDs 7–28) and decreased mean feed consumption (8–21%) from GD 7 to GD 17. There was no treatment-related maternal toxicity for animals in the 30 or 150 ppm dose groups. There was no treatment-related developmental toxicity in any dose group (Table 32).

The daily systemic dose of sulfoxaflor on GDs 27–28 was dose proportional, as indicated by the nearly identical mean dose-corrected $AUC_{24\,h}$ values of 18, 19 and 19 µg·h/ml per milligram per kilogram body weight administered for animals given 30, 150 and 750 ppm, respectively. Levels of sulfoxaflor in maternal and fetal blood were similar (Table 32). The daily systemic dose in this dietary study was similar to that measured in prior gavage studies with sulfoxaflor.

The NOAEL for maternal toxicity was 150 ppm (equal to 6.6 mg/kg bw per day), based on decreased faeces and decreases in body weight gain and feed consumption at 750 ppm (equal to 31.9 mg/kg bw per day).

The NOAEL for prenatal developmental toxicity was 750 ppm (equal to 31.9 mg/kg bw per day), the highest dose tested (Rasoulpour & Brooks, 2009b).

In a special developmental toxicity study conducted to assess the effects of sulfoxaflor on neonatal survival in rabbits, groups of 12 time-mated female New Zealand White (Hra:(NZW) SPF) rabbits that had two previous litters were given diets containing sulfoxaflor (purity 95.6%) at a concentration of 0 or 750 ppm (equal to 0 and 29 mg/kg bw per day) from GD 7 through the initiation of parturition (i.e. 25–26 consecutive days). All diets were provided at 150 g/day during the exposure period (GD 7 through initiation of parturition) and at 200 g/day during LDs 1–4; the control diet was offered to both groups after parturition. The F_0 females were approximately 9–13 months of age at the initiation of test substance exposure. All animals were observed twice daily for mortality and moribundity. Clinical observations, body weights and feed consumption were recorded at appropriate intervals. All F_0 females were allowed to deliver and rear their offspring to LD 4. All F_0 females were necropsied within 24 hours of total litter loss, on LD 4 or on post-mating day 37. All surviving F_1 offspring received a detailed physical examination on PND 4 and were then discarded.

Table 32. Summary of selected findings in a prenatal developmental toxicity study in rabbits

	0 ppm	30 ppm	150 ppm	750 ppm
No. of females pregnant	25	26	25	25
Body weight gain (g)				
- GDs 7–10	28.3	26.5	22.7	9.9
- GDs 10–13	74.8	72.2	73.7	41.9*
- GDs 7–28	356	340	348	315
Terminal body weight (g)	3502	3422	3463	3442
Feed consumption (g/day)				
- GDs 7–8	153	150	154	131*
- GDs 9–10	155	151*	150	142*
- GDs 12–13	150	144	141	122*
Gravid uterine weight (g)	459	420	428	449
No. of litters with viable fetuses	25	26	24	25
No. of live fetuses per litter	8.9	8.3	8.3	8.7
Fetal body weights (g)				
- males	34.8	34.9	34.9	34.8
- females	34.9	33.4	34.7	33.9
External examination (no. of fetuses/litters)	223/25	215/26	199/24	217/25
- total malformed (no. of fetuses/litters)	0/0	0/0	1/1	0/0
Craniofacial examination (no. of fetuses/litters)	117/25	114/26	105/24	114/25
- total malformed (no. of fetuses/litters)	0/0	0/0	0/0	0/0
Visceral examination (no. of fetuses/litters)	223/25	215/26	199/24	217/25
- total malformed (no. of fetuses/litters)	1/1	3/2	3/2	2/2
Skeletal examination (no. of fetuses/litters)	223/25	215/26	199/24	217/25
- total malformed (no. of fetuses/litters)	0/0	0/0	0/0	0/0
Dose in dams (mg/kg bw per day), GD 27	0	1.2	5.6	31.5
Plasma $AUC_{24\,h}$ in dams (µg·h/ml), GD 27	0	20.5	107	599
Plasma concentration (µg/g), GD 28				
- dams	0	0.7	3.7	23.9
- fetuses	0	0.6	3.5	21.2

From Rasoulpour & Brooks (2009b)
AUC, area under the curve; GD, gestation day; * $P < 0.05$

With the exception of one F_0 female in the control and 750 ppm groups euthanized on LD 3 due to total litter loss, all females survived to the scheduled necropsies. No test substance–related maternal macroscopic findings were noted.

Lower mean body weight gains (24.2%) and feed consumption (7.3%) were noted in the 750 ppm group during the gestation exposure period compared with the control group (Table 33). Corresponding incidences of decreased defecation were noted for three females in this group. Although mean body weights remained within 2.9% of control group values throughout gestation, the reductions in mean body weight gains and feed consumption were attributed to test substance exposure. Mean body weights, body weight gains and feed consumption in the 750 ppm group were similar to those of the control group during LDs 1–4.

No test substance–related effects were observed on the mean number of offspring born, offspring survival or the general physical condition of the offspring.

Table 33. Summary of selected findings in a special developmental toxicity study in rabbits

	0 ppm	750 ppm
Body weight change (g)		
- GDs 7–10	−1	−12
- GDs 10–13	81	64
- GDs 13–16	153	121
- GDs 7–28	413	313
- LDs 1–4	33	67
Feed consumption (g/day)		
- GDs 7–10	150	137
- GDs 10–13	150	132
- GDs 13–16	150	136
Gestation length (days)	31.5	31.3
Litter size, PND 0	10.9	10.6
Live litter size, PND 0	10.8	9.8
Postnatal survival (% per litter)		
- PND 0, relative to number born	99.3	93.8
- PND 0 to PND 1	97.8	99.2
- PND 1 to PND 4	74.2	78.4
- Birth to PND 4	72.7	71.6

From Kuhl (2009)
GD, gestation day; LD, lactation day; PND, postnatal day

The NOAEL for maternal toxicity was lower than 750 ppm (equal to 29 mg/kg bw per day), the only dose tested, based on decreased body weight gain and feed consumption.

The NOAEL for neonatal survival was 750 ppm (equal to 29 mg/kg bw per day), the only dose tested (Kuhl, 2009).

2.6 Special studies

(a) Neurotoxicity

In an acute neurotoxicity study conducted in accordance with OECD test guideline 424, groups of 10 male and 10 female F344/DuCrl rats received sulfoxaflor (purity 95.6%) by oral gavage at a dose level of 0, 7.5, 75 or 750 mg/kg bw. The test substance was administered in a 0.5% aqueous Methocel cellulose vehicle at a volume of 10 ml/kg bw. The dose levels were selected on the basis of the results from a pilot study conducted at dose levels of 500 and 750 mg/kg bw using five female rats per dose. Body weights were recorded and a FOB and test for motor activity were conducted pre-exposure (baseline) and on the day of dosing (day 1, time of peak effect), day 8 and day 15. The FOB included hand-held and open-field observations as well as measurements of grip performance, landing foot splay and rectal temperature. Clinical observations were conducted on days 2, 3 and 4. At the end of the study, all rats from the control and high-dose groups and five rats of each sex from the low- and mid-dose groups were perfused for histopathological evaluation of the central and peripheral nervous systems. A second motor activity study at dose levels of 0, 2.5, 7.5 and 25 mg/kg bw was conducted to investigate whether an apparent decrease in motor activity at 7.5 mg/kg bw was reproducible or treatment related and to establish a clear NOAEL. Motor activity was the only end-point examined in this study phase.

In the pilot study at 750 mg/kg bw, two rats had convulsions 1 hour after dosing, and four had decreased activity and muscle tremors beginning 2–4 hours after dosing. Other clinical signs observed in these rats included hindlimb splay, increased reactivity, walking on toes and perineal urine soiling. The highest frequency of these observations occurred 2–4 hours after dosing. One rat given 750 mg/kg bw had no treatment-related clinical signs. At 500 mg/kg bw, treatment-related clinical signs included decreased activity, muscle tremors, hindlimb splay, lacrimation and perineal urine soiling. Similarly, the highest frequency of these observations occurred 2–4 hours after dosing. Based on these results, the time of peak effect was determined to be between 2 and 4 hours after dosing, and the time selected to begin behavioural testing in the full study was 2.5 hours after dosing.

In the main study, one female rat given 750 mg/kg bw died following dosing on day 1, but the cause of death could not be determined. Treatment-related clinical findings were noted in males and females at 750 mg/kg bw on day 2, 3 or 4 and included decreased or absent faeces, red perioral soiling and perineal urine soiling (females only). There was a statistically significant treatment-related decrease in body weight at 750 mg/kg bw when compared with controls on days 8 and 15, which was more prominent in males than in females.

Treatment-related categorical observations on day 1 in males and females at 750 mg/kg bw included increased incidences of muscle tremors and twitches, convulsions, splayed hindlimbs and perineal urine soiling.

Treatment-related ranked FOB observations on day 1 in males and females at 750 mg/kg bw were as follows: increased lacrimation and salivation, decreased pupil size and response to touch, increased level of urination (females only) and decreased level of open-field activity and gait abnormalities. There were no changes in either treatment-related categorical or ranked FOB observations in males or females at 750 mg/kg bw on day 8 or day 15 or in males or females at 7.5 or 75 mg/kg bw during any FOB time point.

There was a treatment-related decrease in rectal temperature at 750 mg/kg bw when compared with controls on day 1, which was not present in the subsequent examinations on days 8 and 15. There were no treatment-related effects in grip performance or landing foot splay.

There was a treatment-related decrease in the day 1 total motor activity and an effect on the distribution of motor activity counts of males and females at 75 or 750 mg/kg bw. The effect on total motor activity of animals at 7.5 mg/kg bw was considered equivocal on day 1. There were no effects on motor activity on day 8 or 15 in rats of any dose group. In the follow-up motor activity study, there were no treatment-related effects on total motor activity or on the distribution of motor activity counts for males and females at 2.5, 7.5 or 25 mg/kg bw when compared with controls (Table 34).

There were no treatment-related gross or histopathological findings in the central or peripheral nervous system.

The NOAEL for acute neurotoxicity was 25 mg/kg bw, based on decreased motor activity at 75 mg/kg bw. The NOAEL for neuropathology was 750 mg/kg bw, the highest dose tested (Marty, Andrus & Stebbins, 2010).

In a developmental neurotoxicity study conducted in accordance with OECD test guideline 426, groups of 25 bred female Crl:CD(SD) rats were given diets containing sulfoxaflor (purity 95.6%) at a concentration of 0, 25, 100 or 400 ppm from GD 6 through LD 21. The mean dose at 25, 100 and 400 ppm was equal to 1.8, 7.1 and 27.7 mg/kg bw per day through gestation and 1.9, 7.6 and 29.8 mg/kg bw per day through lactation, respectively.

All animals were observed twice daily for appearance and behaviour. Clinical observations, body weights and feed consumption were recorded at appropriate intervals during gestation and lactation. In addition, detailed clinical observations were conducted out of the home cage on all dams in each group on GDs 10 and 15 and on LDs 10 and 21. All females were allowed to deliver and rear

Table 34. Summary of selected findings in an acute neurotoxicity study in rats

	Males				Females			
Main study								
Dose (mg/kg bw per day)	0	7.5	75	750	0	7.5	75	750
Body weight (g)								
- day 2	131.3	133.2	132.6	119.7*	89.1	89.6	86.4	82.7
- day 8	141.5	144.8	142.9	127.7*	96.9	97.8	94.5	91.6
- day 15	161.2	164.0	161.7	145.7*	111.1	112.8	108.2	106.4
Rectal temperature (°C), day 1	37.5	37.2	37.2	34.5*	37.9	38.1	38.1	34.7*
Motor activity (activity counts), pre-exposure	17.6	16.1	18.8	17.7	19.3	19.8	17.2	19.1
- day 1	18.1	12.9*	12.4*	3.0*	19.9	17.9*	10.8*	7.3*
- day 8	15.8	16.8	19.6	15.4	20.8	22.8	20.4	19.8
- day 15	16.2	15.3	16.7	15.0	17.9	24.9	23.7	18.4
Follow-up motor activity study								
Dose (mg/kg bw per day)	0	2.5	7.5	25	0	2.5	7.5	25
Motor activity (activity counts), pre-exposure	16.7	13.2	14.0	13.1	14.8	14.6	15.6	16.1
- day 1	12.2	13.0	14.8	13.0	17.3	17.7	17.3	17.9
- day 8	16.9	19.3	19.0	19.6	25,7	23.1	25.0	24.7
- day 15	21.0	18.6	19.6	22.3	25.8	25.6	28.6	26.7

From Marty, Andrus & Stebbins (2010)
* $P < 0.02$ (treatment by time interaction, with male and female data considered together)

their offspring to LD 21. F_0 females that failed to deliver or with total litter loss were necropsied on post-mating day 25 or within 24 hours of total litter loss, respectively. The liver and kidneys from all F_0 females were weighed at necropsy. Clinical observations, body weights and sexes were recorded for the F_1 pups at appropriate intervals. Pre-weaning developmental landmarks (surface righting response and eye opening) were evaluated for all available F_1 pups. On PND 4, litters were culled to eight pups per litter. If a litter consisted of fewer than six pups or failed to meet sex ratio criteria (at least three pups of each sex), the litter was not used for neurobehavioural or neuropathological evaluation, and the dam and litter were necropsied on PND 4. Following culling, a subset of 20 pups of each sex per group (subset A) was assigned to detailed clinical observations (PNDs 4, 11, 21, 35, 45 and 60), auditory startle response (PNDs 20 and 60), locomotor activity (PNDs 13, 17, 21 and 61), learning and memory (PND 62) and brain weight evaluations (PND 72). From this subset, 10 pups of each sex from the control and 400 ppm groups were selected for neuropathological and brain morphometric evaluations on PND 72. A second subset of 20 pups of each sex per group (subset B) was selected for assessment of learning and memory (PND 22). A third subset of 20 pups of each sex per group (subset C) was selected for brain weight evaluations on PND 21; of these, 10 pups of each sex from the control and 400 ppm groups were selected for neuropathological and brain morphometric evaluations on PND 21. Indicators of sexual development (balanopreputial separation and vaginal patency) were evaluated for all F_1 animals in subset A. All F_1 animals not selected for behavioural evaluations were euthanized and necropsied on PND 21. F_1 animals selected for learning and memory assessment on PND 22 were necropsied following completion of these assessments.

There were no treatment-related mortalities in the F_0 maternal animals during the study, and no test substance–related clinical findings were noted during the daily examinations. Total litter loss was noted for two dams in the control group on LDs 0 and 9 and for one dam at 400 ppm on LD 2. Detailed clinical observations as well as maternal body weights and feed consumption during gestation and lactation were unaffected by treatment.

There were no treatment-related differences noted between groups when comparing the mean length of gestation, the process of parturition and internal macroscopic pathological findings. The mean numbers of former implantation sites and sites that were unaccounted for as well as maternal kidney and liver weights were similar across groups.

There were no treatment-related effects on the mean number of pups born, live litter size or the percentage of males at birth at any maternal exposure level. However, F_1 pup toxicity was expressed at 400 ppm by a statistically significant reduction in postnatal survival from birth to PND 4 compared with the control group (Table 35). In addition, malrotation of the left forelimb was noted for two pups in the same litter at 400 ppm during the week prior to weaning (on PNDs 14, 17 and/or 21); this observation was not apparent on PND 1, 4, 7 or 11 for either of these pups, both of which survived to the scheduled euthanasia on PND 21.

Mean pup body weights at 400 ppm were 11.8% and 6.5% lower than those of the control group at birth (PND 1) and on PND 4, respectively. The reduced pup body weights resulted in a statistically significant delay in surface righting response for pups at 400 ppm (Table 35). Pup body weights in the 400 ppm group did not differ from the control group values on PND 7 or later time points. The decrease in postnatal survival at 400 ppm is consistent with results from a previous pilot reproduction study, in which dietary exposures of 500 and 1000 ppm resulted in decreased pup survival. Postnatal survival and pup body weights and body weight gains in the 25 and 100 ppm groups were unaffected by maternal test substance exposure. The ages of attainment of surface righting response at 25 and 100 ppm and eye opening at 25, 100 and 400 ppm were similar to those of the control group. The attainment of sexual developmental landmarks (balanopreputial separation and vaginal patency) was unaffected by maternal test substance exposure.

No remarkable clinical observations or macroscopic findings were noted for F_1 animals at any exposure level. No treatment-related effects were observed for F_1 animals 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 F_1 animals on PNDs 21 and 72.

The NOAEL for maternal toxicity and reproductive toxicity (process of parturition and duration of gestation) was 400 ppm (equal to 28.8 mg/kg bw per day), the highest dose tested.

The NOAEL for developmental neurotoxicity was 400 ppm (equal to 28.8 mg/kg bw per day), as there were no signs of developmental neurotoxicity at the highest dose tested.

The NOAEL for neonatal toxicity was 100 ppm (equal to 7.4 mg/kg bw per day), based on the reduction in postnatal survival and pup body weights at 400 ppm (equal to 28.8 mg/kg bw per day) (Beck, 2010).

(b) Mechanistic studies on liver tumour induction in mice and rats

Mice

A mechanistic study was conducted to obtain information on the potential MOA responsible for the liver weight increases observed in mice and rats after repeated exposure to sulfoxaflor. For this purpose, specific gene expression was assessed by real-time polymerase chain reaction (RT-PCR) in liver samples from female CD-1 mice exposed to 0 or 4500 ppm sulfoxaflor in the diet for 4 days in a previously conducted palatability pilot study (Thomas & Dryzga, 2010). In total, eight genes were selected for this study. Five genes, primarily *Cyp2b10*, but with four additional genes, were chosen to address whether sulfoxaflor induces a phenobarbital-like gene expression response. Two genes that are also induced by phenobarbital were selected specifically to investigate the effect on blood cholesterol seen in sulfoxaflor-treated rodents. One gene, *Cyp4a10*, was included as a marker of peroxisome proliferator-activated receptor alpha (PPARα) to examine this potential MOA.

Table 35. Summary of selected findings in a developmental neurotoxicity study in rats

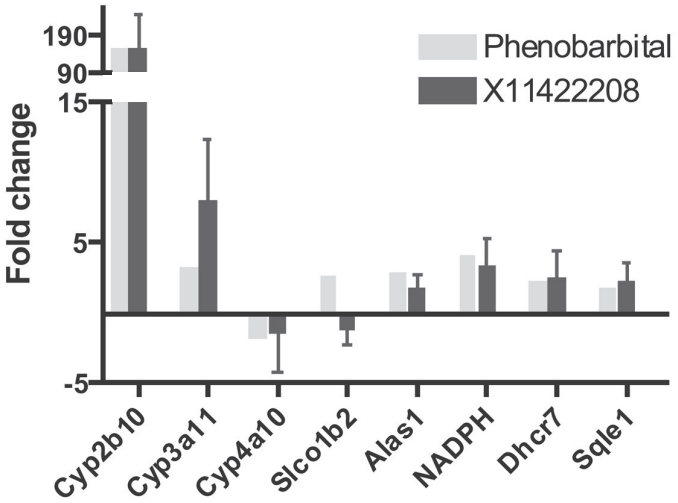
	0 ppm	25 ppm	100 ppm	400 ppm
No. of females delivered	25	25	24	25
Mean litter size, at birth	14.8	15.2	15.3	15.2
Live litter size, PND 0	14.2	15.0	15.1	14.9
Postnatal survival (%)				
- PNDs 0–1	99.5	98.9	96.0	86.9*
- PNDs 1–4	99.8	99.8	98.4	87.2
- birth to PND 4	93.0	97.9	93.2	76.5**
No. of pups/litters found dead or euthanized	24/8	6/5	17/11	59/15
No. of pups/litters with malrotation of left forelimb	0/0	0/0	0/0	2/1
Pup body weight (g)				
- PND 1	6.8	6.7	6.7	6.0**
- PND 4	9.2	9.1	9.2	8.6**
- PND 7	14.3	14.5	14.7	14.0
- PND 21	43.9	45.7	45.2	43.8
Surface righting response (PND)	5.3	5.2	5.2	6.3**
Eye opening (PND)	14.8	15.1	14.8	14.9
Balanopreputial separation (PND) / body weight (g)	46.9/247	46.3/247	46.1/245	47.6/236
Vaginal patency (PND) / body weight (g)	32.7/104	32.9/106	32.7/105	32.6/105
Locomotor activity (counts), both sexes combined				
- PND 13	1585	1499	1688	2411
- PND 17	3130	2687[#]	2855[#]	2339
- PND 21	2498	2198	2937	2790
- cumulative, PNDs 13–21, both sexes combined	7213	6385	7480	7540
- PND 61, both sexes combined	5559	4691	5613	4723
- PND 61, males	5043	4306	4527	4499
- PND 61, females	6050	5076[#]	6698	4947

From Beck (2010)
PND, postnatal day; * $P < 0.05$; ** $P < 0.01$; [#] $P < 0.01$ (treatment by time interaction)

In addition, cell proliferation (seen as an early response to phenobarbital treatment) was assessed by Ki-67 immunohistochemical staining in liver tissue from female CD-1 mice from the mouse palatability study (Thomas & Dryzga, 2010; 0, 3000 and 4500 ppm group) and male and female F344/DuCrl rats from a 28-day rat study (Yano et al., 2009b; 0 and 2000 ppm group).

Sulfoxaflor-treated mice showed gene expression changes similar to those reported in the literature following phenobarbital exposure. The primary phenobarbital-like marker gene, *Cyp2b10*, was induced 148.5-fold. In addition, three of the four remaining phenobarbital-like response genes (*Cyp3a11*, *Alas1* and *NADPH-Cyp-reductase*) were induced 7.85-, 1.15- and 3.18-fold, respectively. The remaining phenobarbital-like gene, *Slco1b2*, was not induced in sulfoxaflor-treated mice. Sulfoxaflor stimulated the cholesterol synthesis–related genes, *Dhcr7* and *Sqle1* (2.42- and 2.05-fold, respectively), at levels similar to those reported in the literature following phenobarbital exposure. The lack of induction for both *Cyp4a10* and an internal homeostasis control gene, *Scd1*, indicates that sulfoxaflor is not acting as a peroxisome proliferator (Figure 2).

Figure 2. Sulfoxaflor (X11422208) and phenobarbital gene expression response in mice (phenobarbital gene expression values are from the literature)



From Geter & Kan (2008)

Cell proliferation in mice was significantly increased in both the centrilobular and midzonal regions at 3000 ppm (19- and 5-fold, respectively), whereas no significant changes in proliferation were seen in the periportal region at 3000 ppm or in any region at 4500 ppm. The lack of significant proliferation response at 4500 ppm was most probably due to the shortened length of exposure (3 days) when compared with the 3000 ppm group (7 days). The proliferation response was accompanied by greater numbers of hepatocyte mitotic figures in the 3000 ppm group than in the 4500 ppm group.

In rats following 28-day treatment at 2000 ppm, a significant increase in proliferation was seen only in the centrilobular region (1.7- or 3-fold in males or females, respectively).

These findings suggest that the MOA responsible for liver weight increases observed in sulfoxaflor-treated mice and rats is phenobarbital-like (Geter & Kan, 2008).

In a subsequent mechanistic study conducted to determine if a phenobarbital-like MOA was responsible for the liver weight increases seen in mice after repeated exposure to sulfoxaflor, groups of five male and five female Crl:CD-1 (ICR) mice per group were given diets containing sulfoxaflor (purity 95.6%) at a concentration of 0, 500 or 750 ppm (males) or 0, 1000 or 1500 ppm (females) for 7 days. The dietary concentrations were equal to 0, 89 and 128 mg/kg bw per day for males and 0, 211 and 323 mg/kg bw per day for females. End-points evaluated were daily cage-side observations, body and liver weights, feed consumption, serum clinical chemistries, targeted gene expression (quantitative RT-PCR using TaqMan gene expression assays), liver cytochrome P450 enzyme activity and hepatocellular proliferation using 5-bromo-2′-deoxyuridine (BrdU) and Ki-67 immunohistochemical techniques. Four nuclear receptors that are primarily responsible for xenobiotic-induced liver weight increase were investigated: the aryl hydrocarbon receptor (AhR; *Cyp1a*), constitutive androstane receptor (CAR; *Cyp2b*), pregnane X receptor (PXR; *Cyp3a*) and PPARα (*Cyp4a*).

In addition, archived liver samples from previously conducted 28-day and 90-day toxicity studies in mice (Thomas et al., 2008, 2010a) were analysed for targeted gene expression, liver enzyme activity and hepatocellular proliferation (Ki-67).

All mice in the 7-day study survived the treatment period. There were no statistically identified differences in body weights of males or females at any dose level. Feed consumption was not affected by treatment in males, but was reduced at 1000 and 1500 ppm in females (12% and 10%, respectively).

Males exposed to 750 ppm had a statistically significant increase (17%) in relative liver weights compared with controls. Females exposed to 1000 and 1500 ppm had a statistically significant increase in relative (38% and 43%, respectively) and absolute (43% and 47%, respectively) liver weights compared with controls (Table 36). These liver weight increases were accompanied by centrilobular and midzonal hepatocyte hypertrophy, with very slightly increased cytoplasmic eosinophilia (altered tinctorial properties consistent with enzyme induction and/or smooth endoplasmic reticulum increase). There were very slight increases in the number of mitotic hepatocytes in some males at 500 or 750 ppm and in the majority of females at 1000 or 1500 ppm. In addition, minimal focal or very slight multifocal individual cell necrosis of hepatocytes occurred in some males at 500 or 750 ppm and in females at 1000 or 1500 ppm, characterized by the presence of one or two necrotic hepatocytes (minimal focal) or five or six scattered necrotic hepatocytes (very slight multifocal) in the entire liver section. Analysis by Oil Red O stain demonstrated slightly increased amounts of lipid in the hepatocytes in males at 750 ppm sulfoxaflor; however, no changes were observed in females.

In the 7-day study, *Cyp2b10* expression, considered to be the prototypical gene response following phenobarbital exposure through activation of CAR, was induced 42.1- and 54.8-fold in males at 500 and 750 ppm, respectively, and 20.0- and 30.8-fold in females at 1000 and 1500 ppm, respectively, relative to controls. In the 28-day and 90-day studies, *Cyp2b10* gene expression in males was increased 61.7-fold (300 ppm) and 56.5-fold (750 ppm), respectively, and in females at 1500 ppm, 93.9- and 53.9-fold, respectively (Table 36).

Cyp3a11, a phenobarbital- and PXR-related gene, was significantly elevated in males at 750 ppm after 7 and 90 days (2.7- and 2.8-fold, respectively), whereas in female mice, it was significantly elevated at all doses and time periods (range of 3.4- to 6.6-fold). 7-Pentoxyresorufin-*O*-deethylase (PROD) and benzyloxyresorufin-*O*-debenzylase (BROD) liver enzyme activities, which give a measure of *Cyp2b* enzyme induction, were elevated in both male and female mice at all time points (range of 2.6- to 9.5-fold).

AhR-related 7-ethoxyresorufin-*O*-deethylase (EROD) liver enzyme activity was slightly elevated at all time points in both male and female mice; however, the degree of induction was mild (none greater than 2.3-fold) and was most likely associated with the large induction of *Cyp2b* enzyme. *Cyp4a10*, a PPARα-related gene, was not significantly altered in this study.

BrdU analysis in the 7-day study showed that males at 500 ppm had elevated proliferation in the centrilobular region and at 750 ppm in both the centrilobular and midzonal regions, whereas females at 1000 and 1500 ppm showed significant induction in all three regions. Using Ki-67 analysis, males at 500 and 750 ppm showed significant induction in the centrilobular region; however, unlike the BrdU analysis, increased proliferation was not observed at 750 ppm in the midzonal region. Ki-67 analysis in females showed no evidence for increased proliferation at any dose or zone.

In the 28- and 90-day studies, Ki-67 analysis of hepatocellular proliferation showed no induction at either time point in male or female mice.

Based upon these results, the MOA responsible for the increased liver weight in mice administered sulfoxaflor was phenobarbital-like, as evidenced by the CAR- and PXR-related molecular, enzymatic and proliferative responses. These data support the conclusion that sulfoxaflor may be an agonist ligand for CAR, and this activation results in the observed phenobarbital-like response and increased liver weight (Geter et al., 2010a).

A mechanistic study was conducted to characterize the induction profile of sulfoxaflor in the livers of C57BL/6J mice in order to provide comparative data to previously conducted studies

Table 36. Summary of selected findings in a mechanistic study in mice

	Males			Females		
7-day study						
Dietary concentration (ppm)	0	500	750	0	1000	1500
Dose (mg/kg bw per day)	0	89	128	0	211	323
Body weight (g)	32.4	31.5	31.5	23.3	24.0	23.8
Liver weight, absolute (g)	1.79	1.80	2.03	1.05	1.50*	1.54*
Liver weight, relative (% of body weight)	5.51	5.72	6.45*	4.50	6.24*	6.45*
Hepatocellular hypertrophy	0/5	0/5	5/5	0/5	4/5	5/5
Mitotic alteration of hepatocytes	0/5	2/5	3/5	2/5	4/5	5/5
Necrosis, single cell, focal/multifocal	0/5	2/5	3/5	0/5	1/5	1/5
Targeted gene expression (fold change)						
- *Cyp 2b10*	1	42.1*	54.8*	1	20.0*	30.8*
- *Cyp 3a11*	1	1.6	2.7*	1	4.0*	6.6*
- *Cyp 4a10*	1	1.2	1.0	1	−5.6	−3.3
Liver enzyme activity						
- EROD (pmol/min per milligram protein)	20.3	30.1*	33.3*	10.5	23.3*	23.0*
- PROD (pmol/min per milligram protein)	1.2	5.1*	5.4*	2.8	13.9*	13.8*
- BROD (pmol/min per milligram protein)	2.7	18.0*	19.7*	2.2	7.7*	8.6*
Liver cell proliferation (BrdU)						
- centrilobular (proliferation index)	2.3	6.2*	7.3*	8.9	36.7*	43.3*
- midzonal (proliferation index)	1.5	4.9	5.6*	8.4	30.0*	39.1*
- periportal (proliferation index)	2.6	3.7	3.8	8.2	22.1*	31.0*
Liver cell proliferation (Ki-67)						
- centrilobular (proliferation index)	0.5	1.1*	1.1*	0.8	2.4	1.8
- midzonal (proliferation index)	0.5	1.0	0.8	0.9	2.0	2.1
- periportal (proliferation index)	0.7	0.5	0.6	0.4	0.8	0.6
28-day study						
Dietary concentration (ppm)	0	300	—	0	—	1500
Dose (mg/kg bw per day)	0	43.9	—	0	—	273
Targeted gene expression (fold change)						
- *Cyp 2b10*	1	61.7*	—	1	—	93.9*
- *Cyp 3a11*	1	1.5	—	1	—	5.6*
- *Cyp 4a10*	1	−1.6	—	1	—	−1.8
Liver enzyme activity						
- EROD (pmol/min per milligram protein)	16.8	23.8*	—	12.7	—	22.9*
- PROD (pmol/min per milligram protein)	1.8	8.8*	—	2.7	—	6.9*
- BROD (pmol/min per milligram protein)	2.3	21.4*	—	3.3	—	14.9*
90-day study						
Dietary concentration (ppm)	0	—	750		—	1500
Dose (mg/kg bw per day)		—	98		—	247
Targeted gene expression (fold change)						
- *Cyp 2b10*	1	—	56.5*	1	—	53.9*
- *Cyp 3a11*	1	—	2.8*	1	—	3.4*
- *Cyp 4a10*	1	—	−3.6	1	—	−2.3
Liver enzyme activity						
- EROD (pmol/min per milligram protein)	20.1	—	24.8*	16.4	—	24.5*
- PROD (pmol/min per milligram protein)	4.0	—	15.5*	5.1	—	20.0*
- BROD (pmol/min per milligram protein)	5.2	—	22.0*	6.1	—	24.4*

From Geter et al. (2010a)

BrdU, 5-bromo-2′-deoxyuridine; BROD, benzyloxyresorufin-*O*-debenzylase; EROD, 7-ethoxyresorufin-*O*-deethylase; PROD, 7-pentoxyresorufin-*O*-deethylase; * $P < 0.05$

in CD-1 mice. If comparable, these preliminary data would be used for an extensive study using C57BL/6J CAR/PXR knockout and "humanized" mice. Groups of five male C57BL/6J mice were given diets containing sulfoxaflor (purity 95.6%) at a concentration of 0, 750 or 1500 ppm (equal to 0, 160 and 310 mg/kg bw per day) for 7 days. End-points evaluated were daily clinical observations, body weights, body weight gain, feed consumption, serum clinical chemistry, targeted gene expression (quantitative RT-PCR using TaqMan gene expression assays; and sodium dodecyl sulfate–polyacrylamide gel electrophoresis [SDS-PAGE] and western blotting), protein quantification of liver microsomes, liver cytochrome P450 enzyme activity and hepatocellular proliferation.

There were no treatment-related clinical observations or effects on body weight or body weight gain throughout the study. There was a treatment-related increase in absolute and relative liver weights following 7 days of exposure to sulfoxaflor. Absolute liver weights at 750 and 1500 ppm groups were 117% and 128% of those of controls, respectively, and relative liver weights were 117% and 140% of those of controls, respectively. Despite this increase in liver weight, there was a lack of overt hepatotoxicity at either dose level of sulfoxaflor, as evidenced by the absence of toxicologically significant increases in plasma AST, cholesterol and triglycerides. ALT was increased in a dose-dependent manner (2-fold at the high dose level); however, this finding was not deemed to be of toxicological significance, as values were all within the historical control range.

Sulfoxaflor at 750 ppm and 1500 ppm elicited a 3- and 5-fold increase in total hepatic P450 content, respectively, a 33-fold increase in PROD activity (at both concentrations), a 47- and 82-fold increase in BROD activity, respectively, and a 4- and 7-fold increase in benzyloxyquinoline debenzylase (BQ) activity, respectively.

Gene expression results indicated the presence of *Cyp2b10* messenger ribonucleic acid (mRNA) in both treatment groups, but not controls. As *Cyp2b10* is not constitutively expressed, a fold change over control values cannot be calculated for treated groups, although it can be seen that there was a large difference from controls, and the change from treatment at 750 ppm to 1500 ppm was 9.2-fold. Unlike *Cyp2b10*, *Cyp3a11* is constitutively expressed in the mouse, and results can be expressed as a relative fold change over control values. Sulfoxaflor at 750 and 1500 ppm elicited a 2.4- and 5.6-fold increase in *Cyp3a11* relative to controls. These gene expression data were confirmed by SDS-PAGE and western blotting, which demonstrated that sulfoxaflor clearly induced *Cyp2b10* and *Cyp3a11*.

In conclusion, it would appear that sulfoxaflor exerts its enzyme induction properties via CAR and possibly also PXR (Elcombe, 2010).

A mechanistic study was conducted to investigate the MOA for sulfoxaflor-induced liver effects by use of dual CAR-PXR knockout and "humanized" mouse models. The aim of the study was to investigate 1) if CAR or PXR mediates sulfoxaflor-induced hypertrophy and hyperplasia in mice and 2) if the human receptors (CAR and PXR) support these processes to a similar extent as the murine receptors. The mouse models used were wild-type C57BL/6J (WT) mice, C57BL/6J mice null for PXR and CAR (PXRKO/CARKO) and C57BL/6J mice humanized for PXR and CAR (hPXR/hCAR).

Groups of 10 male mice of each strain were given diets containing sulfoxaflor (purity 95.6%) at a concentration of 0 or 750 ppm for 7 days. The mean doses at 750 ppm were equal to 116, 120 and 99 mg/kg bw per day for WT, PXRKO/CARKO and hPXR/hCAR mice, respectively. End-points evaluated were daily clinical observations, body weights, body weight gain, feed consumption, plasma clinical chemistry, targeted gene expression (quantitative RT-PCR using TaqMan gene expression assays; and SDS-PAGE and immunoblotting), protein quantification of liver microsomes, liver cytochrome P450 enzyme activity (PROD, BROD, BQ), hepatocellular proliferation using nuclear incorporation of BrdU and liver histopathology.

There were no treatment-related clinical observations or effects on body weight or body weight gain in any strain of mice. There were treatment-related increases in absolute and relative

liver weights in WT and hPXR/hCAR mice (absolute liver weights: 124% and 109% of controls, respectively; relative liver weights: 125% and 112% of controls, respectively), but not in the PXRKO/CARKO animals.

In WT mice, sulfoxaflor treatment increased hepatocellular proliferation (by approximately 4-fold), but no such changes in proliferation were seen in either the hPXR/hCAR or PXRKO/CARKO mice. Treatment-related hepatocyte hypertrophy was observed in WT and hPXR/hCAR mice, whereas increased mitotic figures were observed only in WT mice. Neither hepatocyte hypertrophy nor increased mitotic figures were seen in PXRKO/CARKO mice.

In agreement with results of the pilot study (Elcombe, 2010), sulfoxaflor behaved as a phenobarbital-like inducer in WT mice. This was demonstrated by marked induction of total cytochrome P450 content and markedly increased PROD and BROD activities (approximately 33- and 36-fold, respectively). Under the same treatment, induction of PROD and BROD in hPXR/hCAR mice was marginal (approximate increases of 2- and 3-fold, respectively), whereas neither PROD nor BROD activity was induced in PXRKO/CARKO mice.

These results were confirmed by the increased expression of *Cyp2b10* mRNA demonstrated by RT-PCR and by immunoblotting data showing increases in Cyp2b10 protein. As hepatic *Cyp2b10* is not constitutively expressed in WT C57BL/6J mice, a fold change in response to sulfoxaflor treatment cannot be calculated over control values, although it can be seen that there is a large difference from WT controls (mean threshold cycle [Ct] 29.8 ± 0.5 versus mean Ct > 35, respectively; Ct values > 35 are indicative of mRNA levels below the limits of detection for the assay) (Table 37). In contrast, basal *Cyp2b10* mRNA was expressed in the hPXR/hCAR animals, with a marginal (approximately 3.9-fold) increase in *Cyp2b10* mRNA observed following sulfoxaflor treatment. *Cyp2b10* mRNA levels were undetectable in control PXRKO/CARKO animals and remained so upon exposure to sulfoxaflor. These data indicate that *Cyp2b10* transcription is markedly upregulated by sulfoxaflor in WT mice, but not in hPXR/hCAR or PXRKO/CARKO mice.

Sulfoxaflor-mediated *Cyp3a11* induction, as determined by BQ activity (Cyp3a selective reaction), RT-PCR and immunoblotting, was observed in the humanized and WT mice to similar extents, but was not seen in the PXRKO/CARKO mice (Table 37).

In conclusion, sulfoxaflor exhibited markedly more activity towards the mouse CAR than the human CAR and relatively weak activity towards the mouse and human PXR. Hence, the difference in hepatic response between wild-type and humanized mice in this study is considered to be mediated via CAR. Furthermore, the data show that the human CAR and PXR support sulfoxaflor-induced hypertrophy but not hyperplasia. The study demonstrates that sulfoxaflor, like phenobarbital, acts via a CAR-mediated MOA (Ross, 2010).

Rats

In a mechanistic study conducted to determine if a phenobarbital-like MOA was responsible for the liver weight increases seen in rats after repeated exposure to sulfoxaflor and to obtain information on the time– and dose–response relationships for the effect, groups of five male and five female F344/DuCrl rats per group were given diets containing sulfoxaflor (purity 96.6%) at a concentration of 0, 100, 750 or 1500 ppm for 3 or 7 days. These dietary concentrations were equal to 0, 8.85, 60.3 and 99.2 mg/kg bw per day for males and 0, 7.83, 50.6 and 83.3 mg/kg bw per day for females necropsied on day 3, respectively, and equal to 0, 8.02, 58.6 and 102 mg/kg bw per day for males and 0, 7.74, 53.1 and 94.4 mg/kg bw per day for females necropsied on day 7, respectively. The primary end-points examined in this study were liver weight, targeted gene expression (quantitative RT-PCR using TaqMan gene expression assays), liver cytochrome P450 enzyme activity (EROD, PROD, BROD) and hepatocellular proliferation (Ki-67 immunohistochemical staining). Four nuclear receptors that are primarily responsible for xenobiotic-induced liver weight increase were investigated: AhR, CAR, PXR and PPARα.

Table 37. Summary of selected findings in a mechanistic study in mice

	WT		PXRKO/CARKO		hPXR/hCAR	
	Dietary concentration (ppm)					
	0	750	0	750	0	750
Body weight (g)	22.4	22.2	24.1	24.7	25.5	24.8
Liver weight, absolute (g)	0.99	1.22**	1.13	1.21	1.23	1.35
Liver weight, relative (% of body weight)	4.40	5.50**	4.69	4.89	4.84	5.42**
Hepatocellular hypertrophy	0/10	10/10	0/10	0/10	0/10	10/10
Mitotic alteration of hepatocytes	1/10	7/10	0/10	0/10	0/10	1/10
BrdU-positive hepatocytes (%)	2.41	8.79**	0.38	0.53	0.58	0.88
PROD (pmol/min per milligram protein)	3.3	110**	4.7	4.8	11.2	27.9**
BROD (pmol/min per milligram protein)	7.6	274**	20.3	33.3	83.0	241**
BQ (nmol/min per milligram protein)	1.8	4.8**	3.2	3.3	3.9	8.1**
Total P450 (nmol/mg protein)	0.45	0.86**	0.51	0.51	0.64	0.90**
Cyp2b10; average Ct value	> 35	29.8	>3 5	> 35	33.6	31.7
- fold change, relative to control	—	ND	—	ND	—	3.9
Cyp3a11; average Ct value	21.1	20.1	21.3	22.5	20.2	18.5
- fold change, relative to control	—	2.1	—	−2.5	—	3.4

From Ross (2010)
BQ, benzyloxyquinoline debenzylase; BrdU, 5-bromo-2′-deoxyuridine; BROD, benzyloxyresorufin-*O*-debenzylase; Ct, threshold cycle; ND, not determined; PROD, 7-pentoxyresorufin-*O*-deethylase; ** $P < 0.01$

All rats survived the treatment period. Body weight gains and feed consumption were reduced at 750 and 1500 ppm. Relative liver weights were increased for males and females in the 1500 ppm group at 3 days (14% and 3%, respectively) and at 750 and 1500 ppm at 7 days (11% and 23% for males and 6% and 18% for females, respectively) (Table 38).

Cy*p2b1* gene expression, the prototypical gene response following phenobarbital exposure, was induced over 800-fold in both male and female rats exposed to 1500 ppm sulfoxaflor for 3 and 7 days. To further examine similarities in phenobarbital-like response, *Cyp2b2* and *Cyp3a3* (CAR- and PXR-related genes, respectively) expression levels, together with PROD and BROD enzyme activities, were evaluated at 3 and 7 days. These molecular markers were elevated in males and females exposed to 750 and 1500 ppm at both time periods in a phenobarbital-like manner. At 7 days, male rats exposed to 750 ppm sulfoxaflor and male and female rats at 1500 ppm sulfoxaflor showed significant hepatocellular proliferation; for males, it occurred in the centrilobular and midzonal regions, whereas for females, it was evident in the centrilobular region. Male rats exposed to 750 and 1500 ppm at both time periods and female rats exposed to 1500 ppm at day 7 showed significantly elevated levels of serum cholesterol; targeted gene expression analysis provided little insight into the possible MOA responsible for this increase (Table 38).

Cyp1a1 gene expression in male rats at 750 and 1500 ppm was slightly but significantly elevated at days 3 and 7; however, EROD enzyme activity was only slightly increased on day 3 and returned to control levels by day 7. Female rats showed a slight but significant increase in *Cyp1a1* gene expression at 1500 ppm on day 3, whereas no treatment-related changes in EROD levels were observed at either 3 or 7 days. Furthermore, gene expression of *Cyp4a22* was not elevated in this study. From these data, it is implied that sulfoxaflor is not likely an AhR or PPARα agonist.

Based upon these results, the MOA responsible for the increased liver weight in rats after administration of sulfoxaflor was phenobarbital-like, as evidenced by the CAR- and PXR-related molecular, enzymatic and proliferative responses. With the exception of a very mild induction of

Table 38. Summary of selected findings in a mechanistic study in rats

	Males				Females			
	Dietary concentration (ppm)							
	0	100	750	1500	0	100	750	1500
Body weight (g), day 3	177.8	167.4	168.0	164.9*	144.2	138.4	138.4	131.4*
Body weight gain (g), day 3	15.2	16.3	12.5	6.1	7.8	6.1	4.0	−0.1
Liver weight, absolute (g), day 3	7.47	7.24	7.78	7.84	5.18	5.09	4.95	4.87
Liver weight, relative (% of body weight), day 3	4.19	4.33	4.62	4.76*	3.59	3.68	3.58	3.71*
Body weight (g), day 7	198.7	209.3	195.2	184.6	147.3	150.1	147.8	144.7
Body weight gain (g), day 7	31.6	34.5	29.2	17.8	12.7	13.2	10.4	6.7
Liver weight, absolute (g), day 7	8.37	8.79	9.13	9.54	5.21	5.29	5.56	6.03
Liver weight, relative (% of body weight), day 7	4.20	4.18	4.67*	5.16*	3.54	3.53	3.76*	4.17*
Cholesterol (mg/dl), day 3	62	65	74*	80*	84	83	85	91
Cholesterol (mg/dl), day 7	60	61	80*	112*	75	76	82	106*
Targeted gene expression								
Cyp1a1, day 3	1	1.7	2.8	12.2	1	1.3	1.9	1.7*
Cyp2b1, day 3	1	9.7*	586*	1064*	1	3.6	399*	1204*
Cyp2b2, day 3	1	2.8*	8.2*	17.1*	1	2.5*	10.9*	21.4*
Cyp3a3, day 3	1	1.3	3.4*	8.8*	1	1.4	4.0*	7.8*
Alas1, day 3	1	−1.0	1.8*	2.4*	1	1.0	1.7*	2.6*
NADPH, day 3	1	1.1	2.0*	3.3*	1	1.1	1.7*	2.8*
Cyp1a1, day 7	1	1.1	3.2*	12.3*	1	−1.4	−1.0	2.4
Cyp2b1, day 7	1	7.2*	559*	848*	1	2.1	315*	856*
Cyp2b2, day 7	1	2.9*	10.2*	21.3*	1	1.5	6.7*	11.4*
Cyp3a3, day 7	1	1.2	3.3*	9.3*	1	−1.0	3.2*	6.6*
Alas1, day 7	1	1.2	1.6*	2.9*	1	1.0	1.8*	2.0*
NADPH, day 7	1	1.1	2.0*	3.2*	1	−1.0	1.6*	2.5*
Liver enzyme activity								
EROD (pmol/min per milligram protein), day 3	16.1	20.3*	25.9*	24.9*	26.6	25.3	24.9	28.8
PROD (pmol/min per milligram protein), day 3	2.9	2.7	24.4*	31.7*	3.7	3.3	18.3*	46.4*
BROD (pmol/min per milligram protein), day 3	2.8	3.5	6.4*	4.4*	3.2	3.1	16.4*	17.9*
EROD (pmol/min per milligram protein), day 7	16.3	18.0	18.2	16.3	31.4	30.9	31.7	26.7
PROD (pmol/min per milligram protein), day 7	3.5	4.6	33.9*	35.9*	4.7	5.3	61.4*	75.1*
BROD (pmol/min per milligram protein), day 7	3.5	4.6	16.9*	13.2*	2.6	3.1	27.6*	18.9*
Liver cell proliferation (Ki-67)								
Centrilobular (proliferation index), day 3	2.7	3.5	4.0	2.9	1.9	1.4	3.2	1.7
Midzonal (proliferation index), day 3	4.6	6.5	5.1	4.0	3.3	2.2	3.5	1.6
Centrilobular (proliferation index), day 7	3.3	4.1	7.8*	11.3*	1.7	1.3	2.6	4.5*
Midzonal (proliferation index), day 7	3.9	5.0	8.4*	11.2*	3.0	2.4	3.1	4.1

From Geter & Card (2010)

BROD, benzyloxyresorufin-*O*-debenzylase; EROD, 7-ethoxyresorufin-*O*-deethylase; PROD, 7-pentoxyresorufin-*O*-deethylase; * $P < 0.05$

Cyp2b1 and *Cyp2b2* gene expression in males, all other events occurred at concentrations above 100 ppm. These data support the conclusion that sulfoxaflor may be an agonist ligand primarily for CAR, and this activation results in the observed phenobarbital-like response and increased liver weight (Geter & Card, 2010).

(c) Mechanistic studies on Leydig cell tumour induction in rats

In a mechanistic study conducted to investigate the possible MOA responsible for the increase in bilateral Leydig cell tumour incidences in rats after long-term exposure to sulfoxaflor, groups of 15 male F344/DuCrl and 15 male Crl:CD(SD) rats were given diets containing sulfoxaflor (purity 95.6%) at a concentration of 0, 25, 100 or 500 ppm for up to 8 weeks. These dietary concentrations were equal to 0, 1.41, 5.58 and 27.8 mg/kg bw per day for F344/DuCrl rats and 0, 1.37, 5.59 and 27.7 mg/kg bw per day for Crl:CD(SD) rats. End-points evaluated included daily cage-side observations, weekly clinical observations and weekly examination of body weights and feed consumption. Also, in order to investigate enhanced biliary elimination of testosterone as a potential MOA, three rats per group underwent bile duct cannulation after 2 weeks of treatment to measure levels of [^{14}C]-testosterone-derived radioactivity within the bile and flow rate of the bile over a 2-hour period. In order to investigate dopamine agonism/enhancement as a potential MOA, a serum hormone panel of testosterone, luteinizing hormone (LH), prolactin and 17β-estradiol was evaluated on all available animals after 2, 4 and 8 weeks of treatment. In addition to hormone measurements, targeted gene expression analysis (quantitative RT-PCR using TaqMan gene expression assays) on LH and prolactin receptors in all Fischer 344 rat testes (4 and 8 weeks) as well as immunohistochemistry of LH receptors (LHR) in the testes of all necropsied rats at 4 weeks were performed. Quantification of LHR immunostaining was performed only on Fischer 344 rat testes (4 weeks only). During the 4- and 8-week necropsies, liver samples were collected for possible analysis; however, this was deemed not necessary based on results from the biliary elimination portion of the study indicating that the liver was not involved in the MOA. In addition, portions of testis were frozen during these necropsies as contingencies for immunohistochemistry on frozen sections, which was not necessary, as this was performed on formalin-fixed tissue.

There were no treatment-related clinical observations or effects on body weight or feed consumption in either strain during the study.

There was a treatment-related increase in absolute and/or relative liver weights of rats given 500 ppm sulfoxaflor in both strains at 4 weeks and in Fischer rats at 8 weeks (Table 39). There were no treatment-related effects on liver weights at 25 or 100 ppm or on testis weights at any dose level tested.

Quantification of LHR immunostaining on Fischer 344 rat testes performed at 4 weeks (the time point at which the largest hormone changes were observed) showed no treatment-related effect on the percentage of Leydig cells with cytoplasmic staining of LHR.

In bile duct–cannulated rats, there were no statistically significant or treatment-related differences in the mean [^{14}C]testosterone-derived radioactivity excreted in the bile across all dose groups, per time interval, for Crl:CD(SD) and F344/DuCrl rats. Bile flow was very similar for the respective dose groups, time intervals and strains. Overall, Crl:CD(SD) rats excreted approximately 1.5–3 times the cumulative amount of bile [^{14}C]testosterone-derived radioactivity compared with F344/DuCrl rats from the respective dose groups. The lower plasma radioactivity values for Crl:CD(SD) rats are consistent with having a higher biliary clearance than F344/DuCrl rats.

There was no effect of treatment on Fischer 344 rat serum hormone levels at the 2- or 8-week time points. After 4 weeks at 500 ppm, there was an approximate 2-fold increase in LH levels concomitant with an approximate 1.7-fold dose-dependent decrease in prolactin levels and an approximate 2.8-fold increase in testosterone levels. This hormone profile was somewhat recapitulated in Crl:CD(SD) rats, albeit with a different timing, where an increase in LH levels occurred with a

Table 39. Summary of selected findings in a mechanistic study on Leydig cell tumour induction in rats

	F344/DuCrl				Crl:CD(SD)			
	Dietary concentration (ppm)							
	0	25	100	500	0	25	100	500
Body weight (g), week 4	284	290	284	288	458	489*	457	481
Liver weight, absolute (g), week 4	10.2	11.0	11.0	12.4	17.6	19.7	17.8	21.0*
Liver weight, relative (% of body weight), week 4	3.6	3.8	3.9	4.3*	3.8	4.0	3.9	4.4*
Body weight (g), week 8	303	315	316	315	556	517	527	519
Liver weight, absolute (g), week 8	10.8	11.1	11.7	12.4*	20.7	18.9	19.3	19.9
Liver weight, relative (% of body weight), week 8	3.5	3.5	3.7	3.9*	3.7	3.7	3.7	3.8
Testes, LHR-positive cells (%), week 4	20.4	16.3	23.2	17.0	ND	ND	ND	ND
[^{14}C]Testosterone elimination in bile (%)	25.7	28.7	29.5	31.7	72.3	78.3	43.1	69.8
Serum hormone analysis								
Week 2								
Prolactin (ng/ml)	9.5	12.8	8.2	9.4	14.6	15.7	11.2	15.1
Testosterone (ng/g)	0.76	0.83	0.54	0.90	2.0	4.5*	3.7	3.7*
LH (ng/ml)	0.54	0.81	0.27	0.42	0.29	0.49	0.36	0.78*
Week 4								
Prolactin (ng/ml)	17.9	15.5	16.2	10.4	11.6	11.2	10.3	9.0
Testosterone (ng/g)	0.67	1.00	1.19	0.93	2.42	2.61	4.67	2.50
LH (ng/ml)	0.47	0.54	0.66	0.88*	1.10	0.34	0.36	0.35
Week 4, terminal								
Prolactin (ng/ml)	39.4	57.6	57.1	45.7	60.0	42.5	49.8	32.5
Testosterone (ng/g)	1.17	2.79	2.58	3.27	6.07	5.57	9.35	6.15
LH (ng/ml)	4.41	4.18	4.47	4.49	4.06	2.00	2.10	2.57
Week 8								
Prolactin (ng/ml)	19.3	17.9	19.5	17.5	14.5	16.5	24.3	19.1
Testosterone (ng/g)	0.58	0.67	0.77	0.70	1.75	2.74	2.43	1.94
LH (ng/ml)	0.89	1.04	0.69	1.08	0.25	0.47	0.66*	0.50
Week 8, terminal								
Prolactin (ng/ml)	70.9	60.5	53.7	71.9	35.3	34.0	41.0	33.2
Testosterone (ng/g)	2.56	2.42	2.56	2.68	3.01	3.41	4.28	3.79
LH (ng/ml)	4.37	4.64	3.84	4.75	1.86	1.71	2.50	2.32

From Rasoulpour et al. (2010d)
ND, not determined; * $P < 0.05$

concomitant increase in testosterone levels at 2 weeks, and a decrease in prolactin levels occurred at 4 weeks (Table 39).

Consistent with the decreased prolactin levels in the Fischer 344 rat at 4 weeks (500 ppm), there was a decrease in the LHR and the prolactin receptor gene expression at the 4-week, but not the 8-week, time point at 500 ppm (Table 40).

Table 40. Summary of targeted gene expression analysis in F344/DuCrl rats

	Fraction of control values			
	0 ppm	25 ppm	100 ppm	500 ppm
Week 4				
LHR	1	0.94	0.91	0.64
Prolactin receptor	1	0.79	0.97	0.62
StAR	1	0.80	1.16	0.96
Cyp11a1	1	0.97	1.24	0.87
Cyp17a1	1	0.53	0.91	0.44
HSD3b	1	0.78	1.04	0.73
SDR5a1	1	0.92	1.04	0.73
Week 8				
LHR	1	0.78	1.19	1.12
Prolactin receptor	1	0.79	1.33	1.08
StAR	1	0.79	1.22	1.14
Cyp11a1	1	0.84	1.57	1.45
Cyp17a1	1	0.96	2.12	1.34
HSD3B	1	0.76	1.36	1.06
SDR5a1	1	0.54	0.83	0.90

From Rasoulpour et al. (2010d)
Cyp11a1, P450 side-chain cleavage; *Cyp17a1*, 17α-hydroxylase; *HSD3b*, 3β-hydroxysteroid dehydrogenase; *SDR5a1*, steroid-5α-reductase; *StAR*, steroidogenic acute regulatory protein

There was no dose-dependent effect of treatment on any measured gene in the steroidogenic pathway, including *StAR* (steroidogenic acute regulatory protein), *Cyp11a1* (P450 side-chain cleavage), *Cyp17a1* (17α-hydroxylase), *HSD3b* (3β-hydroxysteroid dehydrogenase) or *SDR5a1* (steroid-5α-reductase) (Table 40).

The authors concluded that the study provides evidence supporting the hypothesis that the Leydig cell tumour promotion seen in the rat chronic toxicity and carcinogenicity study was through weak, but chronic, enhancement of dopamine release and subsequent inhibition of prolactin release from the pituitary gland, leading to increases in LH and testosterone levels, along with decreases in testis LHR gene expression. This MOA could operate through sulfoxaflor-mediated enhancement of dopamine release, potentially though agonism of α4β2 or α4α6β2 central nAChRs, which are known to play a key regulatory role in dopamine release from dopaminergic neurons in the brain (Rasoulpour et al., 2010d).

In a subsequent mechanistic study, sulfoxaflor (purity 95.6%) was evaluated for estrogen receptor (ER) alpha (ERα) and androgen receptor (AR) binding, ER and AR transactivation (agonism and antagonism) and aromatase inhibition. The ER and AR binding potential of sulfoxaflor was assessed in separate fluorescence polarization binding assays, which utilize a fluorescent ligand (Fluormone) and examine the ability of the test material to displace Fluormone from the receptor. The ERα used in these assays was a full-length receptor, whereas the AR consisted of only the ligand binding domain. The biological relevance of the ER and AR binding data was evaluated using a sensitive and reliable in vitro transactivation system. Specifically, the ability of sulfoxaflor was assessed for estrogen and androgen agonism and antagonism using stably transfected hERα-T47D-KBluc and hAR-MDA-kb2 cell lines, respectively. Aromatase inhibition of sulfoxaflor was evaluated by measuring the

conversion of an androgen to an estrogen using microsomes from cells expressing recombinant aromatase (CYP19). During the conversion of [^{3}H]androstenedione to estrone, ^3H_2O was released and quantified as a direct measurement of aromatase activity per unit reaction time. Competitive inhibition of aromatase activity by sulfoxaflor was then evaluated. A minimum of two independent runs of each assay were conducted for ERα and AR fluorescence polarization binding, ER and AR transactivation (agonism and antagonism) and aromatase activity. The duration of exposure was 24 hours for all transactivation assays. A complete concentration–response curve was run for each of the reference compounds and sulfoxaflor each time the transcriptional activation assay was performed. Aromatase inhibition assays were also run as two independent assays; androstenedione was used as the positive reference control.

Sulfoxaflor did not demonstrate any agonism or antagonism in the ER and AR transactivation assays. Reference controls demonstrated that the systems were performing as expected and able to detect mild agonism and antagonism for both ER and AR.

In two independent runs of the AR binding assay, sulfoxaflor exhibited a binding curve that passed 50%, which categorizes this test substance as a potential binder. However, the relative binding affinity of sulfoxaflor compared with dihydrotestosterone (DHT) was weak, with a mean relative binding affinity (compared with DHT) of 0.0014. Furthermore, the AR transactivation assays did not support the binding identified in the fluorescence polarization binding assay. As the AR in the fluorescence polarization binding assay included only the binding domain, it is possible that the binding that occurred in the fluorescence polarization assay could be an event that would not occur with the full-length AR receptor. Other mechanisms of action could also be causing the curve to cross 50% in the fluorescence polarization binding assay, including sulfoxaflor acting directly upon the receptor by denaturation.

In two independent runs of the ERα binding assay, sulfoxaflor did not displace Fluormone from the full-length ER, and no binding occurred. The ER transactivation assay supported the binding data, and sulfoxaflor was determined not to interfere with ER binding. Concurrent positive controls indicated specificity of the system to identify an ER binding compound.

In two independent runs of the aromatase assay, sulfoxaflor did not inhibit CYP19. Concurrent positive controls indicated the specificity of the system to identify an aromatase inhibitor.

Taken together, the results from these five different in vitro screening tests with sulfoxaflor did not indicate changes consistent with endocrine-mediated alterations (Toole, 2011).

A mechanistic study was performed in male Sprague-Dawley rats to determine the effect of sulfoxaflor (purity not stated), administered via intracerebral reverse microdialysis, on extracellular levels of dopamine and its two major metabolites, dihydroxyphenylacetic acid and homovanillic acid, in the hypothalamus. The experiment was performed the day following surgery. Dialysate samples were collected every 20 minutes from 80 minutes before drug administration until 280 minutes after drug administration (18 samples in total from each probe; 4 pre-drug and 14 post-drug). Treatments were administered to eight animals. Sulfoxaflor was infused directly into the hypothalamus at two concentrations (0.4 and 2 mmol/l) for 40 minutes each with the onset of the infusions 120 minutes apart (periods of infusion were 0–40 minutes and 120–160 minutes, respectively). Elevated potassium (50 mmol/l) was infused for 10 minutes to act as a positive control (240–250 minutes).

Sulfoxaflor at 0.4 mmol/l had no effect on the extracellular dopamine levels in the rat hypothalamus at any single time point; however, dopamine efflux averaged over the duration of the sulfoxaflor infusion was significantly ($P = 0.016$) increased. The higher concentration (2 mmol/l) resulted in a significant increase in extracellular dopamine, with a maximal rise of 39% ± 13% 40 minutes after the onset of infusion ($P < 0.001$), and dopamine efflux averaged over the duration of the sulfoxaflor infusion was also significantly ($P = 0.008$) increased. Infusion of potassium ions (50 mmol/l) increased dopamine levels, with a maximum increase of 78% ± 17% 20 minutes after onset of perfusion ($P < 0.001$), and

overall ($P < 0.001$). Both sulfoxaflor and potassium ions produced concomitant significant decreases in the concentration of homovanillic acid 40 minutes after the onset of infusion (18% ± 7%, $P < 0.05$; and 25% ± 6%, $P < 0.01$, respectively), whereas neither sulfoxaflor nor potassium ions altered the extracellular concentration of dihydroxyphenylacetic acid (Rowley & Heal, 2011).

(d) Mechanistic studies on fetal abnormalities and neonatal death in rats

A special study was conducted to examine the binding and the agonist activity of sulfoxaflor (purity 95.6%) on mammalian muscle nAChRs and to test the hypothesis that the high incidence of external limb abnormalities and misshapen clavicles is mediated by the pharmacological agonist action of sulfoxaflor at the fetal neuromuscular junction nAChR. This aim was achieved by use of competition radioligand binding and electrophysiological methods.

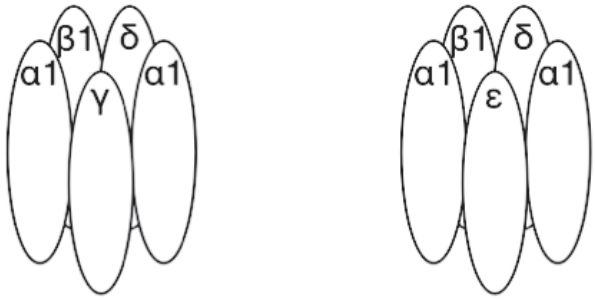
In both invertebrates and vertebrates, nAChRs comprise a diverse family of receptors, being assembled from a variety of distinct subunits. They are oligomeric cell surface proteins in which five subunits co-assemble in a doughnut-shaped arrangement. In the centre of the pentameric arrangement of subunits is a cation-selective ion channel. Binding of the endogenous neurotransmitter acetylcholine (ACh) (or of other agonists) stabilizes the open conformation of the ion channel and allows the influx of cations into the cell. In addition to nAChRs expressed in the mammalian central and peripheral nervous systems, nAChRs are also expressed at the neuromuscular junction. Five nAChR subunits are expressed in mammalian muscle cells (α1, β1, γ, δ and ε). Transcription of the γ and ε subunit genes is regulated developmentally, whereby the γ subunit is expressed in "fetal" muscle and the ε subunit is expressed in "adult" muscle. In rodents, it has been demonstrated that replacement of the γ subunit by the ε subunit initiates late during the 1st postnatal week and is largely complete by the end of the 2nd postnatal week, although in the extraocular muscles, expression of the γ subunit persists into adulthood. In humans, the switch from γ to ε subunit expression occurs predominantly during the late fetal period. Although many subtypes of nAChRs with distinct subunit composition have been identified in the central and peripheral nervous systems (neuronal nAChRs), only two nAChR subtypes are expressed in mammalian muscle (the fetal and adult muscle nAChRs). The subunit composition of the "fetal" and "adult" muscle nAChR can be represented as $(\alpha1)_2\beta1\gamma\delta$ and $(\alpha1)_2\beta1\delta\varepsilon$ (Figure 3).

Radioligand binding studies were performed to examine the ability of sulfoxaflor to bind to nAChRs from three mammalian species (human, rabbit, rat). Fetal muscle tissue was isolated from the rat and rabbit forelimb. Because of difficulty and concerns in obtaining and using human fetal muscle tissue, binding experiments with human nAChRs were performed with recombinant receptors, generated by expression in cultured human embryonic kidney cell lines of cloned complementary deoxyribonucleic acids (cDNAs) encoding human nAChR subunits for the "fetal" ($(\alpha1)_2\beta1\delta\gamma$) and "adult" ($(\alpha1)_2\beta1\delta\varepsilon$) muscle receptors.

The ability of sulfoxaflor to act as an agonist of nAChRs was examined using the technique of two-electrode voltage clamp recording, which allows the flow of current through cell surface nAChRs to be measured in response to agonist application. Receptors were expressed in *Xenopus* oocytes by microinjection of cDNA or complementary ribonucleic acid (cRNA) encoding the appropriate rat or human muscle nAChR subunits. To generate the fetal form of the muscle nAChRs, α1, β1, γ and δ cDNAs or cRNAs were injected, whereas α1, β1, δ and ε were injected to generate the adult form. With this expression system, the potency of agonist-induced nAChR activation can be measured in a quantitative manner, and evidence suggests that recombinant muscle nAChRs expressed in this system accurately mimic the pharmacological properties of native nAChRs. Functional responses (membrane currents) were detected in response to application of the endogenous agonist ACh.

The initial radioligand binding studies showed high levels of nonspecific binding of [^{3}H]sulfoxaflor. Therefore, competition binding was employed to examine whether unlabelled sulfoxaflor was able to displace binding of the high-affinity nAChR radioligand, [^{3}H]epibatidine. By fitting the concentrations of sulfoxaflor causing half-maximal displacement (median inhibitory concentrations

Figure 3. Subunit composition of the "fetal" and "adult" forms of the muscle nAChR



"Fetal" muscle nAChR **"Adult" muscle nAChR**

From Millar (2010)

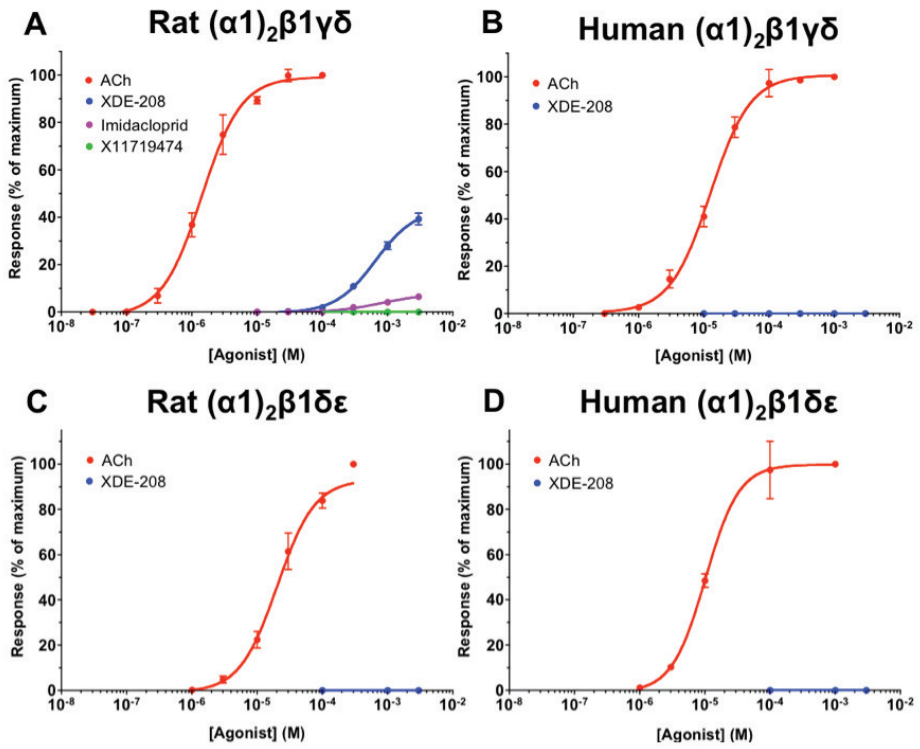
[IC_{50}s]) of 30 nmol/l [^{3}H]epibatidine to a single binding site model, estimates of IC_{50} for sulfoxaflor were 0.2 mmol/l for human, 0.4 mmol/l for rabbit and 2.3 mmol/l for rat. Although data obtained with human nAChRs and with rabbit muscle are fitted well by the single-site model, this was not the case for rat fetal muscle. Therefore, the binding data from rat muscle were fitted with a two-site model that revealed two distinct binding sites of different affinities (0.01 mmol/l and 8.9 mmol/l). The better fit of the rat fetal muscle nAChR experimental data to a two-site model would suggest that sulfoxaflor displaces [^{3}H]epibatidine from the two nAChR agonist binding sites (located at the α–γ and α–δ subunit interfaces) with different affinities.

The electrophysiological studies revealed that sulfoxaflor is a partial agonist of the rat fetal muscle nAChR. However, sulfoxaflor is a relatively low potency partial agonist, as the maximum response observed at the maximum feasible concentration (3 mmol/l, median effective concentration [EC_{50}] > 0.6 mmol/l) was 39% of that detected with a maximal concentration of ACh. In contrast, sulfoxaflor has no detectable agonist activity on the human fetal muscle nAChR or on the adult muscle nAChR from either human or rat (Figure 4). Imidacloprid, a neonicotinoid insecticide, also had partial agonist activity on the rat fetal muscle nAChR, but the maximal response detected with the highest concentration of imidacloprid tested (3 mmol/l) was approximately 7% of that detected with a maximal concentration of ACh and also only about 16% of the much lower maximal response detected with sulfoxaflor. X11719474, a soil metabolite of sulfoxaflor, had no significant agonist activity on the rat fetal muscle nAChR.

The results of this study show that sulfoxaflor is an agonist of the rat fetal muscle nAChR (which contains the rat γ subunit), whereas it has no agonist activity on the equivalent human fetal nAChR (containing the human γ subunit) or on the rat or human adult muscle nAChR (containing the rat or human ε subunit). It seems reasonable to conclude that the selective agonist activity of sulfoxaflor is due to differences in the amino acid sequence of the rat γ subunits compared with that of the human γ subunit (and also with the rat and human ε subunits) (Millar, 2010).

In a special study, a qualitative investigation of the action of sulfoxaflor on isolated phrenic nerve–hemidiaphragm preparations from newborn rats was conducted to assess the assumption that fetal limb contractions and reduced neonatal survival in rats following exposure during gestation result from the activation of the fetal muscle–type nAChR by sulfoxaflor, thereby causing sustained muscle contracture in the fetus and inhibition of nerve-evoked contraction of the diaphragm that would cause impaired respiration after birth, resulting in the previously observed reductions in neonatal survival. In support of this hypothesis, sulfoxaflor has been demonstrated to be an agonist at rat, but not human, embryonic ($(\alpha1)_2\beta1\delta\gamma$)) nAChR, while being without agonist activity at mature ($(\alpha1)_2\beta1\delta\varepsilon$)) muscle–type nAChRs (rat or human).

Figure 4. Agonist activation of nAChRs expressed in* Xenopus *oocytes. *Data are shown for the rat fetal $(\alpha1)_2\beta1\gamma\delta$ nAChR (A), human fetal $(\alpha1)_2\beta1\gamma\delta$ nAChR (B), rat adult $(\alpha1)_2\beta1\delta\varepsilon$ nAChR (C) and human adult $(\alpha1)_2\beta1\delta\varepsilon$ nAChR (D). AChRs were expressed by microinjection of cDNA or cRNA in* ***Xenopus*** *oocytes. Dose–response curves are shown in which agonist-evoked responses are normalized to the maximal response detected with the endogenous agonist, ACh. XDE-208, sulfoxaflor; X11719474, metabolite of sulfoxaflor. Data points are means ± standard error of the mean of 3–7 responses.*



From Millar (2010)

Isolated phrenic nerve–hemidiaphragm preparations from newborn (PND 0) Sprague-Dawley rats (supplier, UCL Biological Services) were used. Recorded measures included 1) changes in muscle twitch and 2) muscle contracture following test material application to the bath perfusion system. Muscle twitch tension reflects phrenic nerve action potential-evoked brief contraction of the diaphragm and is displayed as an upward deflection of the recording trace. Muscle contracture reflects a test material–induced prolonged contraction of the diaphragm associated with increased muscle tension beyond that evoked via electrical stimulation of the phrenic nerve. In each experiment, a period of at least 3 minutes of stable baseline and twitch tension was recorded before application of any test material. Preparations were viable for between 1 and 3 hours and could respond repeatedly to test material application.

Sulfoxaflor at a high concentration (1 mmol/l) consistently produced a reversible, concentration-dependent contracture of the neonatal diaphragm muscle and a decrease in muscle twitch response similar in magnitude to that observed with ACh (100 µmol/l). The contracture was blocked by the selective muscle-type nAChR antagonist, tubocurarine (10 µmol/l), showing that the contracture induced by sulfoxaflor is mediated via nAChR activation, rather than via a post-receptor mechanism. Furthermore, prolonged application of sulfoxaflor caused a sustained muscle contracture. Muscle twitches in response to phrenic nerve stimulation were not affected at low sulfoxaflor

concentration (100 µmol/l) but were reduced at high concentration (1 mmol/l), demonstrating that sulfoxaflor can cause inhibition of nerve-evoked contraction of the diaphragm during sustained contracture, consistent with the observed impairment of respiration in the neonatal rat.

The results of this study demonstrate that sulfoxaflor caused a contracture of the newborn rat diaphragm by acting on the nAChR. Prolonged application caused a sustained muscle contracture and a contracture-associated inhibition of the phrenic nerve–evoked muscle twitch, which is considered analogous to the situation in vivo, which resulted in fetal limb contractions (sustained muscle contractions) and compromised respiration at birth (contracture-associated inhibition of the muscle twitch). The results are consistent with, and add additional support to, the hypothesis that sulfoxaflor causes neonatal death (and fetal abnormalities) via activation of the fetal muscle–type nAChR (Gibb, 2010).

A special histopathological evaluation of fetal lung samples from the prenatal developmental toxicity study in rats (Rasoulpour, Marshall & Saghir, 2010) was conducted to detect any morphological abnormalities (e.g. increased collagen deposition) in any region of the lungs that may have been contributory to the treatment-related increase in neonatal pup mortality. Samples were collected from two formalin-fixed fetuses (one male and one female) per dam from five control and four 1000 ppm litters (18 samples total). Trachea and lungs of the selected fetuses were routinely processed for histology; sections were cut at a thickness of 5–6 µm, stained with haematoxylin and eosin, and examined.

There were no sulfoxaflor-induced lesions in the trachea, bronchi, bronchioles or alveoli in any of the treated fetuses examined. There were no treatment-related increases in collagen deposition around the airways or alveolar walls or any other changes. All observations were considered within normal limits. Thus, histopathological examination of the trachea and lungs of selected fetuses from dams given 1000 ppm sulfoxaflor from GD 6 to GD 21 did not reveal any morphological abnormalities that could have contributed to the sulfoxaflor-induced neonatal mortality in rat pups (Thomas & Marshall, 2010).

(e) Studies with metabolites

The structures of the sulfoxaflor metabolites tested in the toxicological studies described below are given in Table 41.

Studies with X11719474 (soil and plant metabolite of sulfoxaflor)

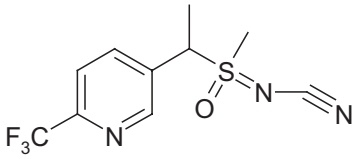
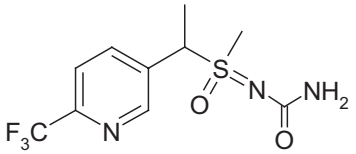
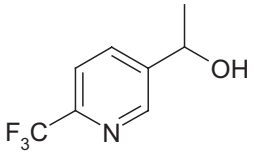
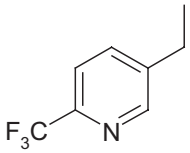
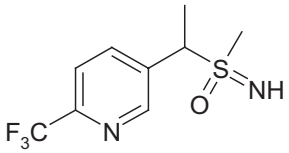
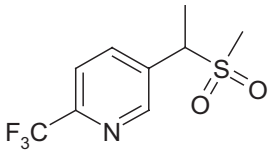
In a non-guideline pilot study to determine toxicokinetic properties, groups of one male and one female (F344/DrCrl) rat were treated with X11719474 (radiochemical purity 98.8%, specific activity 1.73 GBq/mmol) formulated in 0.5% aqueous methylcellulose at 100 mg/kg bw by oral gavage. Toxicokinetics, mass balance and urinary metabolite profile were determined in animals of one group, and the profile of plasma metabolites was determined in a second group.

The test compound was rapidly absorbed upon oral administration. Absorption was almost complete (95%/98% in males/females, respectively). Total recovery was 98%/99% (males/females). The majority of the administered dose (91–95%) was recovered in urine within 12 hours post-administration and increased slightly until 168 hours post-dosing. Low amounts (2–3%) were excreted via faeces over the total observation period. Plasma elimination of test compound was biphasic, with a rapid initial phase ($t_{½} \leq 2$ hours) and a slower second phase ($t_{½} \leq 36$–41 hours). Red blood cell elimination was monophasic and slightly slower than that from plasma.

Absorbed material was essentially unmetabolized in both sexes. In urine, mainly parent compound and two minor (< 1%) metabolites or impurities were detected (Hansen et al., 2010).

In a non-guideline, non-GLP acute oral toxicity study, three female (F344/DuCrl) rats received X11719474 (purity 100%) in 0.5% aqueous methylcellulose at a single dose of 300 mg/kg bw by

Table 41. Structures of sulfoxaflor metabolites tested in toxicological studies

Substance	IUPAC chemical name	Structure
Sulfoxaflor	[Methyl(oxo){1-[6-(trifluoromethyl)-3-pyridyl]ethyl}-λ^4-sulfanylidene]cyanamide	
X11719474 (soil and plant metabolite)	*N*-(Methyl(oxido){1-[6-(trifluoromethyl)pyridin-3-yl]ethyl}-λ^4-sulfanylidene)urea	
X11721061 (plant and animal metabolite)	1-[6-(Trifluoromethyl)pyridin-3-yl]ethanol	
X11596066 (animal metabolite)	5-Ethyl-2-trifluoromethylpyridine	
X11579457 (soil metabolite)	5-[1-(*S*-Methylsulfonimidoyl)ethyl]-2-(trifluoromethyl)pyridine	
X11519540 (soil and animal metabolite)	5-[(1-Methylsulfonyl)ethyl]-2-(trifluoromethyl)pyridine	

gavage at a volume of 10 ml/kg bw. The rats were monitored daily for mortality and clinical signs for a 72-hour period. Animals were not examined for gross pathological changes.

None of the animals died or showed any signs of toxicity. Under the conditions of the study, the estimated acute oral LD_{50} in rats was above 300 mg/kg bw in females (Golden, 2007/2010).

In an acute oral toxicity study conducted according to OECD test guideline 423 ("acute toxic class method"), female Fischer 344 rats received X11719474 (purity 99%) in distilled water at a single dose of 2000 (one animal) or 5000 mg/kg bw (three animals) by gavage at a volume of 24.8 ml/kg bw (because of the high volume, the high-dose group received the dose split into two approximately equal portions). The rats were monitored daily for mortality and clinical signs for a 2-week period, and body weights were recorded on days 1, 7 and 14. On day 14, surviving animals were killed, necropsied and examined for gross pathological changes.

The female dosed with 2000 mg/kg bw and the three females dosed with 5000 mg/kg bw survived. In the high-dose animals, clinical signs following administration included mouth and nasal discharge and/or anogenital staining. Findings regarding detailed clinical observation were noted in mucous membranes, salivation, locomotion, posture, piloerection and/or defecation for all three rats between 3 hours and 2 days post-dosing. However, all animals recovered from the above signs by day 3 and appeared active and healthy for the remainder of the 14-day observation period. No gross abnormalities were observed in any treated animals. The estimated acute oral LD_{50} in female rats was greater than 5000 mg/kg bw (Durando, 2010a).

In a non-guideline, non-GLP acute dermal toxicity/skin irritation screening study, three female F344/DuCrl rats received X11719474 (purity 100%) moistened with 0.5% aqueous methylcellulose at a single dose of 1000 mg/kg bw. The test compound was applied to the back of the animals (which was clipped free of hair on the day before treatment), covered with a gauze patch and wrap and held in place with elastic tape. After 24 hours, the test compound was removed. The rats were monitored daily for mortality and clinical signs for a 72-hour period, and the application site was graded for erythema and oedema within 60 minutes and 24, 48 and 72 hours after removal of the test material. Animals were not examined for gross pathological changes.

None of the animals died. Body weight loss was observed, which was attributed by the study authors to stress caused by semi-occlusive wrapping. Sixty minutes after removal of the test compound, very slight erythema was seen in two animals, and well-defined erythema and slight oedema were observed in the remaining animal. At later skin readings, the skin appeared normal. Under the conditions of the study, the estimated acute dermal LD_{50} in rats was above 1000 mg/kg bw in females. The test compound induced slight dermal irritation, which resolved within 24 hours after removal of the compound (Brooks & Golden, 2008/2010).

In a non-guideline, non-GLP eye irritation screening study, one female New Zealand White rabbit received X11719474 (purity 100%) in an amount of 0.1 ml (69 mg) in the lower conjunctival sac of the right eye. The left eye served as untreated control. Owing to discomfort after dosing, both eyes received local anaesthetic. Eyes were examined for conjunctival redness, chemosis, discharge, corneal opacity and iris irritation at 1, 24, 48 and 72 hours post-dosing.

Slight redness, chemosis and discharge were observed in the treated eye 1 hour after the administration. These findings had resolved by the next reading (24 hours) (Brooks & Golden, 2008/2010).

In a non-GLP reduced local lymph node assay conducted according to OECD test guideline 429 (2002), six female CBA/J mice received X11719474 (purity 100%) dissolved in DMSO at a concentration of 50%. The test compound was applied to both ears in a volume of 25 µl/ear on 3 consecutive days. The dose level was chosen on the basis of preliminary results showing that concentrations of 5%, 25% and 50% caused no effects on body weight and did not induce erythema. An additional group received the vehicle (DMSO) to serve as negative control. Animals were checked for mortality and clinical signs at least daily during the study. In particular, the site of application was examined for signs of local irritation. Individual body weights were measured at study start and at scheduled termination.

No mortality or clinical signs were observed during the study. In particular, no cutaneous reactions were observed at the application site of animals treated with the test compound. Body weight changes were comparable between control and treated groups. A negative response was observed at the tested dose level (stimulation index: 1.3). No concurrent positive control group was included in the study (according to the study result, a previous study with hexylcinnamaldehyde showed the expected result; however, no details on the results were given). On the basis of this study, X11719474 did not show any sensitization potential (Wiescinski & Sosinski, 2008/2010).

In a reverse gene mutation assay in bacteria conducted according to OECD test guideline 471 (1997), *Salmonella typhimurium* (strains TA98, TA100, TA1535, TA1537) and *Escherichia coli* (strain WP2*uvr*A) were exposed to X11721061 (purity 99%), using DMSO as solvent, in the presence and absence of S9 metabolic activation in two independent sets of experiments. Doses were selected based on the guideline limit dose and results of a range-finding experiment. For the main preincubation test using doses of up to and including 5000 µg/plate, three plates were used for each strain, condition and dose. Vehicle and positive controls were included in each experiment. The independent repeat test utilized the same conditions as the initial test. Doses up to and including 5000 µg/plate did not cause any bacteriotoxic effects. Total bacteria counts remained unchanged, and no inhibition of growth was observed.

No evidence for mutagenic activity of X11721061 was seen. No biologically relevant increase in the mutant count, in comparison with the negative controls, was observed. The positive controls induced the appropriate responses in the corresponding strains. Therefore, X11721061 was considered to be not mutagenic in the bacterial strains tested, in either the presence or absence of metabolic activation (Mecchi, 2008).

In an in vitro mammalian cell gene mutation test conducted according to OECD test guideline 476 (1997), X11719474 (purity 100%) dissolved in DMSO was tested for its ability to induce forward mutations at the *HPRT* locus in CHO cells. Two independent sets of experiments were conducted in the presence and absence of S9 metabolic activation. Based on the guideline limit dose (10 mmol/l), concentrations of 184.6–2953 µg/ml were used in the main study both with and without metabolic activation, and the same concentrations were used for the independent repeats. EMS and 20-MCA served as positive controls in the experiments without and with metabolic activation, respectively. The cells were treated for 4 hours in both experiments, without and with metabolic activation. After this, the incubation media were replaced by culture medium, and the cells were incubated (and subcultured every 2–3 days) for about 7–9 days for expression of mutant cells. This was followed by incubation of cells for 6–10 days in selection medium containing 6-thioguanine.

No increase in mutant frequency was observed in either the initial or the confirmatory study. In contrast to this, the positive control substances EMS and 20-MCA resulted in a marked increase in mutant frequency. Based on the results of the study, X11719474 was considered to be not mutagenic in the CHO/HPRT forward mutation assay, in either the presence or absence of metabolic activation (Schisler & Geter, 2008).

In an in vitro mammalian chromosomal aberration test conducted according to OECD test guideline 473 (1997), the clastogenic potential of X11719474 (purity 100%) dissolved in DMSO was tested in rat lymphocytes (whole blood). Based on the results of the mitotic index, concentrations of 369.1, 738.25 and 2953 µg/ml were used with metabolic activation and 738.25, 1476.5 and 2953 µg/ml were used without metabolic activation in an experiment with 4 hours of treatment and harvest at 24 hours (i.e. 20 hours post-exposure). In addition, with 24 hours of treatment without S9 mix, concentrations of 738.25, 1476.5 and 2953 µg/ml were chosen. Vehicle (DMSO) and positive controls (cyclophosphamide and mitomycin C for the test with and without metabolic activation, respectively) were included to demonstrate the sensitivity of the test system. Cultures were set up in duplicate. After slide preparation and staining of the cells, 200 metaphases per dose and treatment condition were analysed for chromosomal aberrations.

None of the cultures treated with X11719474 in the absence or in the presence of S9 mix showed any biologically relevant or statistically significant increase in the numbers of aberrant metaphases. No change in the frequency of polyploid metaphases was observed. The positive controls mitomycin C and cyclophosphamide induced clastogenic effects and demonstrated the sensitivity of the test system and the activity of the S9 mix used. Based on the results of this test, X11719474 was considered

not to be clastogenic for mammalian cells in vitro, in either the presence or absence of metabolic activation (Schisler, Kleinert & Geter, 2008).

In a non-guideline, non-GLP palatability study, groups of three male and three female Fischer 344 rats were fed diets containing X11719474 (purity 99.6%) at a dose of approximately 0, 125, 250, 500 or 1000 mg/kg bw per day for up to 7 days to evaluate palatability, general toxicity and toxicokinetics. Parameters evaluated were daily cage-side observations, body weights, feed consumption, clinical pathology and gross examinations. Liver weight was recorded, and liver sections underwent histopathological examination. Urine and blood plasma were analysed for toxicokinetics.

No mortalities or abnormal clinical observations were found during the treatment period in any treatment group. Animals treated with 1000 or 500 mg/kg bw per day showed low feed intake and body weight and body weight gain. These body weight effects remained for the whole study period in the high-dose groups and in the 500 mg/kg bw per day females group and were reversible by the end of the treatment period in the 500 mg/kg bw per day male group. Feed intake was altered only during the first study days.

The levels of the test compound in blood plasma or in urine increased nearly linearly with the test material intake. Triglyceride levels were lower in 1000 and 500 mg/kg bw per day dose group males and females. Other clinical pathology parameters showed no biologically relevant changes.

No gross abnormalities were observed. Absolute and relative liver weights were increased in males (approximately 15% or 28% increase in relative weight) and females (approximately 9% or 13% increase in relative weight) of the 500 and 1000 mg/kg bw per day dose groups. In sections of liver, midzonal to centrilobular hepatocyte hypertrophy and altered tinctorial properties (cytoplasmic eosinophilia) were observed in 500 and 1000 mg/kg bw per day dose group males (very slight to slight) and in 1000 mg/kg bw per day dose group females (very slight).

Therefore, under the conditions of this study, X11719474 was sufficiently palatable at concentrations up to approximately 500 mg/kg bw per day for studies of repeated administration (Yano et al., 2010a).

In a toxicity study conducted according to OECD test guideline 407 (1995), groups of five male and five female Fischer 344 rats were given diets containing X11719474 (purity 99.5%) at a concentration of 0, 1000, 2000, 3000 or 8000 ppm (equal to 0, 83.4, 167, 244 and 662 mg/kg bw per day in males and 0, 90.1, 184, 278 and 734 mg/kg bw per day in females) for 28 days. Animals were observed daily for mortality and clinical signs. Physical examinations were performed weekly. Body weight and feed consumption were recorded once weekly. During the acclimatization phase, all animals were subjected to an ophthalmic examination; all animals were re-examined prior to scheduled necropsy. Haematology, clinical chemistry and urine parameters were determined at the end of the study. All animals were necropsied, selected organs were weighed and a range of tissues were taken, fixed and examined microscopically. Bone marrow of one femur of each animal was collected, and slides were prepared and archived, but not evaluated for the induction of micronuclei (five males treated with a positive control substance were included, but no details on the compound or the dose level were included in the report). A liver piece from each animal was stored for possible later gene expression analysis.

No dose-related effect on mortality rate was reported. There were no treatment-related effects on clinical signs, ophthalmic observations, or haematological or coagulation parameters. Males and females given 8000 ppm exhibited reduced body weight gain and reduced feed intake compared with controls during the first 5 days; however, body weight and feed intake were comparable to those of controls towards the end of the treatment period. Uric acid crystals were detected in urine of males and females of the top-dose group. Additionally, amorphous urate crystals were noted in top-dose females.

Blood cholesterol levels for males and females given 8000 ppm were higher than those of the controls. Males and females given 8000 ppm had higher absolute (33% and 14%, respectively) and relative (37% and 18%, respectively) liver weights than the controls. Males and females given 8000 ppm and males given 3000 ppm had a slight treatment-related hypertrophy of hepatocytes (with increased cytoplasmic eosinophilia) in the centrilobular/midzonal region of the hepatic lobules. All other organ weight changes and histopathological findings were considered non-adverse.

Absorption of X11719474 from the diet was dose proportional for both sexes, based on plasma concentrations of parent compound. The ingested X11719474 was rapidly eliminated from plasma upon removal of the fortified diet (fasting) and dropped below the limit of quantification at the time of sacrifice in most of the rats. The elimination half-life of X11719474 from plasma of rats with detectable parent test material was between 2.7 and 3.6 hours. Male rats eliminated less of what they consumed during a 24-hour period, when compared with females. Overall, these data show that X11719474 was significantly absorbed following dietary administration and quickly eliminated, primarily via urine, as parent compound. The ratio of diastereomers was not substantially different in the plasma and urine samples when compared with the matrix samples added with the test material.

The NOAEL was 3000 ppm (equal to 244 mg/kg bw per day in males and 278 mg/kg bw per day in females), based on initially reduced body weight gain and feed consumption, liver findings (increased plasma cholesterol levels, organ weight increase) and urinary crystals in males and females at 8000 ppm (Yano et al., 2010b).

In a toxicity study conducted according to OECD test guideline 408 (1998), groups of 10 male and 10 female Fischer 344 rats were given diets containing X11719474 (purity 99.5%) at a concentration of 0, 500, 1000 or 5000 ppm (equal to 0, 32.2, 65.3 and 327 mg/kg bw per day in males and 0, 35.2, 71.8 and 352 mg/kg bw per day in females) for at least 90 days. Animals were observed daily for mortality and clinical signs. Physical examinations were performed weekly. In addition, neurological/functional tests were conducted once during the acclimatization phase and during the last week of the study. Body weight and feed consumption were recorded once weekly. During the acclimatization phase, all animals were subjected to an ophthalmic examination; all animals in the control and treatment groups were re-examined prior to scheduled necropsy. Haematology, clinical chemistry and urine parameters were determined at the end of the study. All animals were necropsied, selected organs were weighed and a range of tissues were taken, fixed and examined microscopically. A liver piece from each animal was stored for possible later gene expression analysis. Compound concentrations (and known major metabolites) were analysed in blood plasma and urine in the last week of treatment for toxicokinetic analysis.

Four days prior to scheduled necropsy, animals were immunized with sheep red blood cells by intravenous injection to determine possible treatment-related effects on the immune system. An additional five animals per sex were treated with cyclophosphamide on the 4 days prior to scheduled necropsy to serve as a positive control for the determination of immunotoxic effects.

No treatment-related effects on mortality, clinical signs, functional tests, body weights, feed intake, ophthalmoscopic examinations, haematology, urinalysis or immune system were detected.

Male and female animals in the top-dose group had higher plasma cholesterol levels (approximately 18–19%) compared with control animals. Additionally, males in the top-dose group had increased relative and absolute liver weights (approximately 16% or approximately 17%, respectively). Livers of top-dose animals showed slight treatment-related hypertrophy of hepatocytes (with increased cytoplasmic eosinophilia) in the centrilobular/midzonal region of the hepatic lobules. Males were affected more severely and also showed very slight, multifocal, individual cell necrosis of centrilobular hepatocytes and vacuolation of hepatocytes, consistent with fatty changes. All other organ weight changes (thyroid and adrenals in males) were considered non-adverse.

Toxicokinetic analysis of plasma after 13 weeks of treatment indicated a dose-proportional systemic exposure across all dose groups in both sexes. The plasma elimination half-life was approximately 7.8 or 7.5 hours in mid-dose animals and 5.2 or 4.1 hours in high-dose males or females, respectively. The test compound was almost completely absorbed and eliminated via urine as unchanged compound.

The NOAEL was 1000 ppm (equal to 65.3 mg/kg bw per day in males and 71.8 mg/kg bw per day in females), based on liver toxicity in both sexes at 5000 ppm (equal to 327 mg/kg bw per day in males and 352 mg/kg bw per day in females) (Yano et al., 2010c).

In a non-guideline palatability study, groups of two male Beagle dogs were administered X11719474 at a dose of 50 or 100 mg/kg bw per day by gavage (10 ml/kg bw per day, formulated in 0.5% aqueous methylcellulose) for up to 7 days to evaluate palatability and general toxicity. Parameters evaluated were daily cage-side observations, body weights and feed consumption.No further examinations of animals after euthanization at study termination were performed.

Oral administration of the test compound at dose levels of 50 or 100 mg/kg bw per day for 7 days was well tolerated by the animals. No adverse effects on body weight or feed intake were observed. One of two males treated with 100 mg/kg bw per day exhibited faecal changes (soft, mucoid and/or discoloured) during the dosing period (Heward, 2009/2010).

In a toxicity study conducted according to OECD test guideline 409, groups of four male Beagle dogs were administered X11719474 (purity 99.5%) by oral gavage in 0.5% aqueous methylcellulose (Methocel A4C) at a dose of 0, 10, 25 or 50 mg/kg bw per day for 90 days. The dose volume was 10 ml/kg bw. The study was intended to compare the properties of the metabolite X11719474 with sulfoxaflor in the available 90-day toxicity study (Stewart, 2010) using as few animals as possible (i.e. only animals of one sex). Hence, the dose levels were selected based on the results of the toxicity study in dogs conducted with sulfoxaflor (LOAEL 10 mg/kg bw per day, NOAEL 6 mg/kg bw per day) and 5-fold and 10-fold increases over the NOAEL. The results in rats (Yano et al., 2009c, 2010c) supported the assumption that the metabolite was less toxic than the parent compound.

Observations for morbidity, mortality, injury and the availability of feed and water were conducted twice daily for all animals. Clinical observations were conducted, and body weights were measured and recorded weekly. Feed consumption was measured weekly. Physical examinations were conducted by a veterinarian pretest to confirm the good health status of each animal placed on study. Blood and urine samples for clinical pathology evaluations were collected from all animals pretest and during weeks 6 and 13. Blood and urine samples were collected from all animals at designated intervals during week 13 for determination of the plasma concentrations of the test material (toxicokinetics). At study termination, necropsy examinations were performed, organ weights were recorded and selected tissues were examined microscopically.

There were no mortalities and no treatment-related clinical observation findings at any dose level. No treatment-related effects were noted on body weight, feed intake, clinical pathology or urinalysis parameters in all dose groups.

There were no treatment-related gross pathological observations, organ weight changes or histopathological changes at any exposure level.

The toxicokinetic analysis after 13 weeks of dosing at 10, 25 or 50 mg/kg bw per day showed that the daily systemic dose ($AUC_{24\ h}$) was 126.2 ± 9.5, 298.4 ± 47.9 and 682.3 ± 82.9 µg·h/ml in males, respectively. The increase in systemic dose was clearly dose proportional in male dogs across all three dose levels. The mean plasma elimination half-life of the test compound at 10, 25 or 50 mg/kg bw per day was 8.4 ± 2.4, 7.8 ± 1.0 and 7.7 ± 1.1 hours, respectively.

The NOAEL was greater than 50 mg/kg bw per day, the highest dose tested (Heward, 2010b).

In a reproduction/developmental toxicity screening study conducted according to OECD test guideline 421, groups of 12 male and 12 female Crl:CD(SD) rats were given diets containing X11719474 (purity 99.5%) at a concentration of 0, 1000, 2000 or 5000 ppm (equal to 0, 80.8, 162 and 396 mg/kg bw per day in males and 0, 81.7, 167 and 451 mg/kg bw per day in females) (pre-mating phase), respectively. Males were fed the test diets for 2 weeks prior to breeding and continuing throughout breeding until termination. The females were fed the test diets for 2 weeks prior to breeding, continuing through breeding (up to 2 weeks), gestation, lactation and weaning; pups were weaned on PND 21. Effects on gonadal function, mating behaviour, conception, development of the conceptus, parturition and postnatal growth and survival were evaluated. In addition, a gross necropsy and histopathological examination of the adults were conducted with an emphasis on organs of the reproductive system. In the offspring, litter size, pup survival, sex, body weight and the presence of gross external morphological alterations were assessed.

All parental animals survived until termination, and there were no treatment-related clinical observations or changes in feed intake at any dose level throughout the study. Females in the 2000 and 5000 ppm dose groups had decreases in body weight and/or body weight gain during isolated gestational and lactational intervals; however, these were considered to be non-adverse.

Males and females of the 5000 ppm dose groups had increased absolute and relative liver weights that were treatment related. Treatment-related histological effects were observed in the livers of males and females given 5000 ppm and consisted of very slight hepatocellular hypertrophy, with altered tinctorial properties, involving the centrilobular to midzonal regions of the hepatic lobule.

There were no reproductive or developmental toxicity effects observed in any group up to PND 21. There were no effects on pup body weight in any dose group.

Toxicokinetic analyses of plasma samples from PND 4 culled pups indicated a dose-proportionate increase in the systemic concentration of the test compound.

The NOAEL for parental toxicity was 2000 ppm (equal to 162 mg/kg bw per day), based on slight signs of liver toxicity. The NOAEL for offspring and reproductive performance was 5000 ppm (equal to 396 mg/kg bw per day), the highest dose tested (Rasoulpour et al., 2010a).

In a prenatal developmental toxicity study conducted according to OECD test guideline 414, groups of 26 time-mated female Crl:CD(SD) rats were given diets containing X11719474 (purity 99.5%) at a concentration of 0, 1000, 2000 or 5000 ppm (equal to 0, 74.4, 152 and 368 mg/kg bw per day) on GDs 6 through 21. In-life maternal study parameters included clinical observations, body weight, body weight gain and feed consumption. On GD 21, all rats were euthanized, and all dams and fetuses were examined for gross pathological alterations. In addition, blood was collected from dams and fetuses to determine blood levels of the test material. Liver, kidneys and gravid uterine weights were recorded, along with the numbers of corpora lutea, uterine implantations, resorptions and live/dead fetuses. All fetuses were weighed, sexed and examined for external alterations. Approximately one half of the fetuses were examined for visceral and craniofacial alterations, whereas skeletal examinations were conducted on the remaining fetuses.

Maternal toxicity at 5000 ppm was limited to transient decreases in body weight gain at the initiation of treatment, relative to controls, with concomitant decreased feed consumption. As a result of the low extent and limited duration, these findings were considered to be non-adverse by the study director.

No dose-related effects on reproductive parameters, gross pathology or organ weights were reported in any dose group.

The external and visceral examination of fetuses revealed no treatment-related effects on development of offspring. In the 5000 ppm dose group, three fetuses from two litters had class I wavy ribs (variation), and two fetuses from two litters had class II wavy ribs (malformation). The former was

within the historical control range, whereas the latter was slightly outside the historical control range. Additionally, slight incidences of calloused ribs and delayed ossification of parietal, interparietal or occipital bones were observed in high-dose fetuses. These findings were within the historical control range. The study director stated that "the incidence of class II wavy ribs was slightly outside historical control range, these findings occurred in foetuses with other rib alterations and occurred at very low incidences therefore they were considered spurious and unrelated to treatment".

The terminal plasma concentrations of test compound in fetal blood were dose proportional throughout the entire range of dietary exposure concentrations.

The NOAEL for maternal toxicity and for prenatal developmental was 5000 ppm (equal to 368 mg/kg bw per day), the highest dose tested (Rasoulpour & Marshall, 2010).

In a non-GLP, non-guideline study, six male F344/DuCrl rats were given test diets formulated to supply 0 or 8000 ppm X11719474 in the diet for 7 days, which corresponded to time-weighted average doses of 0 or 583 mg/kg bw per day, respectively. The purpose of this study was to generate information to aid in understanding the MOA for liver effects in rats induced by X11719474 and to compare the results with those observed following exposure to sulfoxaflor (1500 ppm, equal to 102 mg/kg bw per day; Geter & Card, 2010, see above). The treatment level of 8000 ppm was the high dose level used in the rat 28-day toxicity study. The parameters evaluated were daily cage-side observations, body weight, body weight gain, feed consumption, liver weights, histopathology of the liver, focused gene expression, liver cytochrome P450 enzyme activity and hepatocellular proliferation using BrdU immunohistochemical methods.

At the end of the 7-day treatment period, males exposed to 8000 ppm X11719474 had reduced body weight and body weight gain of approximately 2% and 22% relative to controls, respectively. In addition, males given 8000 ppm X11719474 had increases in liver weights (absolute 16%; relative 19%). X11719474 induced liver weight increases that correlated with treatment-related microscopic observations of very slight centrilobular/midzonal hypertrophy of hepatocytes that were present in all compound-treated animals. At the end of the 7-day treatment period, males exposed to 8000 ppm X11719474 had the same types of treatment-related effects as were observed with the parent compound, but were consistently less marked, despite the almost 6-fold higher dose level.

The CAR-related transcripts, *Cyp2b1* and *Cyp2b2*, along with the PXR-related transcript, *Cyp3a3*, were increased 806-, 32- and 4-fold, respectively. Parent compound–induced gene expression showed a similar profile of 848-, 21- and 9-fold, respectively. The level of hepatocellular PROD enzyme activity, a CAR-associated biomarker, was increased 15-fold following X11719474 exposure. A similar level of induction (10-fold) was seen for parent compound–induced PROD enzyme activity. Hepatocellular proliferation, as measured by BrdU incorporation, was significantly elevated by 4.5-, 2.5- and 2.1-fold in the centrilobular, midzonal and periportal regions, respectively. A hepatocellular proliferation study with parent compound (Geter & Card, 2010), as measured by Ki-67, showed increased proliferation in the centrilobular and midzonal regions of 3.4- and 2.9-fold, respectively.

Based on these results, the MOA responsible for increased liver weight in rats following exposure to X11719474 was sulfoxaflor- and phenobarbital-like, as evidenced by the CAR- and PXR-related molecular, enzymatic and proliferative responses. These data support the conclusion that X11719474 is an agonist ligand for CAR and PXR, and this activation results in the observed phenobarbital-like responses in the liver (Geter et al., 2010b).

Studies with X11721061 (plant and animal metabolite of sulfoxaflor)

In an acute oral toxicity study conducted according to OECD test guideline 425 ("up and down procedure"), female Fischer 344 rats received X11721061 (purity 99%, clear, colourless liquid) at a single dose of 100, 2000 or 5000 mg/kg bw by gavage. Single animals were treated sequentially.

Depending on the survival or death of the treated animal, the dose level administered to the next animal was increased or decreased, respectively. The rats were monitored daily for mortality and clinical signs for a 2-week period, and body weights were recorded on days 1, 7 and 14. On day 15, surviving animals were killed, necropsied and examined for gross pathological changes.

One of the females dosed with 5000 mg/kg bw died; additional mortalities were observed at a dose level of 2000 mg/kg bw (two of three females) within 1 day after the dose administration. Two of two females dosed with 1000 mg/kg bw survived. All animals that died showed clinical signs of toxicity and red intestines upon necropsy. Surviving animals showed clinical signs (e.g. piloerection, hypoactivity, hunched posture), but recovered by day 2, at the latest. No abnormalities were noted in surviving animals. The estimated acute oral LD_{50} in female rats was 2000 mg/kg bw (Durando, 2010b).

In a reverse gene mutation assay in bacteria conducted according to OECD test guideline 471 (1997), *Salmonella typhimurium* (strains TA98, TA100, TA1535, TA1537) and *Escherichia coli* (strain WP2*uvr*A) were exposed to X11721061 (purity 99%), using DMSO as solvent, in the presence and absence of S9 metabolic activation in two independent sets of experiments. Doses were selected based on the guideline limit dose and results of an initial experiment. For the initial preincubation test using doses of up to and including 5000 µg/plate, two plates were used for each strain, condition and dose. Vehicle and positive controls were included in each experiment. The independent repeat test utilized basically the same conditions as the initial test, but the dose levels were adjusted, and three plates were used for each strain, condition and dose. Doses up to and including 5000 µg/plate did not cause any bacteriotoxic effects. Total bacteria counts remained unchanged, and no inhibition of growth was observed.

No evidence for mutagenic activity of X11721061 was seen. No biologically relevant increase in the mutant count, in comparison with the negative controls, was observed. The positive controls induced the appropriate responses in the corresponding strains. Therefore, X11721061 was considered to be not mutagenic in the bacterial strains tested, in either the presence or absence of metabolic activation (Dakoulas & VanDyke, 2009).

In an in vitro mammalian cell gene mutation test conducted according to OECD test guideline 476 (1997), X11721061 (purity 99%) dissolved in distilled water was tested for its ability to induce forward mutations at the *HPRT* locus in CHO cells. Two independent sets of experiments were conducted in the presence and absence of S9 metabolic activation. Based on the guideline limit dose (10 mmol/l), concentrations of 120–1920 µg/ml were used in the main study both with and without metabolic activation, and the same concentrations were used for the independent repeats. EMS and 20-MCA served as positive controls in the experiments without and with metabolic activation, respectively. The cells were treated for 4 hours in both experiments, without and with metabolic activation. After this, the incubation media were replaced by culture medium, and the cells were incubated (and subcultured every 2–3 days) for about 8 days for expression of mutant cells. This was followed by incubation of cells for 7–9 days in selection medium containing 6-thioguanine.

No increase in mutant frequency was observed in either the initial or confirmatory study. In contrast to this, the positive control substances EMS and 20-MCA resulted in a marked increase in mutant frequency. Based on the results of the study, X11721061 was considered to be not mutagenic in the CHO/HPRT forward mutation assay, in either the presence or absence of metabolic activation (Schisler, 2010a).

In an in vitro mammalian chromosomal aberration test conducted according to OECD test guideline 473 (1997), the clastogenic potential of X11721061 (purity 99%) dissolved in distilled water

was tested in rat lymphocytes (whole blood). Based on the results of the mitotic index, concentrations of 480, 960 and 1920 µg/ml were used with and without metabolic activation in an experiment with 4 hours of treatment and harvest at 24 hours (i.e. 20 hours post-exposure). In addition, with 24 hours of treatment without S9 mix, concentrations of 120, 240 and 480 µg/ml were chosen. Vehicle and positive controls (cyclophosphamide and mitomycin C for the test with and without metabolic activation, respectively) were included to demonstrate the sensitivity of the test system. Cultures were set up in duplicate. After slide preparation and staining of the cells, 200 metaphases per dose and treatment condition were analysed for chromosomal aberrations.

None of the cultures treated with X11721061 in the absence or in the presence of S9 mix showed any biologically relevant or statistically significant increase in the numbers of aberrant metaphases. No change in the frequency of polyploid metaphases was observed. The positive controls mitomycin C and cyclophosphamide induced clastogenic effects and demonstrated the sensitivity of the test system and the activity of the S9 mix used. Based on the results of this test, X11721061 was considered not to be clastogenic for mammalian cells in vitro, in either the presence or absence of metabolic activation (Schisler & Geter, 2010).

In a non-guideline, non-GLP palatability study, groups of three male F344/DuCrl rats were fed diets containing X11721061 (purity 98%) at a concentration of 2500, 5000 or 10 000 ppm (equal to approximately 150–175, 323–372 and 547–772 mg/kg bw per day) for up to 7 days to evaluate palatability. Parameters evaluated were daily cage-side observations, body weights, feed consumption and gross examinations. Liver weight was recorded.

All animals survived the treatment period without any clinical signs of toxicity. Body weight development and feed intake were normal. No gross findings were observed. Liver weights were increased dose relatedly (as a result of the lack of a control group, the extent could not be determined).

Therefore, under the conditions of this study, X11721061 was sufficiently palatable at the concentrations tested for studies of repeated administration (Heward, 2010c).

In a study of toxicity conducted according to OECD test guideline 407, groups of five male and five female F344/DuCrl rats were fed diets containing X117721061 (purity 99%) at a concentration of 0, 1000, 3000 or 8000 ppm (equal to 0, 79, 236 and 622 mg/kg bw per day for males and 0, 82, 244 and 649 mg/kg bw per day for females) for 28 days. Animals were observed daily for mortality and clinical signs. A detailed physical examination was performed weekly. Body weight and feed consumption were recorded frequently throughout the study. Ophthalmoscopic examinations were performed pretest and prior to terminal necropsy. Blood samples for haematology and clinical chemistry determinations were collected before final necropsy, whereas urine samples were collected during the last week of the study. At study termination, all animals were necropsied, selected organs were weighed and a range of tissues were taken, fixed and examined microscopically.

No mortalities were observed throughout the treatment period. However, a few animals died during the terminal blood collection. No treatment-related clinical signs of toxicity or other clinical abnormalities were reported.

Body weight loss in the top-dose females and low feed intake in the top-dose males and females (approximately 12–13%) were observed during the first 3 days of treatment. Feed intake and body weight development were normal during later periods in the top-dose animals and in all other dose groups during the whole study period.

No treatment-related effect was noted during ophthalmoscopic examination. Haematological and clinical chemistry analyses and urinalysis showed no consistent changes attributable to treatment.

Table 42. Summary of selected findings in rats in a 28-day study with metabolite X117721061

	Males				Females			
	Dietary concentration (ppm)							
	0	1000	3000	8000	0	1000	3000	8000
Dose (mg/kg bw per day), day 27	0	79	236	622	0	82	244	649
Body weight, terminal (g)	211	212	205	203	131	132	129	128
Liver weight, absolute (g)	6.74	6.68	6.74	7.17	3.88	4.12	3.93	4.09
Liver weight, relative (% of body weight)	3.18	3.16	3.29	3.52	2.97	3.12	3.06	3.19*

From Heward (2010d)
* $P < 0.05$

Macroscopic examination did not reveal treatment-related changes. Terminal body weights of top-dose males were slightly lower than in control animals. Absolute liver weight of top-dose males and females and relative liver weight of top-dose males were slightly, although not statistically significantly, higher, and the relative liver weight of top-dose females was significantly increased (Table 42). No treatment-related histopathological changes were reported.

The NOAEL was 3000 ppm (equal to 236 mg/kg bw per day), based on reduced feed intake in both sexes and a subsequent slight body weight loss in females at 8000 ppm (equal to 622 mg/kg bw per day) during the first 3 days of treatment (Heward, 2010d).

Studies with X11596066 (animal metabolite of sulfoxaflor in hens and goats)

In an acute oral toxicity study conducted according to OECD test guideline 425 ("up and down procedure"), female Fischer 344 rats received X11596066 (purity 98%) in 0.5% aqueous methylcellulose at a single dose of 2000 mg/kg bw by gavage. Single animals were treated sequentially. Depending on the survival or death of the treated animal, the dose level administered to the next animal was increased or decreased, respectively. The rats were monitored daily for mortality and clinical signs for a 2-week period, and body weights were recorded on days 1, 7 and 14. On day 15, surviving animals were killed, necropsied and examined for gross pathological changes.

All five females dosed with 2000 mg/kg bw survived and gained weight during the study. Clinical signs (piloerection) were noted in two animals on the day of dosing. No gross abnormalities were noted during necropsy. The estimated acute oral LD_{50} in rats was greater than 2000 mg/kg bw in females (Durando, 2010c).

In a reverse gene mutation assay in bacteria conducted according to OECD test guideline 471 (1997), *Salmonella typhimurium* (strains TA98, TA100, TA1535, TA1537) and *Escherichia coli* (strain WP2*uvr*A) were exposed to X11596066 (purity 98%), using DMSO as solvent, in the presence and absence of S9 metabolic activation, in two independent sets of experiments. Doses were selected based on the guideline limit dose and results of an initial experiment. For the initial preincubation test using doses of up to and including 5000 µg/plate, two plates were used for each strain, condition and dose. Vehicle and positive controls were included in each experiment. The independent repeat test utilized basically the same conditions as the initial test, but the dose levels were adjusted, and three plates were used for each strain, condition and dose. Doses up to and including 5000 µg/plate did not cause any bacteriotoxic effects. Total bacteria counts remained unchanged, and no inhibition of growth was observed.

No evidence for mutagenic activity of X11596066 was seen. No biologically relevant increase in the mutant count, in comparison with the negative controls, was observed. The positive controls induced the appropriate responses in the corresponding strains. Therefore, X11596066 was considered to be not mutagenic in the bacterial strains tested, in either the presence or absence of metabolic activation (Dakoulas & VanDyke, 2010a).

Studies with X11579457 (soil metabolite of sulfoxaflor)

In an acute oral toxicity study conducted according to OECD test guideline 425 ("up and down procedure"), female Fischer 344 rats received X11579457 (purity 99%) in 0.5% aqueous methylcellulose at a single dose of 2000 mg/kg bw by gavage. Single animals were treated sequentially. Depending on the survival or death of the treated animal, the dose level administered to the next animal was increased or decreased, respectively. The rats were monitored daily for mortality and clinical signs for a 2-week period, and body weights were recorded on days 1, 7 and 14. On day 14, surviving animals were killed, necropsied and examined for gross pathological changes.

All five females dosed with 2000 mg/kg bw survived. Clinical signs were noted in one animal on the day of compound administration. No gross abnormalities were observed when animals were necropsied. The acute oral LD_{50} in rats was greater than 2000 mg/kg bw in females (Durando, 2010d).

In a reverse gene mutation assay in bacteria conducted according to OECD test guideline 471 (1997), *Salmonella typhimurium* (strains TA98, TA100, TA1535, TA1537) and *Escherichia coli* (strain WP2*uvr*A) were exposed to X11579457 (purity 99%), using distilled water as solvent, in the presence and absence of S9 metabolic activation in two independent sets of experiments. Doses were selected based on the guideline limit dose and results of an initial experiment. For the initial preincubation test using doses of up to and including 5000 µg/plate, two plates were used for each strain, condition and dose. Vehicle and positive controls were included in each experiment. The independent repeat test utilized basically the same conditions as the initial test, but the dose levels were adjusted, and three plates were used for each strain, condition and dose. Doses up to and including 5000 µg/plate did not cause any bacteriotoxic effects. Total bacteria counts remained unchanged, and no inhibition of growth was observed.

No evidence for mutagenic activity of X11579457 was seen. No biologically relevant increase in the mutant count, in comparison with the negative controls, was observed. The positive controls induced the appropriate responses in the corresponding strains. Therefore, X11579457 was considered to be not mutagenic in the bacterial strains tested, in either the presence or absence of metabolic activation (Dakoulas & VanDyke, 2010b).

In an in vitro mammalian cell gene mutation test conducted according to OECD test guideline 476 (1997), X11579457 (purity 99%) dissolved in distilled water was tested for its ability to induce forward mutations at the *HPRT* locus in CHO cells. Two independent sets of experiments were conducted in the presence and absence of S9 metabolic activation. Based on the guideline limit dose (10 mmol/l), concentrations of 157.8–2525 µg/ml were used in the main study both with and without metabolic activation, and the same concentrations were used for the independent repeats. EMS and 20-MCA served as positive controls in the experiments without and with metabolic activation, respectively. The cells were treated for 4 hours in both experiments, without and with metabolic activation. After this, the incubation media were replaced by culture medium, and the cells were incubated (and subcultured every 2–3 days) for about 8 days for expression of mutant cells. This was followed by incubation of cells for 7–9 days in selection medium containing 6-thioguanine.

No increase in mutant frequency was observed in either the initial or confirmatory study. In contrast to this, the positive control substances EMS and 20-MCA resulted in a marked increase in mutant frequency. Based on the results of the study, X11579457 was considered to be not mutagenic in the CHO/HPRT forward mutation assay, in either the presence or absence of metabolic activation (Schisler, 2010c).

In an in vitro mammalian chromosomal aberration test conducted according to OECD test guideline 473 (1997), the clastogenic potential of X11579457 (purity 99%) dissolved in distilled water was tested in rat lymphocytes (whole blood). Based on the results of the mitotic index, concentrations

of 631.3, 1262.5 and 2525 µg/ml were used with and without metabolic activation in an experiment with 4 hours of treatment and harvest at 24 hours and in an experiment with 24 hours of treatment without S9 mix. Vehicle and positive controls (cyclophosphamide and mitomycin C for the test with and without metabolic activation, respectively) were included to demonstrate the sensitivity of the test system. Cultures were set up in duplicate. After slide preparation and staining of the cells, 200 metaphases per dose and treatment condition were analysed for chromosomal aberrations.

None of the cultures treated with X11579457 in the absence or in the presence of S9 mix showed any biologically relevant or statistically significant increase in the numbers of aberrant metaphases. No change in the frequency of polyploid metaphases was observed. The positive controls mitomycin C and cyclophosphamide induced clastogenic effects and demonstrated the sensitivity of the test system and the activity of the S9 mix used. Based on the results of this test, X11579457 was considered not to be clastogenic for mammalian cells in vitro, in either the presence or absence of metabolic activation (Schisler, 2010b).

Studies with X11519540 (soil and animal metabolite of sulfoxaflor)

In an acute oral toxicity study conducted according to OECD test guideline 425 ("up and down procedure"), female Fischer 344 rats received X11519540 (purity 98%) in 0.5% aqueous methylcellulose at a single dose of 320, 1000 or 2000 mg/kg bw by gavage. Single animals were treated sequentially. Depending on the survival or death of the animal, the dose level administered to the next animal was increased or decreased, respectively. The rats were monitored daily for mortality and clinical signs for a 2-week period, and body weights were recorded on days 1, 7 and 14. On day 14, surviving animals were killed, necropsied and examined for gross pathological changes.

One animal dosed with 2000 mg/kg bw died within 1 day of test substance administration; additional mortalities were observed at a dose level of 1000 mg/kg bw (three of three females, within 2 days). Clinical signs of toxicity (such as hyperactivity/hypoactivity, abnormal posture or gait, salivation) were observed. At necropsy, red discoloration of intestines was observed in the decedents. All animals ($n = 3$) dosed with 320 mg/kg bw survived, and body weight gain was normal. The estimated acute oral LD_{50} in rats was 565.7 mg/kg bw in females (Durando, 2010e).

In a reverse gene mutation assay in bacteria conducted according to OECD test guideline 471 (1997), *Salmonella typhimurium* (strains TA98, TA100, TA1535, TA1537) and *Escherichia coli* (strain WP2*uvr*A) were exposed to X11519540 (purity 98%), using DMSO as solvent, in the presence and absence of S9 metabolic activation in two independent sets of experiments. Doses were selected based on the guideline limit dose and results of an initial experiment. For the initial preincubation test using doses of up to and including 5000 µg/plate, two (initial experiment) or three plates (second experiment) were used for each strain, condition and dose. Vehicle and positive controls were included in each experiment. The independent repeat test utilized basically the same conditions as the initial test, but the dose levels were adjusted. Doses up to and including 5000 µg/plate did not cause any bacteriotoxic effects. Total bacteria counts remained unchanged, and no inhibition of growth was observed.

No evidence for mutagenic activity of X11519540 was seen. No biologically relevant increase in the mutant count, in comparison with the negative controls, was observed. The positive controls induced the appropriate responses in the corresponding strains. Therefore, X11519540 was considered to be not mutagenic in the bacterial strains tested, in either the presence or absence of metabolic activation (Dakoulas & VanDyke, 2010c).

In an in vitro mammalian cell gene mutation test conducted according to OECD test guideline 476 (1997), X11519540 (98%) dissolved in DMSO was tested for its ability to induce forward mutations at the *HPRT* locus in CHO cells. Two independent sets of experiments were conducted in the presence and absence of S9 metabolic activation. Based on the guideline limit dose (10 mmol/l),

concentrations of 158.8–2540 µg/ml were used in the main study both with and without metabolic activation, and the same concentrations were used for the independent repeats. EMS and 20-MCA served as positive controls in the experiments without and with metabolic activation, respectively. The cells were treated for 4 hours in both experiments, without and with metabolic activation. After this, the incubation media were replaced by culture medium, and the cells were incubated (and sub-cultured every 2–3 days) for about 8 days for expression of mutant cells. This was followed by incubation of cells for 7–9 days in selection medium containing 6-thioguanine.

No increase in mutant frequency was observed in either the initial or confirmatory studies. In contrast to this, the positive control substances EMS and 20-MCA resulted in a marked increase in mutant frequency. Based on the results of the study, X11519540 was considered to be not mutagenic in the CHO/HPRT forward mutation assay, in either the presence or absence of metabolic activation (Schisler, 2010e).

In an in vitro mammalian chromosomal aberration test conducted according to OECD test guideline 473 (1997), the clastogenic potential of X11519540 (purity 98%) dissolved in DMSO was tested in rat lymphocytes (whole blood). Based on the results of the mitotic index, concentrations of 635, 1270 and 2540 µg/ml were used with and without metabolic activation in an experiment with 4 hours of treatment and harvest at 24 hours (i.e. 20 hours post-exposure). In addition, with 24 hours of treatment without S9 mix, concentrations of 158.8, 317.5 and 635 µg/ml were chosen. Vehicle (DMSO) and positive controls (cyclophosphamide and mitomycin C for the test with and without metabolic activation, respectively) were included to demonstrate the sensitivity of the test system. Cultures were set up in duplicate. After slide preparation and staining of the cells, 200 metaphases per dose and treatment condition were analysed for chromosomal aberrations.

None of the cultures treated with X11519540 in the absence or in the presence of S9 mix showed any biologically relevant or statistically significant increase in the numbers of aberrant metaphases. No change in the frequency of polyploid metaphases was observed. The positive controls mitomycin C and cyclophosphamide induced clastogenic effects and demonstrated the sensitivity of the test system and the activity of the S9 mix used. Based on the results of this test, X11519540 was considered not to be clastogenic for mammalian cells in vitro, in either the presence or absence of metabolic activation (Schisler, 2010d).

In a non-guideline, non-GLP palatability study, groups of three male F344/DuCrl rats were fed diets containing X11519540 (purity 98%) at a concentration of 2500, 5000 or 10 000 ppm (equal to 200, 355 and 319 mg/kg bw per day) for up to 7 days to evaluate palatability. Parameters evaluated were daily cage-side observations, body weights, feed consumption and gross examinations. Liver samples of low-dose animals were processed for histopathological evaluation and examined microscopically.

Feed consumption was reduced by 29.6%, 46.4% or 72.3% in animals given 2500, 5000 or 10 000 ppm, respectively, relative to pre-administration days −4 to −1. This was attributed to decreased palatability of the test compound in rodent feed. All animals fed 10 000 ppm had marked body weight loss and low feed intake by day 4 and were humanely euthanized on that day. Body weight losses were observed in the low- and intermediate-dose groups by day 4, which recovered slightly by day 8. Liver weight was increased in low and intermediate dose group animals (by approximately 60–70% compared with historical control values). In liver sections of low-dose animals, the following observations were noted: centrilobular to midzonal hepatocellular hypertrophy with altered tinctorial properties, very slight increase in mitotic figures and very slight multifocal individual cell necrosis.

Therefore, under the conditions of this study, X11519540 was not sufficiently palatable at the concentrations tested for studies of repeated adminstration. Dose levels to be used in future dietary toxicity studies should be below 250 mg/kg bw per day (Sura & Brooks, 2010).

In a toxicity study conducted according to OECD test guideline 407 (2008), groups of five male and five female F344/DuCrl rats were given diets containing X11519540 (purity 98%) at a concentration of 0, 100, 300, 1000 or 2000 ppm (equal to 0, 7.7, 23.1, 74 and 140 mg/kg bw per day in males and 0, 8.5, 24.9, 77.2 and 152 mg/kg bw per day in females) for 28 days. Animals were observed daily for mortality and clinical signs. Physical examinations were performed weekly. Body weight and feed consumption were recorded once weekly. During the acclimatization phase, all animals were subjected to an ophthalmic examination; all animals were re-examined prior to scheduled necropsy. Haematology, clinical chemistry and urine parameters were determined at the end of the study. All animals were necropsied, selected organs were weighed and a range of tissues were taken, fixed and examined microscopically. A liver piece from each animal was collected for gene expression analysis.

No dose-related effect on mortality rate was reported. There were no treatment-related effects on clinical signs, ophthalmic observations or coagulation parameters. Males and females given 1000 or 2000 ppm exhibited reduced body weight gain and reduced feed intake compared with controls.

In animals treated with 1000 or 2000 ppm, parameters for red blood cells/haemoglobin were decreased. In animals treated with 300 ppm, these parameters were decreased too; however, they stayed within the range of historical control data, and hence these changes were considered to be non-adverse. In animals treated with 300 ppm, total protein, albumin and cholesterol levels were increased. In animals dosed with 1000 or 2000 ppm, additionally, the following clinical chemistry parameters were increased: γ-glutamyl transpeptidase, ALT and AST and, additionally in females, globulin level. Urinary pH was decreased in males dosed with 300 ppm and in both sexes at 100 and 2000 ppm. Males treated with 2000 ppm had slightly higher urinary protein levels than controls.

Males and females in all treatment groups had increased (absolute and relative) adrenal and liver weights. Terminal body weight was lower in animals of the 1000 and 2000 ppm dose groups compared with controls. Liver size was increased in animals treated with 300 ppm and above.

Animals in all treatment groups showed centrilobular/midzonal hypertrophy, which increased in severity (very slight to moderate). Males in all treatment groups had an increased number of mitotic figures in liver sections; females showed this finding at 300 ppm and above. Multifocal necrosis of individual hepatocytes was noted in both sexes at 300 ppm and above. At 1000 ppm and above, multifocal hepatocellular necrosis with accompanying inflammation was noted. An increased incidence of multifocal degeneration of tubules in the kidneys in males (300, 1000, 2000 ppm) and females (2000 ppm) was noted. Males from all dose levels and females given 1000 or 2000 ppm had diffuse follicular cell hypertrophy of the thyroid gland. Males and females given 300, 1000 or 2000 ppm had treatment-related very slight or slight hypertrophy of the zona fasciculata of the adrenal glands. In addition, males from all dose levels and females given 300, 1000 or 2000 ppm had treatment-related very slight or slight increased vacuolation of the adrenal cortex (zona glomerulosa, zona fasciculata and zona reticularis). Males given 1000 or 2000 ppm and females given 2000 ppm had very slight diffuse acinar cell hypertrophy of the submandibular salivary gland.

Males and females given 1000 or 2000 ppm had treatment-related very slight or slight erythroid cell hyperplasia of the bone marrow. Males given 2000 ppm had a treatment-related increase in the incidence of very slight extramedullary haematopoiesis (erythroid cell) of the spleen. The bone marrow and spleen effects were interpreted to be reflective of a regenerative response to the lower red blood cell counts, haemoglobin concentrations and haematocrits in males and females given 1000 or 2000 ppm.

Males and females given 1000 or 2000 ppm had treatment-related very slight or slight atrophy of the mesenteric adipose tissue. Females given 1000 or 2000 ppm had treatment-related very slight or slight decreased size of the uterus. Females given 2000 ppm also had treatment-related slightly decreased size of the cervix and vagina. The atrophy of mesenteric adipose tissue and decreased size

of the female reproductive tract were interpreted to be secondary to lower feed consumption and lower body weight gains of animals given 1000 or 2000 ppm.

Toxicokinetic analysis indicated that systemic exposure to the test compound deviated from linearity (plasma AUC versus mean achieved daily dose level) between the 100 and 300 ppm groups. The plasma elimination half-life was between 24 and 35 hours in the 1000 and 3000 ppm group animals. It could not be determined in the 100 and 300 ppm dose groups. Urinary excretion within 24 hours was approximately 47%/51%, 31%/28%, 18%/15% or 13%/11% (males/females) of the calculated mean daily dose in the 100, 300, 1000 or 2000 ppm dose groups.

Targeted gene expression analysis of *Cyp2b1*, *Cyp2b2* and *Cyp3a3* in the liver tissue showed significantly elevated transcripts at 100, 300 and 1000 ppm (2000 ppm was not tested for these end-points). These data suggest that X11519540 may stimulate gene expression consistent with CAR activation.

The NOAEL was less than 100 ppm (equal to 7.7 mg/kg bw per day) in males, based on effects in liver (increased serum cholesterol), thyroid (follicular cell hypertrophy) and adrenals (increased vacuolation of the cortex) (Stebbins et al., 2010b).

3. Observations in humans

There were no reports of adverse health effects in manufacturing plant personnel. Also, there were no reports of poisonings with sulfoxaflor.

Comments

Biochemical aspects

In rats given sulfoxaflor labelled with ^{14}C at the pyridine ring orally by gavage, absorption was rapid and accounted for at least 93% of the total recovered radioactivity after a single dose of 5 mg/kg bw or 100 mg/kg bw; the maximum plasma concentrations of radiolabelled material were reached after 0.5–1.6 hours and after 1.3–2.3 hours, respectively. Radiolabel was widely distributed throughout the body. Elimination of the radiolabel was mainly via the urine (≥ 92%). After intravenous administration, faecal excretion accounted for up to 9% of total excretion. Elimination of the radiolabel from plasma was bi-exponential, with most of the elimination occurring during the α phase, with a half-life of 4–6 hours, whereas the half-life of the β phase was 39–45 hours. Residues in tissues 168 hours after a single oral or intravenous dose as well as after repeated oral dosing accounted for less than 1.3% of the administered dose, with liver, kidney and erythrocytes containing the highest concentrations of residues.

Sulfoxaflor was metabolized to only a very limited extent. The metabolism included oxidative cleavage of the parent molecule, leading to metabolite X11721061, which was subsequently conjugated with glucuronic acid. This was the only metabolite identified in urine, accounting for 3–4% of the administered dose.

Toxicological data

The LD_{50} in rats treated orally with sulfoxaflor was 1000 mg/kg bw. The dermal LD_{50} in rats was greater than 5000 mg/kg bw, and the inhalation LC_{50} in rats was greater than 2.09 mg/l. Sulfoxaflor was not a skin irritant in rabbits, was not irritating to the eye of rabbits and was not a skin sensitizer in the local lymph node assay in mice.

At least in part as a result of its unpleasant smell, sulfoxaflor is of limited oral palatability, so that repeated-dose studies by dietary as well as gavage administration were dose limited by effects on feed intake and consequent body weight reductions.

Following repeated administration of sulfoxaflor to mice and rats, the liver was the main target organ, and males were affected more than females. The effects noted at lower doses (increased liver weights, hepatocellular hypertrophy) were consistent with the induction of hepatic cytochrome P450, whereas effects observed at higher doses included hepatocellular degeneration or necrosis and related clinical chemistry findings (e.g. increased serum levels of liver enzymes, cholesterol or triglycerides). In mice, the adrenals were an additional target, with hypertrophy and/or vacuolization of the zona fasciculata. In dogs, gavage administration gave the highest achievable doses, but the only effects were decreases in feed consumption and body weight gain and increased incidences of soft or watery faeces.

In a 28-day study in mice, the NOAEL was 300 ppm (equal to 43.9 mg/kg bw per day), based on effects in the liver (increased serum ALT, vacuolation/fatty change of hepatocytes) at 1500 ppm (equal to 230 mg/kg bw per day). In a 90-day study in mice, the NOAEL was 100 ppm (equal to 12.8 mg/kg bw per day), based on effects in the liver (vacuolation/fatty change of hepatocytes) and the adrenals (hypertrophy and/or vacuolation of the zona fasciculata) observed in males at 750 ppm (equal to 98 mg/kg bw per day) and in females at 1500 ppm (equal to 247 mg/kg bw per day).

In a 28-day study in rats, the NOAEL was 300 ppm (equal to 24.8 mg/kg bw per day), based on marginal liver toxicity (increased serum cholesterol and total protein levels) in males at 1000 ppm (equal to 79.4 mg/kg bw per day). In a 90-day study in rats, the NOAEL was 100 ppm (equal to 6.36 mg/kg bw per day), based on effects in the liver (increased serum cholesterol level, vacuolation/fatty change of hepatocytes) in males at 750 ppm (equal to 47.6 mg/kg bw per day). After a 28-day recovery phase, very slight histopathological changes in the liver (hypertrophy and fatty change of hepatocytes) were seen in males at 1500 ppm (equal to 94.9 mg/kg bw per day).

In a 90-day oral gavage study in dogs, the NOAEL was 6 mg/kg bw per day, based on decreased feed consumption and decreased body weights during the 1st week of exposure at 10 mg/kg bw per day. After reduction of this dose to 6 mg/kg bw per day on study day 5, no treatment-related adverse effects were observed. In a 1-year oral gavage study in dogs, the NOAEL was 6 mg/kg bw per day, the highest dose tested. The increased incidences of soft/watery faeces in two males at this dose were not considered adverse, as these changes were not accompanied by any other toxicological effect. Also, the slight decreases in feed consumption and body weight in two females during the first 2 weeks of dosing at 6 mg/kg bw per day were not considered adverse, as there were no changes during the remainder of the study. The overall NOAEL for the 90-day and 1-year studies was 6 mg/kg bw per day.

Long-term studies of toxicity and carcinogenicity were conducted in mice and rats. In an 18-month study of carcinogenicity in mice, the NOAEL for carcinogenicity was 100 ppm (equal to 10.4 mg/kg bw per day), based on an increased incidence of hepatocellular adenomas and/or carcinomas in males at 750 ppm (equal to 79.6 mg/kg bw per day). The NOAEL for non-neoplastic changes was 100 ppm (equal to 10.4 mg/kg bw per day), based on liver toxicity (vacuolation/fatty change of hepatocytes) in males at 750 ppm (equal to 79.6 mg/kg bw per day).

In a series of mechanistic studies in mice, including C57BL/6J knockout mice for PXR and CAR and C57BL/6J mice "humanized" for PXR and CAR, it was demonstrated that sulfoxaflor was a relatively potent phenobarbital-like inducer of hepatic P450 enzymes via activation of CAR and possibly, to some extent, PXR. This was apparent at the mRNA, protein and enzyme activity level. Activation of the mouse CAR (and possibly PXR) resulted in increased hepatocyte hypertrophy and proliferation. The human CAR (and possibly PXR) supported modest P450 induction and hepatic hypertrophy by sulfoxaflor, but did not support any effect on hepatocyte proliferation.

In a 24-month study of toxicity and carcinogenicity in rats, the NOAEL for carcinogenicity was 100 ppm (equal to 4.24 mg/kg bw per day), based on an increased incidence of hepatocellular

adenomas in males at 500 ppm (equal to 21.3 mg/kg bw per day). Also at 500 ppm, there was an increased incidence of bilateral Leydig (interstitial) cell adenomas of the testes, whereas there was no effect on the incidence of combined unilateral/bilateral Leydig cell adenomas. The size and weight of the testes and the size of Leydig cell adenomas were increased at 100 and 500 ppm and were associated with the secondary changes in the testes and epididymides listed below. The NOAEL for non-neoplastic effects was 25 ppm (equal to 1.04 mg/kg bw per day), based on changes in the testes (increased testes weights, increased incidence of severe bilateral atrophy of seminiferous tubules) and epididymides (decreased epididymal weights, increased incidence of severe bilateral decreased spermatic elements of the epididymides) in males at 100 ppm (equal to 4.24 mg/kg bw per day). In females, the NOAEL for non-neoplastic effects was 100 ppm (equal to 5.13 mg/kg bw per day), based on hepatocellular degeneration at 750 ppm (equal to 39.0 mg/kg bw per day).

In a mechanistic study on liver tumorigenesis in rats, 3-day or 7-day exposure to sulfoxaflor at dietary concentrations up to 1500 ppm (equal to 83–102 mg/kg bw per day) resulted in increased liver weights, increased cell proliferation in the centrilobular and midzonal regions of the hepatic lobules, marked induction of *Cyp2b1* gene expression and hepatic activities of PROD and BROD, and moderate induction of *Cyp2b2* and *Cyp3a3* expression levels. The pattern of changes was phenobarbital-like, as evidenced by the CAR- and PXR-related molecular, enzymatic and proliferative responses.

The Meeting concluded that for the liver tumours in both mice and rats, there was sufficient evidence to support the proposed phenobarbital-like MOA. In particular, sulfoxaflor exhibited clearly higher activity towards rodent CAR than towards human CAR. The marked qualitative and quantitative species differences in the key events in the MOA for neoplasia in response to CAR activation allowed for the conclusion that the sulfoxaflor-induced liver tumours in rats and mice are not relevant to humans (see Appendix 1).

In a mechanistic study conducted to examine the potential MOA for the Leydig cell effects seen in the rat carcinogenicity study, 8-week exposure of male Fischer 344 rats to sulfoxaflor at dietary concentrations up to 500 ppm (equal to 28 mg/kg bw per day) resulted in decreased serum prolactin and increased serum LH and testosterone levels and in decreased testis LHR and prolactin receptor gene expression at week 4, but not at week 2 or week 8. Treatment had no effect on the percentage of Leydig cells with intracellular staining of LHR, biliary excretion of [^{14}C]testosterone, serum 17β-estradiol level or any measured gene in the steroidogenic pathway. Because Fischer 344 rats are particularly susceptible to effects on Leydig cells, analogous treatment of male Sprague-Dawley rats was performed, resulting in increased serum LH and testosterone levels at week 2 and a decrease in serum prolactin level at week 4.

In a mechanistic study using intracerebral microdialysis in rats, sulfoxaflor infusion (0.4 and 2 mmol/l) evoked dose-related increases in the extracellular level of dopamine in the mediobasal hypothalamus, with a maximal rise of 39%, 40 minutes after the onset of infusion at 2 mmol/l.

In a further mechanistic study on Leydig cell effects, sulfoxaflor did not bind to the ERα and had weak binding affinity to the AR, whereas it did not show any agonism or antagonism in the ER and AR transactivation assays. In addition, there was no evidence for aromatase inhibition by sulfoxaflor.

Although the proposed MOA—that sulfoxaflor can act as a dopamine agonist in the central nervous system and may inhibit prolactin release in the pituitary (an MOA for the induction of Leydig cell tumours that is considered to be not relevant to humans)—has not been completely demonstrated, the Meeting concluded that the increased incidences of bilateral Leydig cell adenomas in male rats are of low relevance to humans, as there are large qualitative and quantitative differences between rats and humans regarding Leydig cell responses to hormonal stimuli (see Appendix 1). In addition, these effects occurred only at high doses, did not occur in mice and would be anticipated to exhibit a threshold. As a consequence, the secondary changes in the testes and epididymides would not be relevant to the dietary risk assessment of sulfoxaflor.

Sulfoxaflor was tested for genotoxicity in vitro and in vivo in an adequate range of assays. It was not found to be genotoxic in mammalian or microbial test systems.

The Meeting concluded that sulfoxaflor was unlikely to be genotoxic.

On the basis of the absence of genotoxicity, the human non-relevance of the liver tumours in both mice and rats and the fact that the Leydig cell responses observed in rats are unlikely to be relevant to humans (see Appendix 1), the Meeting concluded that sulfoxaflor is unlikely to pose a carcinogenic risk to humans at dietary exposure levels.

In a reproduction/developmental toxicity screening study in rats, the NOAEL for both parental toxicity and effects on offspring was 100 ppm (equal to 8.26 mg/kg bw per day), based on decreased body weight gains in females during the 1st week of gestation and reduced pup survival at 500 ppm (equal to 40.7 mg/kg bw per day).

In a two-generation reproductive toxicity study in rats, the NOAEL for effects on fertility was 400 ppm (equal to 24.6 mg/kg bw per day), the highest dose tested. The NOAEL for parental toxicity was 100 ppm (equal to 6.07 mg/kg bw per day), based on liver toxicity (increase in vacuolation/fatty change of centrilobular hepatocytes) in F_0 males at 400 ppm (equal to 24.6 mg/kg bw per day). The NOAEL for offspring toxicity was 100 ppm (equal to 6.07 mg/kg bw per day), based on reduced pup survival and delayed preputial separation (puberty onset) in F_2 males at 400 ppm (equal to 24.6 mg/kg bw per day).

In a cross-fostering study conducted to assess whether the observed effects of sulfoxaflor on neonatal survival in rats resulted from in utero and/or lactational exposure, all offspring from dams exposed to sulfoxaflor (1000 ppm, equal to 60–81 mg/kg bw per day) prior to birth died by PND 4, irrespective of whether they were cross-fostered to control or treated foster dams. There was no effect on survival for pups exposed only after birth. Thus, the effect of sulfoxaflor on pup survival was due to in utero, not lactational, exposure.

In a developmental toxicity study in rats, the NOAEL for maternal toxicity was 150 ppm (equal to 11.5 mg/kg bw per day), based on decreased body weight and body weight gain and decreased feed consumption at 1000 ppm (equal to 70.2 mg/kg bw per day). The NOAEL for developmental toxicity was 150 ppm (equal to 11.5 mg/kg bw per day), based on increases in several fetal abnormalities (forelimb flexure, hindlimb rotation, bent clavicle, fused sternebrae, convoluted ureter and hydroureter) at 1000 ppm (equal to 70.2 mg/kg bw per day).

A series of special studies conducted to determine the critical window of developmental susceptibility of rat fetuses demonstrated that late gestational exposure (i.e. from GD 20 to GD 21 or 22) of dams to sulfoxaflor (1000 ppm, equal to 36–39 mg/kg bw per day) resulted in reduced neonatal survival and limb abnormalities seen in pups at PNDs 1–3, whereas no limb abnormalities were observed at PND 4 in the same litters. Offspring from dams exposed (at 1000 ppm, equal to 43–77 mg/kg bw per day) up to GD 19 did not show any limb abnormalities or reduced neonatal survival.

Histopathological evaluation of fetal lung samples from the prenatal developmental toxicity study in rats did not reveal any morphological abnormalities that could have contributed to the sulfoxaflor-induced neonatal mortality in rat pups.

In mechanistic studies conducted to test the hypothesis that the limb abnormalities and bent clavicles in rat fetuses are mediated by the pharmacological agonist action of sulfoxaflor at the fetal neuromuscular junction nAChR, radioligand binding and electrophysiological examination revealed that sulfoxaflor is an agonist of the rat fetal muscle nAChR (which contains the rat γ subunit), whereas it has no agonist activity on the equivalent human fetal nAChR (containing the human γ subunit) or on the rat or human adult muscle nAChR (containing the rat or human ε subunit). In rodents, replacement of the γ subunit by the ε subunit commences late during the 1st postnatal week and is largely complete by the end of the 2nd postnatal week, whereas in humans, the switch from γ to ε subunit expression occurs predominantly during the late fetal period. These results were considered

to support the hypothesis that sulfoxaflor induces fetal abnormalities and neonatal death in rats via its pharmacological action on the fetal muscle nAChR. This receptor develops functional expression between GDs 16 and 17 in the rat, resulting in synchronized fetal limb movements and diaphragmatic responsiveness important for the transition to extrauterine respiration.

Two developmental toxicity range-finding studies in rabbits demonstrated that administration of sulfoxaflor in the diet afforded a greater applied maximally tolerated dose (1000 ppm, equal to 36.6 mg/kg bw per day) relative to gavage (15 mg/kg bw per day caused excessive maternal toxicity). Thus, dietary administration of sulfoxaflor was chosen for the main developmental toxicity study.

In a developmental toxicity study in rabbits, the NOAEL for maternal toxicity was 150 ppm (equal to 6.6 mg/kg bw per day), based on decreased faeces and decreases in body weight gain and feed consumption at 750 ppm (equal to 31.9 mg/kg bw per day). The NOAEL for prenatal developmental toxicity was 750 ppm (equal to 31.9 mg/kg bw per day), the highest dose tested.

In a special study conducted to assess the effects of sulfoxaflor on neonatal survival in rabbits, dams were exposed to sulfoxaflor (750 ppm, equal to 29 mg/kg bw per day) from gestation day 7 through the initiation of parturition and allowed to deliver and rear their offspring to lactation day 4. Dams showed decreased body weight gains and feed consumption, whereas no treatment-related effects on the mean number of offspring born, offspring survival or the general physical condition of the offspring were observed.

The Meeting concluded that for the limb abnormalities and bent clavicles observed in rats, there is sufficient evidence that these effects were induced by pharmacological action of sulfoxaflor at the rat fetal muscle nAChR, whereas sulfoxaflor has no agonist activity on the equivalent human fetal nAChR or on the rat or human adult muscle nAChR. This allowed for the conclusion that these effects are not relevant to humans. Regarding the reduced neonatal survival observed in rats, the Meeting noted that the human relevance for this effect cannot be excluded, as the underlying MOA is unclear.

In an acute neurotoxicity study in rats, the NOAEL for neurotoxicity was 25 mg/kg bw, based on decreased motor activity at 75 mg/kg bw. There was no evidence for neuropathological effects up to the highest dose tested (750 mg/kg bw).

In a developmental neurotoxicity study in rats, the NOAEL for maternal and reproductive toxicity was 400 ppm (equal to 28.8 mg/kg bw per day), the highest dose tested. The NOAEL for developmental neurotoxicity was 400 ppm (equal to 28.8 mg/kg bw per day), as there were no signs of developmental neurotoxicity at any exposure level. The NOAEL for neonatal toxicity was 100 ppm (equal to 7.4 mg/kg bw per day), based on the reduction in postnatal survival and pup body weights at 400 ppm (equal to 28.8 mg/kg bw per day).

Toxicological data on metabolites

X11719474, the major soil and plant metabolite of sulfoxaflor, was of low acute oral toxicity in rats (LD_{50} > 5000 mg/kg bw) and showed no genotoxic potential in vitro in mammalian or microbial test systems. In a 90-day oral toxicity study in rats, the NOAEL was 1000 ppm (equal to 65.3 mg/kg bw per day), based on effects in the liver (vacuolation/fatty change) at 5000 ppm (equal to 327 mg/kg bw per day). In a reproduction toxicity screening study in rats, the NOAEL for reproductive and offspring performance was 5000 ppm (equal to 396 mg/kg bw per day), the highest dose tested. In a prenatal developmental toxicity study in rats, the NOAEL for developmental toxicity was 5000 ppm (equal to 368 mg/kg bw per day), the highest dose tested.

X11721061, a plant and animal (rat) metabolite of sulfoxaflor, was of low acute oral toxicity in rats (LD_{50} = 2000 mg/kg bw) and showed no genotoxic potential in vitro in mammalian or microbial test systems. In a 28-day oral toxicity study in rats, the NOAEL was 3000 ppm (equal to 236 mg/kg bw per day), based on reduced feed consumption at 8000 ppm (equal to 622 mg/kg bw per day).

X11596066, a metabolite of sulfoxaflor identified in hens and goats, was of low acute oral toxicity in rats (LD_{50} > 2000 mg/kg bw) and showed no genotoxic potential in vitro (Ames test).

X11579457, a soil metabolite of sulfoxaflor, was of low acute oral toxicity in rats (LD_{50} > 2000 mg/kg bw) and showed no genotoxic potential in vitro in mammalian or microbial test systems.

X11519540, a soil and animal (hen) metabolite of sulfoxaflor, was of moderate acute oral toxicity in rats (LD_{50} = 565 mg/kg bw) and showed no genotoxic potential in vitro in mammalian or microbial test systems. In a 28-day oral toxicity study in rats, the NOAEL was less than 100 ppm (equal to 7.7 mg/kg bw per day) in males, based on effects in liver (increased serum cholesterol), thyroid (follicular cell hypertrophy) and adrenals (increased vacuolation of the cortex).

All metabolites were less toxic than the parent compound, except for X11519540, which had higher acute and higher short-term toxicity than the parent.

There were no reports of adverse health effects in manufacturing plant personnel. Also, there were no reports of poisonings with sulfoxaflor.

The Meeting concluded that the existing database on sulfoxaflor was adequate to characterize the potential hazards to fetuses, infants and children.

Toxicological evaluation

The Meeting established an acceptable daily intake (ADI) for sulfoxaflor of 0–0.05 mg/kg bw, based on a NOAEL of 5.13 mg/kg bw per day for hepatocellular degeneration in female rats in a 2-year toxicity and carcinogenicity study and application of a safety factor of 100. The ADI is supported by the NOAEL of 6.07 mg/kg bw per day for systemic toxicity (increased vacuolation/fatty change of centrilobular hepatocytes in F_0 males) and offspring toxicity (reduced neonatal survival) at 24.6 mg/kg bw per day in a two-generation rat study, the NOAEL of 6.36 mg/kg bw per day, based on effects in the liver (increased serum cholesterol, vacuolation/fatty change of hepatocytes) in a 13-week study in rats, and the overall NOAEL of 6 mg/kg bw per day in the 90-day and 1-year dog studies.

The Meeting established an acute reference dose (ARfD) for sulfoxaflor of 0.3 mg/kg bw, based on the NOAEL of 25 mg/kg bw for decreased motor activity at 75 mg/kg bw in an acute neurotoxicity study in rats. A 100-fold safety factor was applied.

Levels relevant to risk assessment

Species	Study	Effect	NOAEL	LOAEL
Mouse	Thirteen-week study of toxicity	Toxicity	100 ppm, equal to 12.8 mg/kg bw per day	750 ppm, equal to 98 mg/kg bw per day
	Eighteen-month study of toxicity and carcinogenicity	Toxicity	100 ppm, equal to 10.4 mg/kg bw per day	750 ppm, equal to 79.6 mg/kg bw per day
		Carcinogenicity	100 ppm, equal to 10.4 mg/kg bw per day	750 ppm, equal to 79.6 mg/kg bw per day
Rat	Thirteen-week study of toxicity	Toxicity	100 ppm, equal to 6.36 mg/kg bw per day	750 ppm, equal to 47.6 mg/kg bw per day
	Two-year study of toxicity and carcinogenicity	Toxicity	100 ppm, equal to 5.13 mg/kg bw per day	750 ppm, equal to 39.0 mg/kg bw per day
		Carcinogenicity	100 ppm, equal to 4.24 mg/kg bw per day[a]	500 ppm, equal to 21.3 mg/kg bw per day

Species	Study	Effect	NOAEL	LOAEL
	Two-generation study of reproductive toxicity	Reproductive toxicity	400 ppm, equal to 24.6 mg/kg bw per day[b]	—
		Parental toxicity	100 ppm, equal to 6.07 mg/kg bw per day	400 ppm, equal to 24.6 mg/kg bw per day
		Offspring toxicity	100 ppm, equal to 6.07 mg/kg bw per day	400 ppm, equal to 24.6 mg/kg bw per day
	Developmental toxicity study	Maternal toxicity	150 ppm, equal to 11.5 mg/kg bw per day	1000 ppm, equal to 70.2 mg/kg bw per day
		Embryo and fetal toxicity	150 ppm, equal to 11.5 mg/kg bw per day	1000 ppm, equal to 70.2 mg/kg bw per day
	Acute neurotoxicity study[c]	Neurotoxicity	25 mg/kg bw	75 mg/kg bw
	Developmental neurotoxicity study	Developmental neurotoxicity	400 ppm, equal to 28.8 mg/kg bw per day[b]	—
Rabbit	Developmental toxicity study	Maternal toxicity	150 ppm, equal to 6.6 mg/kg bw per day	750 ppm, equal to 31.9 mg/kg bw per day
		Embryo and fetal toxicity	750 ppm, equal to 31.9 mg/kg bw per day[b]	—
Dog	Thirteen-week and 1-year studies of toxicity[c,d]	Toxicity	6 mg/kg bw per day	10 mg/kg bw per day

[a] Not considered relevant for human risk assessment.
[b] Highest dose tested.
[c] Gavage administration.
[d] Two studies combined.

Estimate of acceptable daily intake for humans

0–0.05 mg/kg bw

Estimate of acute reference dose

0.3 mg/kg bw

Information that would be useful for the continued evaluation of the compound

Results from epidemiological, occupational health and other such observational studies of human exposure

Critical end-points for setting guidance values for exposure to sulfoxaflor

Absorption, distribution, excretion and metabolism in mammals	
Rate and extent of oral absorption	Rapid; ≥ 93%
Distribution	Extensive; highest concentrations in liver, kidney and erythrocytes
Rate and extent of excretion	≥ 93% within 168 h (≥ 92% in urine; 5–9% in faeces)
Potential for accumulation	None
Metabolism in animals	Very limited, oxidative cleavage of the molecule, followed by conjugation with glucuronic acid; one metabolite identified in urine (conjugate of X11721061, 3–4% of administered dose)
Toxicologically significant compounds (animals, plants and the environment)	Sulfoxaflor, X11519540 (soil metabolite)

Acute toxicity	
Rat, LD_{50}, oral	1000 mg/kg bw
Rat, LD_{50}, dermal	> 5000 mg/kg bw
Rat, LC_{50}, inhalation	> 2.09 mg/l (4 h, nose-only exposure)
Rabbit, dermal irritation	Not irritating
Rabbit, eye irritation	Not irritating
Mouse, dermal sensitization (local lymph node assay)	Not sensitizing
Short-term studies of toxicity	
Target/critical effect	Liver (liver cell vacuolation/fatty change) in mice and rats; adrenals (cortical hypertrophy and vacuolation) in mice; decreased feed consumption and body weight gain in dogs
Lowest relevant oral NOAEL	6.36 mg/kg bw per day (90-day study in rats)
Lowest relevant dermal NOAEL	500 mg/kg bw per day (28-day study in rats)
Lowest relevant inhalation NOAEC	No data
Long-term toxicity and carcinogenicity	
Target/critical effect	Liver (liver cell vacuolation/fatty change) in mice and in female rats
Lowest relevant NOAEL	5.13 mg/kg bw per day (2-year study in rats)
Carcinogenicity	Unlikely to pose a carcinogenic risk to humans at levels of dietary exposure
Genotoxicity	
	Not genotoxic
Reproductive toxicity	
Reproductive target/critical effect	No effects on fertility at highest dose tested; reduced pup survival and delayed preputial separation at parentally toxic dose
Lowest relevant reproductive NOAEL	6.07 mg/kg bw per day for offspring toxicity (two-generation study in rats)
Developmental target/critical effect	Fetal abnormalities (forelimb flexure, hindlimb rotation, bent clavicle, fused sternebrae, convoluted ureter) at maternally toxic dose
Lowest relevant developmental NOAEL	11.5 mg/kg bw per day (rat)
Neurotoxicity	
Acute neurotoxicity	Decrease in motor activity; NOAEL: 25 mg/kg bw
Developmental neurotoxicity	No evidence of developmental neurotoxicity at highest dose tested
Other toxicological studies	
Mechanistic studies	Studies on liver tumorigenesis (rats, mice) demonstrate non-relevance to humans
	Studies on Leydig cell effects in rats suggested evidence for an MOA unlikely to be relevant to humans
	Studies demonstrated that limb and clavicle abnormalities are due to rat-specific agonist activity on the fetal muscle nAChR not relevant to humans

Studies on metabolites	X11719474: lower toxicity than parent compound, not genotoxic in vitro, no developmental toxicity X11721061, X11596066 and X11579457: lower toxicity than parent compound, not genotoxic in vitro X11519540: higher toxicity than parent compound, not genotoxic in vitro

Medical data

	Limited data; no adverse health effects reported in manufacturing plant personnel

Summary

	Value	Study	Safety factor
ADI	0–0.05 mg/kg bw	Two-year study in rat (supported by two-generation study in rats, 90-day study in rats and 1-year study in dogs)	100
ARfD	0.3 mg/kg bw	Acute neurotoxicity study in rat	100

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Appendix 1: Application of the IPCS conceptual framework for cancer risk assessment

(IPCS framework for analysing the relevance of a cancer mode of action for humans)

This framework, developed by an International Programme on Chemical Safety (IPCS) working group, provides a generic approach to the principles commonly used in evaluating a postulated MOA for tumour induction by a chemical (Sonich-Mullin et al., 2001; Boobis et al., 2006). Thus, the framework was used by the 2011 JMPR to provide a structured approach to the assessment of the overall weight of evidence for the postulated MOA for the increased incidences of hepatocellular

adenoma and carcinoma in male mice, the increased incidences of hepatocellular adenoma in male rats and the increased incidences of bilateral Leydig cell adenomas in male rats observed after long-term administration of sulfoxaflor.

A. Hepatocellular tumours in mice and rats

A.1 Introduction

In the 18-month study of carcinogenicity in mice, increased incidences of hepatocellular adenoma and carcinoma were observed in male mice after administration of sulfoxaflor at a dietary concentration of 750 ppm (equal to 79.6 mg/kg bw per day).

In the 24-month study of chronic toxicity and carcinogenicity in rats, increased incidences of hepatocellular adenoma were observed in male rats after administration of sulfoxaflor at a dietary concentration of 500 ppm (equal to 21.3 mg/kg bw per day) (see section 2.3).

A.2 Postulated mode of action (theory of the case)

The postulated MOA for the development of liver cell tumours in rodents after long-term administration of sulfoxaflor is considered to be similar to the MOA for phenobarbital, which induces liver cell tumours in rodents by a non-genotoxic mechanism (LeBaron et al., 2010). A key effect of phenobarbital is the induction of hepatic cytochrome P450 enzymes, which is mediated through activation of nuclear receptors, in particular CAR in hepatocytes (and possibly, to some extent, PXR), which is followed by altered expression of CAR- (and PXR-)regulated genes and subsequent induction of liver enzymes (including CYP isoenzymes), hepatocellular hypertrophy, increase of cell proliferation and suppression of apoptosis and perturbation of liver function. The increased cell replication and suppression of normal apoptotic processes are associated with clonal expansion of potentially mutated cells, which leads to foci of altered hepatocytes. Such altered foci ultimately progress to neoplasia (Bannasch, Haertel & Su, 2003).

A.3 Key events

Analysis of the available toxicological data for sulfoxaflor, including extensive mechanistic data, suggested a phenobarbital-like induction of hepatic CYP enzymes as the most likely MOA for the liver cell tumour development, which would operate through the following key events:

- *Activation of nuclear receptors, in particular CAR*: Activation of nuclear receptors, in particular CAR, is most probably an early step, although no *direct* evidence has been presented that demonstrates an interaction of sulfoxaflor with any nuclear receptor. However, the activation of CYP2B genes is commonly considered to be a diagnostic, surrogate response to CAR activation. This response was demonstrated in mice treated for 7–90 days and in rats treated for 3–7 days by increased expression of CYP2B10 or CYP2B1 mRNA (in mice or rats, respectively) and by increases in CYP2B10 proteins in mice.
- *Induction of hepatic CYP enzymes*: Induction of hepatic CYP2B enzymes, particularly BROD and PROD, was observed in mice treated for 7–90 days (≥ 44 mg/kg bw per day) and in rats treated for 3–7 days (≥ 59 mg/kg bw per day) and may be considered to be indicative of a CAR-mediated response.
- *Increase of liver cell proliferation*: Increased liver cell proliferation was demonstrated in mice (BrdU labelling, Ki-67 staining; ≥ 89 mg/kg bw per day) and in rats (Ki-67 staining; ≥ 59 mg/kg bw per day) after 7 days of sulfoxaflor administration.
- *Hepatocellular hypertrophy and hyperplasia*: Hepatocellular hypertrophy was observed in mice after 7 or 90 days and 18 months of treatment (128, ≥ 98 or 80 mg/kg bw per day, respectively)

and in rats after 28 or 90 days and 12–24 months of treatment (≥ 79, ≥ 48 or 21 mg/kg bw per day, respectively).

- *Increase in liver weight*: Increased liver weight was observed in mice after 7 or 90 days and 18 months of treatment (128, ≥ 98 or 80 mg/kg bw per day, respectively) and in rats after 7, 28 or 90 days and 12–24 months of treatment (≥ 59, ≥ 79, ≥ 48 or 21 mg/kg bw per day, respectively).
- *Development of altered hepatic foci*: Foci of altered hepatocytes (eosinophilic and vacuolated foci) were increased in male mice after administration of sulfoxaflor for 18 months at 80 mg/kg bw per day. In male rats, the incidence of eosinophilic foci (quantified as more than five foci in the three standard liver sections examined microscopically) was marginally increased at 21 mg/kg bw per day when compared with controls (19 versus 9, respectively).

The key events as described above have been observed in mice and rats in mechanistic studies as well as in short-term and long-term toxicity studies. The dose–response relationships and the temporal analyses of the key events and tumour response are presented below.

A.4 Concordance of dose–response relationships

All of the features considered to contribute to the key events occurred in dose–response relationships, and it was obvious that key events regarded as occurring later in the MOA sequence did not occur at doses lower than those regarded as occurring earlier in the MOA sequence. The NOAELs and lowest-observed-adverse-effect levels (LOAELs) for the key events in the MOA for sulfoxaflor-induced liver tumours in mice and rats are provided in Tables A1 and A2, respectively.

Table A1. NOAELs/LOAELs for key events in the MOA for sulfoxaflor-induced liver cell tumours in mice

Key event	NOAEL/LOAEL (mg/kg bw per day)
Activation of CAR (*indirect* evidence based on activation of CYP2B10 genes)	—/89 (7-day mechanistic study)
	—/44 (28-day study)
	—/98 (90-day study)
Induction of CYP2B enzymes (PROD, BROD)	—/89 (7-day mechanistic study)
	—/44 (28-day study)
	—/98 (90-day study)
Increase in liver cell proliferation	—/418 (7-day palatability study; Ki-67 staining)
	—/89 (7-day mechanistic study; increased BrdU labelling, mitotic alteration)
	44/230 (28-day study; mitotic alteration)
	98/166 (90-day study; mitotic alteration)
	10/80 (18-month study; mitotic alteration)
Hepatocellular hypertrophy	—/89 (7-day mechanistic study)
	44/230 (28-day study)
	13/98 (90-day study)
	10/80 (18-month study)
Increase in liver weight	89/128 (7-day mechanistic study)
	44/230 (28-day study)
	13/98 (90-day study)
	2.5/10 (18-month study)
Increase in hepatic foci	10/80 (18-month study; increase in eosinophilic and vacuolated)
Increase in liver cell tumours	10/80 (18-month study; increase in adenoma and carcinoma)

Table A2. NOAELs/LOAELs for key events in the MOA for sulfoxaflor-induced liver cell tumours in rats

Key event	NOAEL/LOAEL (mg/kg bw per day)
Activation of CAR (*indirect* evidence based on activation of CYP2B1 genes)	—/8.9 (3-day mechanistic study) —/8.0 (7-day mechanistic study)
Induction of CYP2B enzymes (PROD, BROD)	8.9/60 (3-day mechanistic study) 8.0/59 (7-day mechanistic study)
Increase in liver cell proliferation	99/— (3-day mechanistic study; Ki-67 staining) 8.0/59 (7-day mechanistic study; Ki-67 staining) —/192 (28-day study; Ki-67 staining)
Hepatocellular hypertrophy	25/79 (28-day study) 6.4/48 (90-day study) 4.2/21 (24-month study)
Increase in liver weight	60/99 (3-day mechanistic study) 8.0/59 (7-day mechanistic study) 25/79 (28-day study) 6.4/48 (90-day study) 4.2/21 (24-month study)
Increase in hepatic foci	4.2/21 (24-month study; marginal increase in eosinophilic foci)
Increase in liver cell tumours	4.2/21 (24-month study; increase in adenoma)

In mice, activation of CYP2B10 genes as well as increases of hepatic CYP2B enzyme activities (PROD, BROD) and of liver cell proliferation were observed in a dose-related manner in a 7-day mechanistic study (at ≥ 89 mg/kg bw per day). Also, the increases in the incidence and/or severity of hepatocellular hypertrophy and of liver weights were dose related in the 28- and 90-day toxicity studies and in the 18-month carcinogenicity study.

In rats, dose-related activation of CYP2B1 genes as well as increases of hepatic CYP2B enzyme activities (PROD, BROD), of liver cell proliferation and of liver weights were demonstrated in both a 3-day and 7-day mechanistic study (activation of CYP2B1 genes at ≥ 8 mg/kg bw per day; other effects at ≥ 59 mg/kg bw per day). Also, the increases in the incidence or severity of hepatocellular hypertrophy and of liver weights were dose related in the 28- and 90-day toxicity studies (at 48–79 mg/kg bw per day and above), whereas these effects were noted in the 24-month toxicity and carcinogenicity study only at the highest dose tested (21 mg/kg bw per day).

A.5 Temporal association

Within the limitations of study design, the temporal relationships followed a logical pattern. The key events occurring earlier in the MOA sequence, such as activation of CAR, induction of CYP2B enzymes, increased liver cell proliferation, liver cell hypertrophy and increased liver weights, were observed after only a 7-day or 28-day exposure to sulfoxaflor. In mice, increased incidences of foci of altered hepatocytes (and adenoma/carcinoma) first appeared after 18 months of exposure, whereas in rats, a marginal increase of eosinophilic foci and increased incidences of liver cell adenoma were observed after 24 months of exposure.

A.6 Strength, consistency and specificity of association of tumour response with key events

Strength, consistency and specificity of the association can be established from the studies described previously. The quantifiable precursor events, fundamental to the proposed MOA, are

relatively consistent with the emergence of liver cell tumours. Induction of CYP enzyme activity is a well-known MOA for rodent hepatocarcinogens, and phenobarbital is a standard example of a CAR-mediated CYP inducer (Whysner, Ross & Williams, 1996; Holsapple et al., 2006; Lake, 2009; Cohen, 2010). The key events for this MOA are CAR activation, with associated CYP isozyme induction, and increase in hepatocellular proliferation, which result in subsequent adenomas and carcinomas. In addition to these key events, reversibility of hepatic effects upon discontinuance of treatment is considered necessary information to support this MOA (Cohen, 2010).

Overall, the mechanistic studies for both mice and rats clearly demonstrate a sulfoxaflor-induced, 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, 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 (equal to 99–120 mg/kg bw per day). 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 plausible sulfoxaflor MOA, while simultaneously ruling out other nuclear receptor–mediated MOAs for rodent hepatic carcinogens, such as PPARα or AhR agonism.

A.7 Biological plausibility and coherence

The proposed MOA for the induction of liver tumours in male mice and rats by sulfoxaflor is plausible and cohesive, as the data show a substantial similarity to the MOA that has been proposed (and which is widely accepted) for phenobarbital.

In addition, the specificity for the MOA was demonstrated for sulfoxaflor using genetically engineered mouse models. As described above, the CARKO/PXRKO mice were refractory to the CAR-mediated hepatic effects demonstrated for sulfoxaflor in wild-type mice. Moreover, and most importantly, "humanized" CAR/PXR (hCAR/hPXR) mice demonstrated a similar, although quantitatively lower, response for most end-points directly associated with CAR activation, but no increase in hepatocellular proliferation was noted. These data are consistent with the known MOA for phenobarbital and other CAR activators and are considered to be supportive of why humans are refractory to the hepatocarcinogenic effects of phenobarbital (Holsapple et al., 2006; Lake, 2009; Cohen, 2010).

A.8 Other modes of action

a) Genetic activity, including DNA reactivity, is always one possible MOA to consider, but no genotoxic potential was demonstrated for sulfoxaflor in the following tests:

- bacterial reverse mutation test,
- mammalian cell gene mutation test,
- mammalian chromosomal aberration test, in vitro,
- mammalian erythrocyte micronucleus test, in vivo.

Therefore, the available evidence indicates that genotoxicity is not an alternative MOA for sulfoxaflor.

b) There was no indication of hepatotoxic effects such as peroxisome proliferation or chronic degeneration in the general toxicity studies performed on sulfoxaflor that might suggest cycles of degeneration and regenerative hyperplasia.

c) Also, none of the studies of general toxicity or toxicity to reproduction have suggested that there might be perturbation of estrogenic hormone homeostasis that could result in a mitogenic stimulus to the liver.

d) An unknown is whether CYP induction is a surrogate for a wider pleiotrophic response (Ueda et al., 2002); it is known that CAR is involved in the epigenetic alteration of a large number of different genes, many of which may be involved in tumorigenesis (Phillips & Goodman, 2009; Phillips, Burgoon & Goodman, 2009).

A.9 Uncertainties, inconsistencies and data gaps

No direct evidence for binding to CAR or rather activation of CAR by sulfoxaflor has been provided. However, this data gap might be considered to be of low relevance given the close similarities in the MOA between sulfoxaflor and phenobarbital, which is considered to be the prototype for rodent liver tumour formation by CAR-mediated induction of hepatic CYP2B forms.

Also, the MOA evaluation focused mainly on observations in male mice and rats (which were more sensitive than female mice and rats to hepatic effects of sulfoxaflor), although a statistically not significantly increased incidence of hepatocellular tumours was identified in female mice treated for 18 months at 1250 ppm (equal to 176 mg/kg bw per day). Histopathological examination of the liver of those animals with hepatocellular tumours (and of liver tissue in the shorter-duration studies) revealed a phenotype entirely consistent with that identified in males of increased CYP induction and eosinophilia. Although inclusion of females in the MOA studies and MOA evaluation may have been informative, the MOA data provide compelling evidence that the sulfoxaflor liver tumour MOA is not sex specific. Thus, restricting the MOA investigations to the more sensitive sex significantly reduced the number of animals used for the studies.

Reversibility of sulfoxaflor-induced hepatic effects was investigated in a standard, repeated-dose 90-day rat toxicity study. Animals administered the top dietary concentration of 1500 ppm (equal to 95 mg/kg bw per day, i.e. more than 4-fold greater than the hepatocarcinogenic dose level of 21 mg/kg bw per day in the 2-year rat study) for 90 days had a relative liver weight increase of 41% with clear microscopic hepatocellular hypertrophy identified. A subset of these animals was then subsequently switched to a control diet for an additional 28 days, and the data indicated that those animals did not have significantly increased relative liver weights or microscopic hepatocellular hypertrophy compared with controls. A complete evaluation of the molecular reversibility for sulfoxaflor-induced hepatic effects across all MOA studies was not undertaken in an effort to restrict animal usage, as the most definitive experiment for specificity of sulfoxaflor-induced liver effects was demonstrated with the use of CARKO/PXRKO (knockout) and hCAR/hPXR (humanized) mice.

A.10 Assessment of postulated mode of action

The data provided support a non-genotoxic, threshold-based MOA for the development of liver cell tumours in mice and rats following chronic exposure to sulfoxaflor. The key events for the proposed MOA of sulfoxaflor have been identified and documented, and they illustrate a strong dose–response relationship and temporal relationship to tumour formation. The proposed MOA of sulfoxaflor is consistent with the well-known MOA for phenobarbital-induced liver tumours in rodents and is consistent with the current understanding of cancer biology and nuclear receptor–mediated carcinogenesis.

Human relevance analysis

1. Is the weight of evidence sufficient to establish a mode of action in animals?

The toxicological data provided for sulfoxaflor support a non-genotoxic, threshold-based MOA for the development of liver cell tumours in mice and rats, and the weight of evidence supports a phenobarbital-like MOA.

2. Can human relevance of the MOA be reasonably excluded on the basis of fundamental, qualitative differences in key events between experimental animals and humans?

The key events in CAR-mediated hepatocellular carcinogenesis include activation of CAR and induction of CYP isozymes, leading to increased hepatocellular proliferation with subsequent induction of proliferative lesions in the liver, including foci, adenomas and carcinomas. Activation of rodent CAR produces a cascade of alterations in gene transcription that leads to increased hepatocellular proliferation, a critical event in the development of liver tumours (Whysner, Ross & Williams, 1996; Cohen, 2010).

In contrast, phenobarbital-induced enzyme induction in human liver may act more through PXR than through CAR (Moore et al., 2003), and different enzymes are induced in humans compared with rodents (Lambert et al., 2009). Also, and more importantly, there is no evidence of increased hepatocellular proliferation in humans or primary human hepatocytes in vitro after exposure to phenobarbital (Lake, 2009). Furthermore, recent research has strongly suggested that human CAR and PXR introduced to mouse knockout models lacking the mouse genes for these functions result only in hepatic hypertrophy and not hepatic hyperplasia when these modified mice are treated with either phenobarbital or chlordane (Ross et al., 2010). This finding was confirmed in studies with sulfoxaflor, where humanized CAR/PXR knock-in mice were refractory to the hepatocellular proliferative effect of sulfoxaflor, whereas wild-type mice demonstrated increased proliferation.

Extensive epidemiological studies in humans exposed to levels of phenobarbital comparable to those in rodent bioassays did not find an increased risk of cancer (Whysner, Ross & Williams, 1996; Lamminpaa et al., 2002). Based on the MOA assessment, phenobarbital is not a hepatocarcinogen in humans. Furthermore, a hepatocarcinogenic response in rodents for compounds that have data to support a phenobarbital-like MOA is not relevant to humans (Holsapple et al., 2006). On this basis, the rodent liver tumours associated with administration of high dose levels of sulfoxaflor would not pose a cancer hazard to humans.

3. Can human relevance of the MOA be reasonably excluded on the basis of fundamental, quantitative differences in key events between experimental animals and humans?

As human relevance of the experimental animal MOA can be reasonably excluded on the basis of qualitative differences in key events (Question 2), a quantitative assessment of kinetic or dynamic factors is not necessary.

B. Leydig cell adenomas in rats

B.1 Introduction

In the 24-month study of chronic toxicity and carcinogenicity in Fischer 344 rats, increased incidences of Leydig cell adenoma were observed in male rats after administration of sulfoxaflor at a dietary concentration of 500 ppm (equal to 21.3 mg/kg bw per day) (see section 2.3).

B.2 Postulated mode of action (theory of the case)

The postulated MOA for the increase of Leydig cell tumours in rats after long-term administration of sulfoxaflor is based on the hypothesis that sulfoxaflor can act as a dopamine agonist in the brain and reduces prolactin release by the anterior pituitary gland (Rasoulpour et al., 2011). This results in decreased serum prolactin levels and decreased binding of prolactin to prolactin receptors on Leydig cells, which leads to a downregulation of LHR on Leydig cells. The LHR downregulation results in decreased testosterone production, which feeds back to induce LH release from the pituitary and causes a compensatory increase in circulating LH. The elevation in LH produces Leydig cell hyperplasia and Leydig cell tumours if the elevations are over a sustained period.

B.3 Key events

Following analysis of the available toxicological data for sulfoxaflor, including extensive mechanistic data, dopamine agonism was proposed as the most likely MOA for the Leydig cell tumour development in Fischer 344 rats, which would operate through the following key events:

1) increased neuronal dopamine release via specific dopaminergic neuron-based nAChR agonism,
2) decreased serum prolactin levels,
3) downregulation of LHR gene expression in Leydig cells,
4) transient decreases in serum testosterone,
5) increased serum LH levels,
6) increase in Leydig cell proliferation.

This hypothesis was evaluated in a specific MOA study in which these key events were examined to determine the causality of sulfoxaflor's promotion of Fischer 344 rat Leydig cell tumours in the carcinogenicity study. Additionally, studies were conducted to consider whether other known potential MOAs were involved in the Leydig cell tumour promotion effect of sulfoxaflor and to examine the effect of sulfoxaflor on extracellular levels of dopamine in the mediobasal hypothalamus.

B.4 Concordance of dose–response relationships

With respect to dose–response relationships, owing to the subtle nature of the effects, no precursor key events were observed at 100 ppm (equal to 4.2–5.6 mg/kg bw per day), only at 500 ppm (equal to 21–28 mg/kg bw per day). A dose–response relationship for these apical end-point effects existed with increased testis size and increased incidence of bilateral Leydig cell tumours at 500 ppm. Because of the high background incidence of these tumours in Fischer 344 rats, the lack of a response for precursor key events with the MOA analysis at the 100 ppm dose level is not surprising.

The LOAELs for the key effects in the MOA of sulfoxaflor in the Leydig cells are provided in Table B1.

Key event #1 (increased dopamine release) has been confirmed in a study using intracerebral microdialysis in rats. In this study, sulfoxaflor infusion (0.4 and 2 mmol/l) evoked dose-related increases in the extracellular level of dopamine in the mediobasal hypothalamus, with a maximal rise of 39% 40 minutes after the onset of infusion at 2 mmol/l.

Key event #2 (decreased serum prolactin levels) was demonstrated in the 8-week mechanistic study at the highest dose tested, 28 mg/kg bw per day.

Table B1. NOAELs/LOAELs for key events in the MOA for sulfoxaflor-induced Leydig cell tumours in rats

Key event	NOAEL/LOAEL (mg/kg bw per day)
Increased dopamine release	—/0.4 mmol/l by intracerebral microdialysis
Decreased serum prolactin levels	5.6/28 (8-week mechanistic study: effect observed at week 4, not at week 2 or 8)
Downregulation of LHR gene expression in Leydig cells	5.6/28 (8-week mechanistic study: effect observed at week 4, not at week 8)
Transient decrease in serum testosterone levels	Not demonstrated (8-week mechanistic study)
	6.1/25 (two-generation study: delayed preputial separation, probably due to reduced testosterone levels)
Increased serum LH levels	5.6/28 (8-week mechanistic study: effect observed at week 4, not at week 2 or 8)
Increase in Leydig cell proliferation	1.0/4.2 (24-month study: indirect evidence from increased testes weights)
Increase in Leydig cell tumours	4.2/21 (24-month study)

The subsequent key event #3 (downregulation of LHR gene expression in Leydig cells), key event #4 (transient decrease in serum testosterone levels) and key event #5 (increased serum LH levels) were observed also in the same dose range of 25–28 mg/kg bw per day. Key event #4 (transient decrease in serum testosterone levels), however, was not demonstrated directly, but was deduced from indirect evidence (delayed pubertal onset in male rats).

The ultimate key event #6 (Leydig cell proliferation) was also not demonstrated directly; however, a dose–response relationship for this apical end-point effect existed in the 24-month toxicity and carcinogenicity study with increased testes weights at dose levels of 4.2 mg/kg bw per day and above and increased incidence of bilateral Leydig cell adenoma at 21 mg/kg bw per day.

5. Temporal association

Temporal relationships for each of the key events are difficult to ascertain when evaluating hormone data or hormone-dependent changes in target organs, due to the inherent variability, feedback compensation and very long latency for the apical end-point effect of Leydig cell hyperplasia and tumours. Owing to the persistent compensatory nature of the hypothalamic–pituitary–gonadal (HPG) axis, it is not surprising that the changes observed in the hormone data were temporal in nature, whereas conclusive Leydig cell effects occurred only after 2 years of exposure to sulfoxaflor.

6. Strength, consistency and specificity of association of tumour response with key events

The biological processes resulting in rat Leydig cell tumours have been reviewed extensively (Prentice & Miekle, 1995; Clegg et al., 1997; Cook et al., 1999). Leydig cell tumours initially appear as hyperplasia of interstitial cells that can grow with age to the diameter of a single normal seminiferous tubule, at which point they are classified as adenomas per guidance from the United States National Toxicology Program (NTP) (Boorman, Hamlin & Eustis, 1987; Boorman, Chapin & Mitsumori, 1990).

In the 8-week mechanistic study in Fischer 344 rats, there was a dose-dependent increase in LH concentrations concomitant with a dose-dependent decrease in prolactin levels at the 4-week time point, whereas no effects of treatment on prolactin, LH or testosterone levels were observed at all other time points. Consistent with the decreased prolactin levels was a dose-dependent decrease in LHR gene expression at the 4-week, but not 8-week, time point. Although not statistically significant,

the magnitude of gene expression changes is consistent with the dynamic range of these genes in vivo and likely represents a biologically meaningful effect based on alterations in hormone levels.

The specificity of the data for the proposed MOA is the decrease in circulating serum prolactin levels and decreased LHR gene expression. These findings would be observed only with the proposed MOA, but would not be associated with the other possible MOAs leading to Leydig cell tumours (see 8: Other modes of action). Furthermore, the decrease in serum prolactin was associated with a compensatory increase in serum LH, which in turn could act as the primary trophic stimulus over a 2-year Fischer 344 rat carcinogenicity study leading to Leydig cell tumour promotion.

7. Biological plausibility and coherence

Dietary administration of sulfoxaflor to Fischer 344 rats resulted in the early key events (decrease in serum prolactin and LHR gene expression) that lead to an increase in serum LH levels. The MOA demonstrated for sulfoxaflor is for the most part consistent with well-known MOAs for dopamine agonists/enhancers and is consistent with current understanding of hormone-based Leydig cell tumorigenesis. The data for sulfoxaflor are consistent with a non-genotoxic, threshold MOA.

8. Other modes of action

a) Genetic activity, including DNA reactivity, is always one possible MOA to consider, but no genotoxic potential was demonstrated for sulfoxaflor in the following tests:

- bacterial reverse mutation test,
- mammalian cell gene mutation test,
- mammalian chromosomal aberration test, in vitro,
- mammalian erythrocyte micronucleus test, in vivo.

Therefore, the available evidence indicates that genotoxicity is not an alternative MOA for sulfoxaflor.

b) AR antagonists compete with testosterone and DHT for binding to the AR. This competition reduces the androgenic signal to the hypothalamus and adenohypophysis, resulting in an increase in LH secretion with a concomitant elevation of testosterone secretion, leading to the development of Leydig cell tumours (Cook et al., 1999). Direct data obtained with sulfoxaflor showed that although there was an indication that sulfoxaflor was a potential binder to a fragment (i.e. ligand binding domain) of the AR in a non-cell-based binding assay, there was no effect on agonism or antagonism within the AR transactivation assay.

c) ER agonists/antagonists result in changes in estradiol levels, which ultimately cause an increase in LH levels, resulting in the development of Leydig cell tumours. Direct data obtained with sulfoxaflor showed no evidence for ER binding and transactivation (agonism and antagonism), even when tested up to very high concentrations in vitro.

d) 5α-Reductase inhibitors result in decreased conversion of testosterone to DHT. This reduces the net androgenic signal received by the hypothalamus and pituitary, thereby causing a compensatory increase in LH levels, resulting in the development of Leydig cell tumours (Cook et al., 1999). The prostate is differentially sensitive to effects on DHT; for example, DHT has 5-fold greater affinity for AR compared with testosterone (Feldman & Feldman, 2001). Because of this, the prostate would be the most sensitive organ to be affected compared with other accessory sex glands. 5α-Reductase inhibitors can reduce prostate size by 20–30%, although testosterone levels can remain normal (Steers, 2001). Based on the weight of evidence, considering both

direct data that show that sulfoxaflor had no effect on testes 5α-reductase gene expression and indirect data generated from the toxicology package that indicated no prostate effect, a 5α-reductase inhibition MOA is not a plausible alternative MOA for the Leydig cell effects seen in Fischer 344 rats after 2 years of treatment with sulfoxaflor.

e) Aromatase inhibition would result in decreased conversion of androstenedione to estrone and testosterone to estradiol. This would result in an increase in LH levels, leading to the development of Leydig cell tumours (Cook et al., 1999). Direct data obtained with sulfoxaflor showed that it was negative for aromatase inhibition when tested up to very high (i.e. super-physiological) concentrations in vitro. In addition, aromatase inhibitors cause effects on mating and fertility indices as well as female reproductive organ weights and histopathology (Cook et al., 1999; Turner et al., 2000). There were no effects on mating, sperm parameters (counts, motility, morphology) or fertility indices in the two-generation reproductive toxicity study.

f) Inhibition of testosterone biosynthesis would result in lower testosterone and estradiol levels and increased LH levels, resulting in the development of Leydig cell tumours (Cook et al., 1999). Direct data were provided for sulfoxaflor from the Leydig cell tumour MOA study, which showed that there was no treatment-related effect on any measured gene in the steroidogenic pathway, including *StAR* (steroidogenic acute regulatory protein), *Cyp11a1* (P450 side-chain cleavage), *Cyp17a1* (17α-hydroxylase), *HSD3b* (3β-hydroxysteroid dehydrogenase) and *SDR5a1* (5α-reductase). If reduced testosterone biosynthesis were the operant MOA, one or more of these genes would be affected. Also, the hormone panel data would have shown a sustained decrease in circulating levels of testosterone, which was not observed in this study. Taken together, these data, as well as a lack of female reproductive effects, refute decreased steroidogenesis as the operant MOA.

g) Increased biliary elimination of testosterone would cause lower testosterone levels and increased LH levels, resulting in the development of Leydig cell tumours. Based on known nuclear receptor–mediated liver effects with sulfoxaflor administration, this MOA was assessed, and direct data were provided from the Leydig cell tumour MOA study. There were no statistically significant or treatment-related differences in the mean [^{14}C]testosterone-derived radioactivity excreted in the bile across all dose groups, per time interval, and bile flow was very similar for the respective dose groups and time intervals. Thus, these data clearly refute biliary elimination of testosterone as the operant MOA.

h) Gonadotropin releasing hormone (GnRH) (luteinizing hormone releasing hormone, or LHRH) agonism would cause both reduced accessory sex gland weights (due to negative feedback HPG axis compensation) as well as histopathological effects in the pituitary gland, as this is the primary site of functional GnRH receptor expression. As mentioned previously, there were no effects on accessory sex gland weights in the sulfoxaflor two-generation reproductive toxicity study, as well as no treatment-related effects on the pituitary gland in any rat toxicity study, including the 2-year rat carcinogenicity study. Based on the weight of evidence, considering indirect data showing no effect on the pituitary gland, a GnRH agonism MOA is not plausible for the Leydig cell effects seen in Fischer 344 rats after 2 years of treatment with sulfoxaflor.

9. Uncertainties, inconsistencies and data gaps

a) Key event #1 (increased dopamine release via agonism on central dopaminergic neuron nAChRs): Owing to the technical and biological complexity of measuring neurotransmitters within the hypothalamic–hypophyseal portal vein system, key event #1 has been confirmed only after intracerebral infusion of sulfoxaflor into the hypothalamus at relatively high concentrations (0.4 and 2 mmol/l). It remains unclear whether and to what extent increases in dopamine release would occur at the dose levels that were associated with increased Leydig cell tumours in the 24-month study of chronic toxicity and carcinogenicity in rats (21.3 mg/kg bw per day).

b) Key event #4 (transient decreases in serum testosterone): Under the conditions of the 8-week mechanistic study in Fischer 344 rats, there were no measurable decreases in serum testosterone. However, the delay in balanopreputial separation in the two-generation reproductive toxicity study may be considered to provide indirect evidence for a transient decrease in testosterone. Although these data are supportive, there are no hormone measurement data that confirm a decrease in serum levels of testosterone.

c) The dose–response relationships for most of the key events have been established only incompletely, as effects were observed only at 500 ppm (equal to 21–28 mg/kg bw per day), but not at 100 ppm (equal to 4.2–5.6 mg/kg bw per day). However, as a result of the high background incidence of Leydig cell tumours in Fischer 344 rats, the lack of precursor key events for this subtle, hormone-based MOA at the lower dietary concentration of 100 ppm is not surprising, given the transient and compensatory nature of hormone regulation in the HPG axis.

d) The temporal relationships for the key events have not been established adequately, as in the 8-week mechanistic study in Fischer 344 rats, effects on serum prolactin levels, LHR gene expression in Leydig cells and serum LH levels were demonstrated only at the 4-week time point, but not at the 2-week and/or 8-week time points. In addition, effects on serum testosterone levels have not been demonstrated at any time point.

10. Assessment of postulated mode of action

The data provided support a non-genotoxic, threshold-based MOA for the development of Leydig cell tumours in rats following chronic exposure to sulfoxaflor. However, the key events for the proposed MOA of sulfoxaflor, the dose–response relationships and the temporal relationships to tumour formation have been identified or demonstrated only in part. For that reason, the weight of evidence is not sufficient to establish the proposed MOA (dopamine agonism) for the Leydig cell tumours induced in rats after chronic exposure to sulfoxaflor.

11. Conclusion

The relevance of the increased Leydig cell tumours in male Fischer 344 rats to humans cannot be discounted, as the results of the mechanistic studies were only partly sufficient to support the proposed hypothesis that sulfoxaflor can act as a dopamine agonist in the central nervous system. In particular, the increased dopamine release at the median eminence of the hypothalamus into the hypothalamic–hypophyseal portal veins to inhibit prolactin release in the anterior pituitary has been demonstrated only after intracerebral infusion of sulfoxaflor into the hypothalamus at relatively high concentrations (0.4 and 2 mmol/l), and there is a lack of a dose–response relationship and/or temporal concordance with key precursor events (e.g. decrease in serum prolactin level, downregulation of LHR on Leydig cells, decrease in serum testosterone and compensatory increase in serum LH level) and tumour incidence. However, the Leydig cell tumours occurred only at high doses, did not occur in mice and would be anticipated to exhibit a threshold.

For the risk assessment of sulfoxaflor, it must therefore be considered that the effect in question is subtle in nature, and the background incidence of Fischer rat Leydig cell tumours is 75–100% in 2-year studies compared with 1–5% in CD rats, even less in CD-1 mice, and orders of magnitude lower (in ranges of less than 0.01%) for 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 more than 10-fold more LHR than those of 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 prolactin and GnRH receptors on their surface. Stimulation of rat Leydig cells through both prolactin and GnRH receptors is a rat-specific mechanism

by which Leydig cell tumour formation can occur. For prolactin receptor involvement in Leydig cell tumours, dopamine agonists (e.g. muselergine) reduce prolactin release by the anterior pituitary gland. This results in decreased binding of prolactin to prolactin receptors on Leydig cells, leading to downregulation of the LHR and transient reductions in testosterone production, which feeds back to induce LH release from the pituitary, leading to Leydig cell stimulation and hyperplasia over time.

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